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The Importance of Keeping the Big Ones: Harvest Slot Limits and Marine Protected Areas for the Management of the Caribbean Spiny Lobster

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THE IMPORTANCE OF KEEPING THE BIG ONES: HARVEST SLOT LIMITS
AND MARINE PROTECTED AREAS FOR THE MANAGEMENT OF THE
CARIBBEAN SPINY LOBSTER

by

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Mark J. Butler, IV (Director)
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ABSTRACT

THE IMPORTANCE OF KEEPING THE BIG ONES: HARVEST SLOT LIMITS AND MARINE PROTECTED AREAS FOR THE MANAGEMENT OF THE CARIBBEAN SPINY LOBSTER

Gayathiri Gnanalingam
Old Dominion University, 2018
Director: Dr. Mark J. Butler IV

Fishing typically removes the oldest and/or largest individuals from populations undermining stability and reproductive success. Traditional fisheries management tools fail to protect these oldest and/or largest individuals, but two less conventional tools: marine protected areas (MPAs), and harvest slot limits have the potential to do so. Here I tested the possible use of these tools for the Caribbean spiny lobster, *Panulirus argus*, an iconic and economically valued species. After decades of intense fishing, the largest lobsters have largely been wiped out. The loss of the largest lobsters is significant as large lobsters have considerably greater reproductive potential than their smaller counterparts. I had four main objectives (1) developing a technique for directly ageing *P. argus* using banding in the gastric ossicles, (2) examining the possibility of reproductive senescence as it relates to body size in *P. argus*, (3) modeling the potential use of harvest slot limits and MPAs using a two-sex stage-structured matrix model, and (4) assessing the possible ecological consequences in terms of interactions with prey-species, of increasing the abundance and size of *P. argus* through a series of cafeteria trials. This work provides some necessary background information to support using MPAs and harvest slot limits in the management of *P. argus* in the Caribbean - calls for which has grown appreciably in recent years. Direct ageing of *P. argus* using bands in the gastric ossicles proved successful as it was possible to validate the ages of wild caught lobsters with lobsters of known age. The success of
this technique opens up the potential for age based stock assessment and consideration of the relationship between size, age and reproduction. Lobsters were not found to exhibit reproductive senescence and for several of the metrics tested there was a positive relationship with parental size, confirming the biological value of retaining the largest lobsters in populations. The modeling demonstrated clearly the potential for MPAs and slot limits combined to increase the sizes and densities of *P. argus* in the marine environment, while the cafeteria trials demonstrated that larger lobsters did not have any appreciable preference for species of high ecological value.
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# TABLE OF CONTENTS

| LIST OF TABLES | ix |
| LIST OF FIGURES | x |

## Chapter

1. **INTRODUCTION**
   1.1 GENERAL INTRODUCTION .................................................. 1
   1.2 REPRODUCTION IN MARINE ORGANISMS ................................... 1
   1.3 CONSERVING SPAWNING STOCK ............................................. 6
   1.4 THE CARIBBEAN SPINY LOBSTER ........................................... 10

2. **DIRECT AGEING**
   2.1 INTRODUCTION .............................................................. 18
   2.2 METHODS .......................................................................... 20
   2.3 RESULTS ............................................................................ 25
   2.4 DISCUSSION ........................................................................ 33

3. **REPRODUCTIVE SENESCENCE**
   3.1 INTRODUCTION ................................................................. 38
   3.2 METHODS ............................................................................ 43
   3.3 RESULTS ............................................................................. 50
   3.4 DISCUSSION ........................................................................ 60

4. **MODELING MPAS AND HARVEST SLOT LIMITS**
   4.1 INTRODUCTION ................................................................. 68
   4.2 METHODS ............................................................................ 72
   4.3 RESULTS ............................................................................. 83
   4.4 DISCUSSION ........................................................................ 99

5. **FEEDING ECOLOGY**
   5.1 INTRODUCTION ................................................................. 105
   5.2 METHODS ............................................................................ 107
   5.3 RESULTS ............................................................................. 109
   5.4 DISCUSSION ........................................................................ 113

6. **CONCLUSIONS** ................................................................. 118

**LITERATURE CITED** .............................................................. 123
APPENDICES
A. A NON-INVASIVE TECHNIQUE FOR ESTIMATING FECUNDITY .............148
B. CO-AUTHORSHIP STATEMENT .................................................................160

VITA .............................................................................................................161
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Summary of management tools currently in place for <em>Panulirus argus</em> in the Caribbean and the Atlantic coast of South America</td>
<td>15</td>
</tr>
<tr>
<td>2. Coefficient of Variation (CV) and Pearson’s Correlation Coefficient (R) estimates for readers estimating age of 34 randomly selected <em>Panulirus argus</em> sections</td>
<td>32</td>
</tr>
<tr>
<td>3. Coefficient of Variation (CV) and Pearson’s Correlation Coefficient (R) estimates for readers estimating age of 22 known age <em>Panulirus argus</em> sections</td>
<td>32</td>
</tr>
<tr>
<td>4. Effect sizes and p-values for models testing spermatophore attributes</td>
<td>52</td>
</tr>
<tr>
<td>5. Effect sizes and p-values for models testing female attributes</td>
<td>56</td>
</tr>
<tr>
<td>6. Effect sizes and p-values for models testing larval attributes</td>
<td>59</td>
</tr>
<tr>
<td>7. Data sources and biological parameters</td>
<td>75</td>
</tr>
<tr>
<td>8. Life history stages and codes for <em>Panulirus argus</em> used in the matrix model</td>
<td>78</td>
</tr>
<tr>
<td>9. Management scenarios and fishing restrictions applied to each model run for the 10 <em>P. argus</em> populations</td>
<td>81</td>
</tr>
<tr>
<td>10. Uncertainty used in stochastic simulations</td>
<td>83</td>
</tr>
<tr>
<td>11. Summary table from deterministic model runs incorporating management scenario and fishing effort on total abundance (N), spawning stock abundance, reproductive output, number of migrants, and λ for the 10 populations combined over 30 years</td>
<td>91</td>
</tr>
<tr>
<td>12. Results of GLMs testing relationship between lobster carapace length (CL; mm) and prey size (mm) relative to consumption</td>
<td>114</td>
</tr>
<tr>
<td>13. Fecundity estimates using non-invasive and traditional methods</td>
<td>156</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Cutting axes used for each of the structures examined: mesocardiac, pterocardiac, and zygocardiac gastric ossicles and eyestalks</td>
<td>21</td>
</tr>
<tr>
<td>2. Sections from a 106.4 mm carapace length female from the Florida Keys</td>
<td>27</td>
</tr>
<tr>
<td>3. Sections from the zygocardiac ossicles of two known age individuals: (A) Female 165 mm carapace length, age 10; (B) Male 180 mm carapace length, age 7</td>
<td>27</td>
</tr>
<tr>
<td>4. Sections from the pterocardiac ossicles of two individuals tagged with calcein</td>
<td>28</td>
</tr>
<tr>
<td>5. (A) Postmolt lobster dissected within hours of ecdysis, (B) Gastric mill dissected from exuviae within hours of molting, (C) Gastric mill dissected from an individual 12 hours after molting, (D) Gastric mill dissected from an individual 72 hours after molting</td>
<td>29</td>
</tr>
<tr>
<td>6. (A) Reader bias between four independent readers using the smaller lobe (B) of the zygocardiac ossicles (n = 14). (B) Known age and estimated age (n = 22) from readings of zygocardiac ossicles by five independent readers</td>
<td>31</td>
</tr>
<tr>
<td>7. The relationships between size of <em>P. argus</em> males and spermatophore attributes including: (A) spermatophore weight, (B) spermatophore area, (C) sperm density per gram, and (D) the proportion of non-viable sperm cells</td>
<td>51</td>
</tr>
<tr>
<td>8. Mean protein content for spermatophores relative to male carapace length (mm) and spermatophore number (A); and protein content of eggs relative to female carapace length (mm) and clutch number (B)</td>
<td>53</td>
</tr>
<tr>
<td>9. (A) Estimated fecundity relative to female carapace length (mm) per clutch</td>
<td>55</td>
</tr>
<tr>
<td>10. The relationships between female carapace length (mm) and larval attributes per clutch: (A) larval carapace length (mm), (B) swimming distance cm per 10s, (C) days to 50% mortality, and (D) days to 100% mortality</td>
<td>58</td>
</tr>
<tr>
<td>11. Stage based matrix model of reproductive and non-reproductive cycles used for ten countries that harvest <em>P. argus</em> in the Western Atlantic. Country codes: HAI = Haiti, BEL = Belize, DR = Dominican Republic, HON = Honduras, MEX = Mexico, BAH = Bahamas, CUB = Cuba, VEN = Venezuela, NIC = Nicaragua, USA = United States of America</td>
<td>74</td>
</tr>
<tr>
<td>12. Reproductive matrix used in stage based model</td>
<td>79</td>
</tr>
<tr>
<td>13. Example of how fishing effort influenced total abundance (N) using Scenario 1 – Fishing, for the 10 populations combined</td>
<td>85</td>
</tr>
</tbody>
</table>
14. Effect of management scenario and fishing effort on total abundance ($N$) for the 10 countries combined over 30 years .......................................................................................................................... 87

15. Effect of management scenario and fishing effort on spawning stock abundance for the 10 countries combined over 30 years ......................................................................................................................... 88

16. Effect of management scenario and fishing effort on total egg production (reproductive output) for the 10 populations combined over 30 years ........................................................................................................... 89

17. Biomass of fishery sized lobsters (> 75 mm carapace length) at Year 30 for each management scenario and the 10 populations combined. Models were run at mid fishing effort ($F = 0.34$) .................................................................................................................................................. 90

18. Relative change in total abundance ($N$) as Year 30/Year 0 for each of the management scenarios at low, mid, and high fishing efforts ........................................................................................................................................ 93

19. Relative change in spawning stock abundance as Year 30/Year 0 for each of the management scenarios at low, mid, and high fishing efforts ........................................................................................................................................ 94

20. Relative change in total egg production (reproductive output) as Year 30/Year 0 for each of the management scenarios at low, mid, and high fishing efforts ........................................................................................................................................ 95

21. Stock structure of fisheries sized lobsters (> 85mm CL) at Year 30 for each of the management scenarios run at mid fishing effort ($F = 0.34$) ........................................................................................................................................ 96

22. Box plot of variation in (A) total abundance ($N$), (B) spawning stock abundance, (C) reproductive output and (D) emigrant mortality as a result of stochasticity (natural mortality ($M$), larval mortality, fecundity, emigrant mortality, combination of the previous four). ........................................................................................................... 98

23. Organic content of prey species (in grams; g) relative to prey size: panel A: Mangrove oyster, *Crassostrea gasar*; panel B: Long spined sea urchin *Diadema antillarum*; panel C: Pencil urchin *Eucidaris tribuloides*; panel D: Clams *Mercenaria mercenaria*; panel E: West Indian Star Snail *Lithopoma tectum* .................................................................................................................................................. 111

24. Mean Rogers’ Index values for the six prey species offered to large lobsters (> 100 mm CL; $n = 31$) and small lobsters (< 90 mm CL; $n = 23$) .................................................................................................................................................. 112

25. Measurements taken to estimate fecundity: length and height of segments ........................................ 151

26. Estimated mean clutch size (non-invasive method) and carapace length (log transformed) for 102 female *Panulirus argus* (63–141 mm carapace length) sampled from the Florida Keys, FL .................................................................................................................................................. 155
27. Estimated mean clutch size for different sizes of female *Panulirus argus* using the non-invasive method and previously published studies for Mexico (Fonesca-Larios & Briones-Fourzán, 1998), Florida (Cox & Bertelsen, 1997), Brazil (Nascimento & Araújo, 1984), and Cuba (Cruz *et al.*, 1987).
CHAPTER 1

INTRODUCTION

1.1 General Introduction

Globally, the landings of marine fisheries have been declining since the 1980s (Pauly et al. 2002, Worm et al. 2006). The percentage of fish stocks that are within biologically sustainable levels also continues to decline such that 58% of all stocks assessed by the Food and Agriculture Organization of the United Nations are considered ‘fully fished’ (FAO 2016). The majority of the world’s fisheries are unregulated, open access fisheries, or de-facto open access fisheries given the lack of compliance or enforcement (Agnew et al. 2009, FAO 2016). Overfishing is thus common. Even where attempts are made to regulate fisheries, data deficiencies, socio-economic conditions, and/or political apathy result in arbitrary regulations that are ineffective or detrimental to long term stock sustainability. This is especially true in developing countries where resources for rigorous research do not exist (Júnior et al. 2016). Ecological data are essential to the long term management of marine fisheries (Pikitch et al. 2004, Beddington et al. 2007). Without a clear understanding of a species’ life history and its role in the broader ecosystem, we cannot understand the true impact of fishing nor modify or adapt our management to better suit ecosystem and fishery stability (Zukowski et al. 2012).

1.2 Reproduction in Marine Organisms

One of the key life history traits that is often poorly understood, resulting in mismanaged fisheries, is reproduction. Given the importance of reproduction to the sustainability of fisheries
and targeted species, it is alarming that we attempt to manage fisheries without a robust understanding of reproduction and its nuances. Fishing typically removes the oldest and/or largest individuals from exploited populations, a tendency compounded by commonly used minimum harvest size restrictions (Rowe and Hutchings 2003, Williams and Shertzer 2005, Tsikliiras and Polymeros 2014). This differential selection creates severe size/age truncation of wild populations resulting in populations in which the largest/oldest individuals are conspicuously absent - often with negative consequences for the production of offspring (Berkeley et al. 2004, Birkeland and Dayton 2006). Furthermore, fishing induced size selectivity can induce the evolution of negative traits associated with growth, maturation, and reproduction (Law 2000, Conover and Munch 2002). Where fishing differentially removes more of one sex than the other, negative consequences for mate selection or fertilization success often ensue (Kendall and Quinn 2013).

**Effect on mating structure**

The targeted removal of an exploited population’s largest or oldest individuals can disrupt social hierarchies, mate choice, and sexual competition (Whitman et al. 2004, Lane et al. 2011), eventually undermining the population's stability and reproductive success. For example, Atlantic cod (*Gadus morhua*) form leks for reproduction, in which larger more aggressive males defend territories separate from female aggregations (Nordeide and Folstad 2000, Windle and Rose 2007). If these larger, more aggressive males are more likely to be fished, then the functionality of these leks and thus their reproductive success is compromised (Rowe and Hutchings 2003). For species with sequential hermaphrodites (i.e., the majority of parrotfishes; Scaridae), in which females change into terminal phase males, fishing that targets the largest
sizes disproportionately removes terminal phase males (DeMartini and Howard 2016). This can have several consequences including a reduction in size at maturity for both males and females, a reduced size at sex change, skewed sex ratios, sperm limitation, and reproductive failure (Hamilton et al. 2007).

**Allee effects**

If a substantial number of individuals are removed from a population those that remain may be unable to reproduce if their population has fallen below some critical threshold density (Gascoigne and Lipcius 2004). This phenomenon, termed an Allee effect in ecological literature (Allee 1931) and depensation in fisheries literature (Hutchings 2014), describes a decline in fitness as it relates to a decline in population size or density (Stephens et al. 1999; Gascoigne et al. 2009). The mechanisms underscoring Allee effects include an inability to find a mate, low fertilization success, and reduced genetic variation in offspring (Rowe and Hutchings 2003). Eventually this inhibits population growth, making biological extinction a real possibility. Allee effects are particularly worrisome for broadcast spawners with limited or no mobility because at low densities the potential for gametes to meet in the water become increasingly less likely (Gascoigne and Lipcius 2004). After decades of sustained harvest, several abalone species on the west coast of North America including the white abalone, *Haliotis sorensi*; the black abalone *Haliotis cracherodii*, and the northern pinto abalone *Halitois kamstchatkana*, have been reduced to extremely low densities and recruitment failure and Allee effects have been identified as being one of the main threats to the species’ long term sustainability (Rothaus et al. 2008, Stierhoff et al. 2012). Examples of Allee effects, however, are not limited to broadcast spawners. Small aggregations of the spotted spiny lobster (*Panulirus guttatus*) stranded on isolated patch reefs
due to the lobster’s strong site fidelity, experience Allee effects in the form of highly variable reproductive success due to the availability of suitable sized mates (Robertson and Butler 2009). In the heavily fished Caribbean Queen conch *Lobatus gigas*, which reproduces by way of internal fertilization, there is a positive relationship between density and per capita reproductive activity (Stoner and Ray-Culp 2000). A number of studies also suggest that Allee effects have accelerated rates of population decline in the heavily fished Atlantic cod, *G. morhua* (Gascoigne et al. 2009, Kuparinen et al. 2014).

*Sperm limitation*

Mate availability is compromised in heavily fished populations not only by mate abundance, but also by the availability of mates of the appropriate size. Some marine organisms maximize their reproductive output by preferentially coupling with large males, or multiple males, so as to ensure sufficient sperm for fertilization (e.g. Sato and Goshima 2007). This poses problems in fished populations where the oldest or largest individuals have been removed, so that females are unable to maximize their reproductive output. Sperm limitation has been documented in a number of exploited populations, particularly those in which males are selectively fished (e.g. blue crab *Callinectes sapidus* in the Chesapeake Bay; Hines et al. 2003, Carver et al. 2005, Ogburn et al. 2014), the Chilean rock crab *Metacarcinus edwardsii* in southern Chile (Pardo et al. 2015), and the American clawed lobster *Homarus americanus*. In the latter case, the absence of large males has resulted in females mating multiple times in what is hypothesized as an attempt to deal with a reduced sperm supply (Goldstein et al. 2014).

*Reductions in size at maturity, egg production, offspring quality*
The sustained and excessive fishing of a population’s largest and typically fastest growing individuals often observed in sperm limited populations also imposes a selective pressure on these individuals. Over time, selective harvesting can favor the selection of genotypes with slower growth or early sexual maturity, resulting in a reduction in population productivity over time (Law 2000, Conover and Munch 2002, Jorgensen et al. 2007, Olsen et al. 2011). In Atlantic silversides, *Menidia mendia*, the harvesting of the largest 90% of individuals over four generations resulted in the evolution of fishes with a reduced biomass and slower growth (Conover et al. 2005). The reduction in size at maturity has one obvious effect, particularly for species where egg production scales relative to body size: the fecundity of individuals that mature at a smaller size will be lower than the fecundity of individuals that mature at a larger size (Baskett et al. 2005, Green 2008). Additionally, in some species the quality of offspring may be detrimentally affected. Maternal effects in which a mother’s phenotype directly affects offspring fitness (Bernado 1996), while is by no means universal, has been documented in a diverse taxonomic range of species including rockfishes *Sebastes spp* (Berkeley et al. 2004, Stafford et al. 2014), ascidians *Ciona intestinalis* (Marshall and Keough 2003), bryozoans *Bugula neritina* (Marshall et al. 2003) and haddock *Melanogrammus aegelfinus* (Hislop 1988). In the rockfish, *Sebastes melanops*, older mothers produce offspring of a higher quality because their larvae contain larger oil globules, hence better larval provisioning and survival (Berkeley et al. 2004). The fishing of larger individuals therefore results in the removal of older mothers who produce larvae that are more likely to survive. Larger/older females also often have earlier or longer spawning seasons than smaller/younger females (Wright and Trippel 2009, Hixon et al. 2014), facilitating temporal and sometimes spatial bet hedging that aids larval survival in variable environmental conditions (Hsieh et al. 2010).
1.3 Conserving Spawning Stock with Fisheries Management

Fisheries management tools that specifically aim to conserve spawning stocks of older/larger marine organisms are uncommon. Traditional fisheries management measures control catch (i.e. quotas, bag limits) or effort (i.e., number and size of vessels, closed seasons, temporary closures) but these approaches are typically applied uniformly across organism size classes and have failed time and again to guard against overfishing (Roberts and Polunin 1991). Mechanisms that aim to protect spawning stock, such as seasonal closures during breeding seasons and prohibitions on the take of ovigerous females meanwhile, usually only provide protection to those individuals for that particular breeding season. A notable exception being the ‘V-notching’ of the telson of ovigerous female lobsters caught and released by fishermen in the American Clawed Lobster (*Homarus americanus*) fishery in New England (DeAngelis 2010, Acheson and Gardner, 2011), a practice that prohibits the harvest of V-notched, reproductive females. However, two mechanisms are broadly designed to address these issues: size limits and marine protected areas (MPAs), both of which can demonstrably provide lasting protection for older/larger individuals.

*Size Limits*

Regulations that restrict catch based on size are common in fisheries management (FAO 2012). While they do not explicitly limit the total number of individuals caught, they can influence catch composition (Liu et al. 2016). Minimum size limits where only individuals above a designated size can be harvested are primarily designed to protect juveniles and prevent recruitment overfishing (the reduction of a spawning stock beyond a point at which it can
replenish itself) by allowing fish to spawn at least once prior to harvesting (Allen et al. 2013). They are especially well-suited to populations with low recruitment where juvenile mortality is high (FAO 2012). Minimum size limits are simple regulations to implement in that they are easily understood and enforced (Hill 1992). Their broad appeal is demonstrated by the number of fisheries that use them.

Maximum size limits, where only individuals below a stated size can be harvested are less common in marine commercial fisheries although they are widely employed in freshwater and marine shallow water recreational fisheries. Maximum size limits aim to reduce abundance and competition among small fish as well as protect large fecund spawners (FAO 2012). They are particularly well suited to species that exhibit high recruitment, slow growth and moderate natural mortality (FAO 2012). Their use is limited however, for species with low post-capture survival (e.g., deep water species that suffer barotrauma during harvest; Kerwath et al. 2013).

Combinations of both minimum and maximum size limits result in ‘slot limits’ where individuals of an intermediate range maybe harvested (harvest slot limit, open-slot) or protected (protected slot limit, closed-slot) (Gwinn et al. 2013). Harvest slot limits are designed to protect both young recruits and spawning stock, and are particularly useful when size-dependent maternal effects influence recruitment, or when fishing depletes spawning biomass (McPhee 2008, Arlinghaus et al. 2010, FAO 2012). They may also provide a means of maintaining a high harvest – an important consideration for commercial and recreational fisheries (Gwinn et al. 2013). Slot limits are not common however and are considered difficult to implement (Hixon et al. 2014). In some instances, their use has been controversial (e.g., protected slot limits for walleye, Sander vitreus, and smallmouth bass, Micropterus dolomieu in some freshwater systems
in the United States) because they are seen as prohibiting anglers from harvesting fish of the size they most prefer (Carlin et al. 2012, Fincel et al. 2015).

*Marine Protected Areas*

Harvest size limits are a management tool specifically designed to regulate the fishing of a single species. Marine protected areas, on the other hand, have a multitude of uses (e.g., preservation of cultural artifacts, sensitive habitats, or biologically important locations such as spawning aggregation sites) and are an ecosystem-based form of multi-species management (Halpern et al. 2010). Although MPAs are typically not considered a fisheries management tool, their use as a method of controlling harvests and conserving marine resources is increasing worldwide (Roberts and Polunin 1991, Dayton et al. 2000, Pande et al. 2008). MPAs have been credited with increasing the density, biomass, size, and diversity of a number of target species (see Halpern and Warner 2002, Halpern 2003 for reviews). Of relevance to the conservation of spawning biomass, MPAs can rebuild and protect larger mature individuals especially for sedentary or philopatric species such as abalone, limpets, and lobster (Rogers-Bennett et al. 2002, Branch and Odendall 2003, Shears et al. 2006, Jack and Wing 2013). As a consequence, egg production within protected populations ought to increase because of higher densities of individuals with a more mature age/size structure (Jack and Wing 2010).

*Slot Limits and MPAs together*

Despite the growing acceptance and implementation of MPAs and evidence that they harbor larger individuals than what normally occur in heavily fished areas, MPAs alone are not likely to be the solution to rebuilding spawning stocks depleted by decades of overfishing.
Marine protected areas are likely to remain too small and too few to provide adequate replenishment of populations outside of reserves (Goñi et al. 2011). Slot-limits alone may also be ineffective in rebuilding stocks of large, reproductive individuals if the population is heavily fished and thus the probability of surviving the intense fishing gauntlet while of a smaller, legal size is minimal. What is more likely to provide long lasting protection is a suite of regulations working in concert including a combination of slot limits with MPAs and controls on fishing mortality. This combination may serve to rebuild spawning stock throughout the entire seascape, and not just within the confines of MPAs (Steneck et al. 2009).

**Spiny lobsters and slot limits**

Spiny lobsters (Palinuridae) are a family of morphologically, ecologically, and behaviorally diverse species, widely distributed through temperate and tropical systems from shallow waters to extreme depths (Ptacek et al. 2001, Lavalli and Spanier 2010). The family consists of eight genera and over 47 species, 33 of which are commercially harvested (Holthius 1991, Lipcius and Eggleston 2000). Fisheries for panulirids are some of the most economically valuable in the world with major fisheries located in Australia, USA, South Africa, New Zealand, and the Caribbean (Lavalli and Spanier 2010, Phillips et al. 2013). Panulirids additionally support a number of artisanal and recreational fisheries worldwide (Lipcius and Eggleston 2000). Given that spiny lobsters are large, long lived coastal species supporting intense and valuable fisheries globally, and are robust to handling and trap capture, they are good candidates for exploring the application of slot limits and MPAs for their management. To that end, my focus here, is on the Caribbean spiny lobster, *Panulirus argus*. 
1.4 The Caribbean Spiny Lobster

This species is distributed throughout the Western Atlantic Ocean, Caribbean Sea, and Gulf of Mexico (Holthius 1991). The wide distribution of the species is a result of the widely dispersing phyllosoma larvae which spend 6-10 months in the plankton and thus are capable of being transported long distances by oceanic currents until they are large enough to settle (Goldstein et al. 2008, Kough et al. 2013). As a result, *P. argus* has long been considered as forming a single, Pan-Caribbean meta-population. Genetic analyses generally support this hypothesis (Silberman et al. 1994, Naro-Maciel 2011) although the most recent genetic techniques have revealed some genetic structure in areas of the Caribbean where oceanographic conditions favor self-recruitment (Truelove et al. 2015, 2017).

Juvenile *P. argus* have three ecologically distinct phases following settlement: algal, post-algal, and adult (Herrnkind and Butler 1986, Butler and Herrnkind 1997, Butler and Herrnkind 2001) and display ontogenetic habitat shifts from shallow macroalgal nursery habitat to offshore reefs as they mature (Saul 2004). The shift in habitat also marks a shift in sociality: asocial juveniles become gregarious as they move out of the algal nursery habitat (Butler et al. 1997, Childress and Herrnkind 1997). As adults they form aggregations in dens provided by rock or coral ledges (Butler et al. 2006). *Panulirus argus* are ecologically important as large abundant predators in benthic habitats (Cox et al. 1997, Briones-Fourzán et al. 2003, Nizinkski 2007). Their selective predation plays a major role in influencing species composition and the size frequency distributions of invertebrates such as sea urchins, mussels, isopods, ostracods, and gastropods (Herrnkind et al. 1988, Nizinksii 2007). In turn, *P. argus* are prey for larger predators including finfish, sharks, and octopus (Smith and Herrnkind 1992, Berger and Butler 2001, Butler and Lear 2009).
Panulirus argus are sexually dimorphic with males distinguishable from females by their broader sternum, elongated second walking legs, curved dactyls, and raised genital openings (gonopores) at the base of the fifth pair of walking legs (Holthius 1991). Size at sexual maturity varies throughout the Caribbean and this variance is generally attributed to differences in environmental conditions, density, and fishing intensity (Chubb 2000). For example, ovigerous females smaller than 80mm carapace length (CL) are typically not observed in the Dry Tortugas National Park (Florida, USA) where fishing is prohibited, but are commonplace in fished areas (Bertelsen and Matthews 2001, Maxwell et al. 2009). Although individuals as small as 57mm CL have been observed with eggs (Maxwell et al. 2009) estimates for size at 50% maturity vary by location and range from 75mm CL in Florida to 92mm CL in Colombia (Hunt and Lyons 1986, FAO 2001).

Individual fecundity is closely tied to body size, with larger females producing exponentially more eggs (Ehrhardt 2005, MacDiarmid and Sainte-Marie 2006). Females typically move to the edges of reefs or coastal shelves to incubate and release larva (Nemeth 2009). Throughout much of the Caribbean spawning occurs year-round (Butler et al. 2010), though in more subtropical areas where spawning is correlated to temperature and photoperiod P. argus has a more defined breeding season during the spring-summer or summer-autumn (Chubb 2000). During this defined spawning period, lobsters may produce multiple clutches, with larger females typically producing more clutches and spawning earlier in the season than smaller females (MacDiarmid and Butler 1999, Butler et al. 2015).

Fisheries for P. argus are some of the largest and most economically valuable in the Caribbean, with an estimated annual regional value in excess of $450 million USD (CRFM 2013). It forms the major fishery of 24 Caribbean nations, employing an estimated 50,000 fishers
and an additional 200,000 in fishery related jobs in the region (CRFM 2011). Four countries (The Bahamas, Cuba, Brazil and Nicaragua) are currently responsible for 76% of total global production (Chavez 2009) with major import markets being the US, European Union, and more recently China (FAO 2017). Capture methods vary, even within the same country, and include: traps, pots, scuba, nets, spears, hooks, noose and artificial structures known as casitas, condominiums or pesqueros (CRFM 2011, Gutzler et al. 2015). As a consequence of their high value and market demand, many regional populations are currently fully-capitalized or overfished (Ehrhardt et al. 2010). Regional landings peaked in the early 1990s around 36,000 metric tons but have since declined by 55% (CRFM 2011). Exacerbating the decline is a lack of scientific and institutional capacity, poor socio-economic conditions, open access fishing, and limited enforcement capability (Ehrhardt 2005, Chavez 2009).

**Fishery effects on *P. argus* reproduction**

The effects of decades of intense fishing of *P. argus* throughout the Caribbean has taken its toll on the mating dynamics and reproductive success of the species. Size selective fishing has all but wiped out the largest individuals in a large swath of the Caribbean with the exception of well enforced marine protected areas such as the Dry Tortugas National Park. It is only in these unfished zones that the polygynous, lek-like mating structure in which large males defend a den from other large males and females chose among them, are still preserved (MacDiarmid and Butler 1999, Butler et al. 2015). For the majority of lobsters, this structured mating system has been replaced by scramble competition for mates among smaller animals. The loss of large individuals has impacts that go beyond the breakdown of this ancestral mating structure: sperm limitation, a reduction size at maturity, and reduced fecundity are also evident in heavily
exploited *P. argus* populations. Large males produce larger, heavier spermatophores and have the ability to scale spermatophore size relative to their female partner (MacDiarmid and Butler 1999, Butler et al. 2015). Large females that produce exponentially more eggs than their smaller counterparts also preferentially select large males and in the absence of these individuals are forced to mate with smaller males resulting in reduced fertilization (MacDiarmid and Butler 1999, Butler et al. 2015). The fishing effect on size at sexual maturity in *P. argus* is no more obvious than when one compares the unexploited population in the Dry Tortugas National Park with the heavily exploited population in the Florida Keys (Bertelsen and Matthews 2001, Maxwell et al. 2009). Females from the Dry Tortugas become sexually mature at a much larger size (close to 100mm CL) than those from the Florida Keys, which typically mature at around 75 mm CL, although egg-bearing females as small as 57 mm CL have been observed (Chubb 2000). Such differences are unlikely to be the result of differential genetic selection between the two populations given the species' protracted larval duration and distant dispersal (Goldstein et al. 2008). Instead, the more likely hypothesis is that intense fishing removes the fastest growing early maturing individuals from the population as they reach the minimum harvestable size. With a smaller size at maturity and the absence of large males to fertilize the clutches of large females, a reduction in individual and population fecundity is the likely consequence (Ehrhardt 2005).

Added to this is evidence of positive maternal effects that link increased maternal size to enhanced offspring fitness (Gnanalingam and Butler 2018a) and the detrimental impact that over-fishing has in removing the largest individuals from the population, further diminishing reproductive output.

*Current management of P. argus fisheries*
Management of *P. argus* fisheries is complicated by the species’ long postlarval duration, distant dispersal, and hence widespread distribution of the species in the Caribbean. At present there are no standardized management measures specific to *P. argus* on a regional scale. Instead, most Caribbean nations that target *P. argus* do so unilaterally with a range of regulations (FAO 2015; Table 1). In recent years more emphasis has been placed on regional cooperation culminating with the Spiny Lobster Declaration 2015, which has been heralded as a roadmap for closer regional collaboration on lobster management (CRFM 2015). Thus far, the most common regulations applied in the management of *P. argus* fisheries include: seasonal closures, minimum harvest sizes, and prohibitions on the harvesting of lobsters that are ovigerous or recently molted. Though two of the three of these (seasonal closures and prohibitions on the fishing of ovigerous females) aim to protect spawning stock, they do not provide adequate long-term protection. Meanwhile, minimum harvest sizes compel harvesters to target large mature individuals.
Table 1. Summary of management tools currently in place for *Panulirus argus* in the Caribbean and the Atlantic coast of South America. Color indicates the presence of the regulatory measure. MLS = Minimum legal size.

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Protecting the Big Ones: Size slot limits and MPAs for P. argus

Harvest slot limits and MPAs when applied in concert offer a potential means of protecting the most fecund P. argus and increasing reproductive output, whilst allowing fishers to continue fishing (Steneck et al. 2009). Although no-take MPAs have the potential to offer blanket protection to spawning individuals, pragmatically their implementation and enforcement are mired by the socio-economic conditions of the region. No take MPAs alone are likely to be too few, too small, and lacking in adequate enforcement to substantially increase population densities. No take MPAs applied in conjunction with harvest slot limits, however, extend protection from harvest to larger spawning individuals that move into, or that are resident in, unprotected areas (Steneck et al. 2009). Within this context I set out to examine the potential for implementing MPAs and harvest slot limits in tandem for the management of P. argus, and the implications of doing so from ecological and management perspectives.

First (Chapter 2), I outline development of a technique for directly ageing P. argus using banding in the gastric ossicles located within the lobster stomach. Given the importance of age to understanding stock structure and reproductive capability, development of this technique is a key advance to enhancing the management of this species.

Second (Chapter 3), I examine the possibility of reproductive senescence relative to body size in P. argus using a series of mating experiments. If increasing the reproductive potential of lobster stocks is the key goal, and the protection of the largest lobsters the key strategy, then determining whether there is a decrease in gamete or larval quality relative to body size over multiple clutches is a necessary first step.

Third, (Chapter 4) I evaluate the potential use of harvest slot limits and MPAs on lobster harvest, biomass, and fecundity. To accomplish this, I use a two-sex matrix model that links 10
different populations to explore different management scenarios relative to lobster population dynamics and larval connectivity.

Finally, (Chapter 5), I assess the possible ecological consequences, in terms of interactions with prey-species, of increasing the abundance and size of *P. argus* to that expected if management resulted in a greater abundance of large individuals in the population. Through a series of cafeteria-style experiments, I test whether lobster consumption, prey preference, and prey size-specific mortality differ relative to lobster body size.
CHAPTER 2

DIRECT AGE ESTIMATION IN *PANULIRUS ARGUS*

2.1 Introduction

Size-at-age is integral to understanding fisheries population dynamics and forms a key component of fisheries stock assessments (Quinn and Deriso 1999, Crone and Valero 2014). Life history traits such as growth, mortality, and reproduction are often age- not size-dependent, therefore age structure can produce more accurate stock assessments than those based on size alone (Campana 2001). Considerable efforts have thus been made to determine the age of marine organisms, especially those subject to fishing, using annuli in structures as diverse as: otoliths, statoliths, fins, teeth, scutes and skeletons (Campana 2001, Campana et al. 2006, Evans et al. 2007). Direct age estimates for one taxon - crustacea - have proven particularly elusive, however, because growth occurs via ecdysis, or molting, of the calcified exoskeleton (Travis 1954). It had long been believed that molting resulted in the complete loss and replacement of calcified structures, precluding the use of conventional methods for ageing marine organisms. To fill the gap, research on crustaceans has relied on indirect methods to estimate age such as modal analysis of size frequency distributions (e.g., France et al. 1991), approximations of size and growth from tag-recapture studies (e.g., Ehrhardt 2008), and the accumulation of the pigment lipofuscin in neural tissues (e.g., Maxwell et al. 2007; for a review of methods see Kilada and Driscoll 2017). These indirect methods however, are heavily influenced by environmental conditions which brings their accuracy and widespread applicability into question (Vogt 2012, Wahle et al. 2013).
In 2011, Leland et al. described the presence of bands in the gastric mill of five decapod crustaceans. Located within the anterior chamber of the foregut, the gastric mill consists of four calcified ossicles that are used to grind food (Patwardhans 1935). The bands consist of broad translucent zones bordered by narrow opaque zones and were hypothesized to be a record of growth (Leland et al. 2011). The use of bands as a measure of chronological age was corroborated by Kilada et al. (2012) who used them to estimate age in four temperate decapod species. Since 2012, the technique has been applied to several other crustacean species, including freshwater crayfish (Orconectes propinquus, Procambarus clarkia), crabs (Portunus pelagicus), and lobsters (Nephrops norvegicus, Panulirus cygnus, Panulirus ornatus, Jasus edwardsii, Sagmariasus verreauxi) (Clore 2014, Sheridan et al. 2015, Kilada and Acuna, 2015; Kilada and Ibrahim 2016, Leland and Bucher 2017) - although validation of the method is still required for many of these species.

Until now, the technique had not been attempted on the Caribbean spiny lobster, Panulirus argus (Latreille 1804), which supports some of the largest and most economically valuable fisheries in the Caribbean (CRFM 2013). Despite their wide distribution and economic importance, few stock assessments have been conducted for P. argus and those that exist use age-length keys that are based on growth trajectories derived from tagging studies (Forcucci et al. 1994, Muller et al. 1997, Ehrhardt 2008), and lipofuscin (Maxwell et al. 2007, SEDAR 2010). However, the growth and survival of Panulirus argus is influenced by a suite of factors including temperature, salinity, food availability, predation, injury, and disease (Field and Butler 1994, Behringer and Butler 2006, Smith and Herrnkind 1992, Butler and Lear 2009, Behringer et al. 2011). Size can therefore be a misleading and biased estimator of age in P. argus, especially when data are extrapolated beyond their original temporal and spatial contexts (Vogt 2012).
Furthermore, existing growth models fail to estimate growth of lobsters beyond a maximum size of 140 mm carapace length (Muller et al. 1997). Yet, *P. argus* can exceed this size in populations protected from fishing or with low fishing pressure, attaining carapace lengths over 200 mm (MacDiarmid and Butler 1999, Bertelesen and Matthews 2001, Butler et al. 2015), thus the age of the largest lobsters are unknown.

The aim of this study was to investigate the use of banding in the gastric ossicles and eyestalks as a direct measure of age in *P. argus*. First, we sought to identify whether banding in the gastric ossicles and eyestalks of *P. argus* occurs and is consistent with that previously described in other species. Second, we attempted to validate band counts in wild caught lobsters with known age lobsters reared in the laboratory for up to 10 years – the first time that the age of any crustacean has been validated over so long of a timeframe using this technique. Third, we examined the retention of gastric ossicles through ecdysis and the deposition of bands over time in an experiment using lobsters marked with a fluorescent tag (calcein). Finally, we assessed precision (reproducibility) of band counts between independent readers.

2.2 Methods

*Identifying banding and structure selection*

Lobsters for this study were primarily caught from around Long Key, Florida (USA) by divers in 2014-2016. The carapace length (CL; measured by calipers to the nearest mm), sex, and molt stage of individuals were recorded as well as the presence of any injuries or disease. Lobsters were euthanized via rapid cold exposure and their eyestalks and gastric mills were dissected. Dissected structures were kept frozen until processing. Immediately prior to embedding, as much tissue as possible was removed from the ossicles and the structures stored in
a solution of 4% glycerol, 26% water, and 70% ethanol to prevent them from becoming dry and brittle. We adopted the methods of Kilada et al. (2015) to embed, serially section, and image the structures. The only modification was the addition of longitudinal sections of the pterocardiac ossicles (300-400 µm thickness) viewed under reflected light (Fig 1).

Figure 1. Cutting axes used for each of the structures examined: mesocardiac, pterocardiac, and zygocardiac gastric ossicles and eyestalks. Longitudinal sections were used for mesocardiac and pterocardiac gastric ossicles and eyestalks. Transverse sections were used for zygocardiac ossicles.
Validation

Known-Age animals

Of particular value to this study was the availability of *P. argus* of known-age (1.5 - 10 years) that had been maintained at the Fish and Wildlife Research Institute, South Florida Regional Laboratory in Marathon, Florida. Known-age lobsters were collected as pueruli (approximately 6 mm CL and considered age 0 for this study) from Witham collectors deployed 100 m offshore of Long Key (24°48’N, 80°50’W) and Big Munson Island (24°37’N, 81°23’W) in the Florida Keys (Maxwell et al. 2007). After metamorphosis from planktonic pueruli to the benthic juvenile stage, lobsters were initially raised in 1500 L (2m dia) tanks and as they grew larger were transferred to 9500L (6m dia) tanks equipped with flow-through seawater and subject to ambient seawater temperatures and daylight conditions. Lobsters were fed frozen shrimp or squid ad libitum daily and fresh oysters, crabs, snails, or urchins once a week. We sampled ossicles from these known-age lobsters opportunistically when individuals died naturally with the exception of two individuals (9 years old) that were sacrificed in 2017 for this study. In September 2015, an additional 72 pueruli were collected for captive rearing for 18 months to provide younger known-age individuals for this study. Band counts from both younger and older sets of known-age lobsters were used to validate band counts in individuals of unknown age. Bands characterized by paired light and dark zones in sections 300-400 µm in thickness were counted according to Kilada et al. (2012) from the basal to distal regions of the endocuticle. Bands were counted from images taken with an Olympus SZX16 microscope fitted with an Olympus DP74 camera with reflected light, and the images were enhanced with Adobe Photoshop (Version 19.1.0) to improve their readability.
Calcein tagging

To determine whether material from the gastric ossicles is retained through ecdysis and to assess the frequency with which growth increments are added to ossicles, lobsters were tagged with the fluorescent marker calcein (Kilada et al. 2012, Leland et al. 2015; Leland and Butcher 2017) and reared in the laboratory up to 18 months. Calcein is a calcium binding fluorochrome dye commonly used to tag fish otoliths (Thomas et al. 1995, Campana 1999, Mohler et al. 2002). Delivered through immersion, injection, or feeding it typically produces a permanent tag visible under fluorescent light in the growth increment formed at the time of tagging (Campana 2001). The tag not only enables confirmation of tissue retention over time, but it is commonly used to validate band formation - making it possible to assess growth increment formation relative to time at liberty post-tagging (Campana 2001). We initially immersed juveniles (< 50 mm CL; n = 50) in a seawater-calcein bath (500mg l⁻¹) in an aerated aquarium for 48 hours prior to, or during ecdysis at a neutral pH maintained with the addition of NaOH. In later treatments, lobsters were injected with a 10-15mg/kg calcein solution at intermolt (n = 183). Juveniles were then separated into two temperature treatments, ambient (16 – 33.5°C) and constant (30°C ± 1°C), and reared in the laboratory (under conditions described above) at ambient photoperiod for 1.5 years.

Based on ageing work using lipofuscin (Maxwell et al. 2009), ovigerous females collected from the Florida Keys reef tract were believed to be the oldest individuals in the Florida Keys fishery (up to 5 years old). Therefore, we obtained 18 ovigerous adult females (>60 mm CL) from the wild, injected them with a 10mg/kg calcein solution, and held them at ambient temperature and daylight conditions for up to 13 months. This group was thus used to facilitate comparisons of banding between juveniles and other wild caught adults. Combined with the calcein tagged juveniles, these ovigerous females were considered to be of ‘partially known-age’.
Calcein tagged lobsters were fed frozen squid and shrimp daily ad libitum and that diet was supplemented with fresh oysters, crabs, snails or urchins once a week. Lobsters were tagged and monitored and their molt histories recorded. Gastric ossicles from lobsters that died naturally or that were sacrificed at 6 monthly intervals were prepared, stained, and sectioned as above. Sections were viewed using a Zeiss LSM700 spectral confocal microscope (10-20x magnification). Images were taken at red (555 nm) and green (488 nm) emission wavelengths.

Ecdysis experiment

Sheridan and colleagues questioned the utility of this direct ageing technique based on their discovery of loose ossicles in the stomach of the Norway lobster (*Nephrops norvegicus*) immediately following ecdysis (Sheridan et al. 2016). The implication of this finding being that ossicles are lost during the molting process and thus could not retain a record of chronological age. Therefore, in July-August of 2017 we assessed postmolt *P. argus* and their exuviae for the relative amount of ossicle material retained through ecdysis. Adopting the method of Sheridan et al. (2016), we stained gastric material with a solution of 10% potassium hydroxide and saturated Alizarin Red S, and then assessed the gastric ossicles of 11 lobsters from 1 h to 1-wk postmolt and the exuviae of another 9 lobsters within 12 h of ecdysis.

Reader Precision

Because sections from zygocardiac and pterocardiac ossicles provided the clearest bands, these structures were used to assess the precision (i.e., repeatability) of age estimates. Thirty-four randomly selected samples (zygocardiac ossicles n = 19; pterocardiac ossicles n =15) were used
to assess precision among four independent readers who had no knowledge of the lobster’s size or the other readers’ estimates. The level of experience among readers varied. One had several years’ experience in crustacean ageing while the remaining three had received 2-3 training sessions. When both lobes of the zygocardiac ossicles were present, readers made separate counts for each lobe to assess differences in readability within the same structure. The precision of counts between ossicles of the same lobster and among the four readers was assessed using the mean coefficient of variation (CV) (Campana 2001), as has been done for other crustaceans assessed via this technique. Twenty-two additional images of zygocardiac ossicles from known-age lobsters (n = 12) and partially known-age lobsters (n = 10) were also read by five readers (an additional reader with 2-3 training sessions was added) to assess precision.

2.3 Results

Identification of banding

As has been observed in the other decapods for which this technique has been applied, all four layers of the cuticle - the epicuticle, exocuticle, endocuticle, and membranous layer (growing edge) - were visible in the sections. Growth bands comprised of alternating light and dark zones (varying in width from 200-11 μm) in the endocuticle layer of the cuticle were consistently identified in longitudinal sections of the mesocardiac and pterocardiac ossicles and in transverse sections of the zygocardiac ossicles of the gastric mill (Fig 2). Banding was not observed in the eyestalks. Narrow microlamellae in the endocuticle and exocuticle were also observed (< 5μm width); particularly in the mesocardiac ossicles and were distinguishable from the primary growth bands by their smaller size, regularity, and placement.
Validation with known age animals

Animals reared in captivity since settlement as postlarvae (Years: 1.5, 2, 7, 9, 10; n = 9) were used to validate the periodicity of growth bands. An additional 27 animals with partially known histories (i.e., the juveniles and ovigerous females from the calcein tagging experiment) were also used. In the nine known-age animals the number of growth bands counted corresponded to their known ages (Fig 3). Sections from individuals with partially known histories also had band counts that matched age estimates based on their combined time in captivity and published age-length relationships (Sharp et al. 2000).

Retention of ossicles through molts

Lobsters tagged with calcein, and known age lobsters for which we had complete molt histories, had band counts that were lower than their instar number, demonstrating that bands were not just a record of molt history. For example, a juvenile lobster (initial CL 38.8 mm) tagged with calcein and kept for 13 mo (final CL 86 mm), molted 6 times in captivity. Its estimated age based on band counts was 2. Calcein tagged lobsters sacrificed after 3-14 months of laboratory rearing in constant and ambient temperature treatments had clear bands when viewed under reflected light. However, the calcein did not appear as a discrete band in the sections. Fluorescence in the endocuticle, signifying the presence of calcein, varied in its position (Fig 4). Multiple samples displayed a calcein tag at the growing edge of the ossicle despite months of grow-out following tagging, whereas others had multiple bands of fluorescence through the endocuticle (Fig 4). Thus, we found calcein to be an unreliable method for marking a baseline growth band.
Figure 2. Sections from a 106.4 mm carapace length female from the Florida Keys. Estimated age was 3+ based on all three structures: (A) zygocardiac ossicle, (B) pterocardiac ossicle, (C) mesocardiac ossicle. Growth bands are indicated by dots in each image.

Figure 3. Sections from the zygocardiac ossicles of two known age individuals: (A) Female 165 mm carapace length, age 10; (B) Male 180 mm carapace length, age 7. Growth bands are indicated by dots in each image.
Figure 4. Sections from the pterocardiac ossicles of two individuals tagged with calcein. (A) Male 93.4 mm carapace length, 13 months post-tagging; note the obvious green calcein tag and subsequent growth. (B) Male 91 mm carapace length, 11 months post-tagging; note the diffuse green calcein tag throughout the endocuticle.

Ecdysis experiment

In postmolt lobsters examined immediately after ecdysis, the ossicles of the cardiac foregut were found intact and attached to the cardiac sac. Structures were soft and translucent, and staining with Alizarin red showed reduced calcification (Fig 5). After 24-48 h, structures remained pliable but staining indicated increased calcification of the ossicles particularly the mesocardiastic and pterocardiac ossicles (Fig 5). By the end of one week, ossicles appeared fully calcified and had hardened completely. In the nine exuviae examined after ecdysis, remnants of the foregut were found within the cardiac sac. However, these consisted primarily of the lateral teeth; the ossicles themselves were not present in the foregut exuviae (Fig 5).
Figure 5. (A) Postmolt lobster dissected within hours of ecdysis. The cardiac sac with gastric ossicles is visible in the center of the image indicated by the arrow. (B) Gastric mill dissected from exuviae within hours of molting. Note the absence of the ossicles. (C) Gastric mill dissected from an individual 12 hours after molting. Note calcification in the mesocardiac and pterocardiac ossicles indicated by the dark red staining. (D) Gastric mill dissected from an individual 72 hours after molting. Dark red coloration of structures shows that calcification of ossicles is complete.
**Reader Precision**

Samples used to estimate reader bias, ranged in age from 1.5 to 9.6 years, and included individuals 55 to 180mm CL. Band counts of pterocardiac ossicles produced a CV value of between 22% - 27% for individual readers and 26% for all four readers combined. Pearson’s correlation coefficients ranged between 0.483 and 0.515 for readers against the most experienced reader (Reader 1) (Table 2). The larger lobe (A) in the zygocardiac ossicle had a CV value of 24% for all four readers combined (Pearson’s r = 0.790-0.847), whereas the smaller lobe (B) had a CV value of 15% for all four readers combined (Pearson’s r = 0.478-0.858). The best CV estimates obtained for lobe B readings as compared to the most experienced reader was 11% and 13% (Fig 6, Table 2).

Known-age lobsters ranged in age from 10 months to 9.6 years, and carapace lengths from 25.5 to 180mm. For the 22 known-age individuals, the CV estimate was 33% for the five readers (Table 3). The two most experienced readers had a CV value of 24% (Table 3). Overall, there was greater variation in estimates for younger lobsters less than 2yrs old, and the CV estimates were considerably influenced by two samples overestimated by three readers: a 1.5-year-old which was overestimated by 3.5-5.5 years and a 3.5-year-old which was overestimated by 1.5-4.5 years (Fig 6).
Figure 6. (A) Reader bias between four independent readers using the smaller lobe (B) of the zygocardiac ossicles (n = 14). Readers 2-4 are compared to Reader 1 (R. Kilada) who was the most experienced reader. R = Pearson’s correlation coefficient. (B) Known age and estimated age (n = 22) from readings of zygocardiac ossicles by five independent readers. Error bars represent 95% confidence intervals around the age estimated by the five readers.
Table 2. Coefficient of Variation (CV) and Pearson’s Correlation Coefficient (R) estimates for readers estimating age of 34 randomly selected *Panulirus argus* sections. Readers are compared to Reader 1 (the most experienced). Readers 2-4 received 2-3 training sessions.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Reader</th>
<th>CV</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pterocardiac ossicle</td>
<td>2</td>
<td>22%</td>
<td>0.501</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>22%</td>
<td>0.515</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>27%</td>
<td>0.483</td>
</tr>
<tr>
<td></td>
<td>Readers combined</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td>Zygocardiac ossicle (Lobe A)</td>
<td>2</td>
<td>29%</td>
<td>0.816</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20%</td>
<td>0.847</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>28%</td>
<td>0.790</td>
</tr>
<tr>
<td></td>
<td>Readers combined</td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td>Zygocardiac ossicle (Lobe B)</td>
<td>2</td>
<td>13%</td>
<td>0.858</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>11%</td>
<td>0.795</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>17%</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td>Readers combined</td>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Coefficient of Variation (CV) and Pearson’s Correlation Coefficient (R) estimates for readers estimating age of 22 known age *Panulirus argus* sections. Reader 1 was the most experienced, Readers 2-5 received 2-3 training sessions.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Reader</th>
<th>CV</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zygocardiac ossicle</td>
<td>1</td>
<td>24%</td>
<td>0.974</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24%</td>
<td>0.969</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>40%</td>
<td>0.923</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>32%</td>
<td>0.894</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>37%</td>
<td>0.872</td>
</tr>
<tr>
<td></td>
<td>Readers combined</td>
<td>33%</td>
<td></td>
</tr>
</tbody>
</table>
2.4 Discussion

In step with recent advances in the ageing of crustaceans, the primary goal of this study was to examine the feasibility of using the gastric ossicles as a means of directly estimating age in *P. argus*. The strong correlation between band counts and the known age of multiple individuals across the largest range of ages tested for a crustacean species indicates that this is possible. The bands observed in the gastric ossicles of *P. argus* were similar to those seen in other decapod crustaceans (e.g., Kilada and Ibrahim 2015, Kilada et al. 2015, Sheridan et al. 2015) and were not strongly influenced by molting, given that instar number was always much higher than the number of primary growth bands. Banding was most easily observed in the pterocardiac and zygocardiac ossicles of the gastric mill and was harder to discern in the mesocardiac ossicle. Bands were not seen at all in the eyestalks. Other studies using this technique have also found that the eyestalks do not always contain clear bands (Kilada et al. 2012, Leland et al. 2015) and, as is the case with fish otoliths, the usefulness of a given structure may vary relative to the species being examined (Campana 2001).

Few studies have benefitted from the availability of older, known-age individuals for age validation as we did. Although captive rearing of individuals for age validation has potential biases resulting from growth in an artificial environment, it is an accepted method of validating annulus formation (Campana 2001). The known-age *P. argus* available to us were captured from the plankton as postlarvae, then reared in ambient seawater and photoperiod conditions, and fed ad libitum a combination of live and frozen feed. Given the ready source of food, and lack of predation pressure it is likely that these individuals had growth rates that were artificially high. We suspect that this perhaps changed the width of each band laid down (i.e., Bestgen and Bundy 2011), but not the number of paired light and dark bands laid down in a year. If so, the estimated
ages of the laboratory reared lobsters that we evaluated would still be accurate but the size-at-age relationships would be overestimates of those likely to be found in nature.

Previous studies (i.e., Kilada et al. 2012, Leland et al. 2015, Leland and Bucher 2017) used calcein tagging to assess the retention of material in the cuticle through ecdysis. Kilada et al. (2012) demonstrated the deposition of discrete calcein bands that persisted through ecdysis, but our results and those of others (Leland et al. 2015, Leland and Bucher 2017) were not as clear. Although calcein was evident in samples up to 13 months post-tagging, the calcein did not form a discrete band. Furthermore, in many samples calcein was visible at the growing edge with no ossicular material beyond the tag, which does not make sense in terms of the grow-out period that followed tagging. Leland et al. (2015) similarly had inconsistent results with calcein tagging. Of nine individuals they tagged, only one showed band growth proportional to the time spent in grow-out following tagging. Four others had variable band growth after tagging and the remaining four either had calcein along the outer edge, or an indistinguishable discrete tag. Leland and Bucher (2017) also documented calcein distributed throughout the entire endocuticle or no growth beyond tagged material in *P. cygnus* and *Sagmariasus verreauxi*. Being a calcium-binding fluorochrome dye, there is no obvious reason to think that calcein would not bind to gastric ossicles given that they are primarily composed of calcium hydroxyapatite (Kilada et al. 2012), an inorganic compound primarily composed of calcium. Perhaps the calcein tagged material is being remobilized during ecdysis and is therefore not sequestered in a discrete band (Sheridan et al. 2016).

In contrast to results reported by Sheridan et al. (2016), we did not find the ossicles of the gastric mill loose within the stomach of the lobster immediately after ecdysis. Instead, they were attached to the cardiac sac. However, the ossicles were soft and translucent indicating
decalcification of the structures, and it took 24-48 h for these to re-harden. Ossicles were absent in the exuviae where only lateral teeth were found. It is not entirely surprising that these results differ from those observed in *N. norvegicus* given differences in decapod physiology and morphology (e.g., *N. norvegicus* has a gastrolith while *P. argus* does not) (Farmer 1973, Góes and Lins-Oliveira 2009), and their environments (i.e., carbonate-rich Caribbean and carbonate-limited North Eastern Atlantic (Einsele 2013)). It is possible that the physiological processes orchestrating ecdysis differ between the two species. Studies by Vatcher et al. 2015, and Roer and Dillaman 2018, also document the loss of the endocuticle during ecdysis in blue crabs, *Callinectes sapidus* thus there is clearly some uncertainty as to what is creating the bands observed in several crustaceans. The mechanism by which bands are formed in the gastric ossicles of *P. argus* is not understood and further research on the physiological mechanism underpinning this process is clearly warranted.

The CV estimates for this study suggest moderate to high reproducibility of repeated measurements. The values we obtained (11% - 29%) varied among the structures assessed and fall outside of the boundaries of what is generally accepted for fish otoliths (5-12%) and bivalves (5-7%) (Campana 2001, Kilada et al. 2009), but are within the range observed for other crustaceans in which this technique has been applied. Estimates in crustaceans range from 5% in Antarctic krill (*Euphausia superba*) (Kilada et al. 2017) to 19% in the blue swimmer crab (*Portunus pelagicus*) (Kilada and Ibrahim 2016). The precision of band readings in calcified structures varies among species and the structure being examined, so it is not currently possible to designate a target precision for *P. argus*. However, with a larger sample size and increased training, it is likely that precision will increase. Counts using the smaller lobe of the zygocardiac ossicle (lobe B) had the least bias. This, in addition to easier embedding and sectioning, and the
duplication in this structure, suggests that the zygocardiac ossicles may be the best structure to use when aging *P. argus*.

Band counts using the known age and partially known age animals alone suggested that the greatest variability in counts were for small lobsters, particularly those smaller than the minimum size limit of 76 mm CL in Florida. The initial age of lobsters reared in the laboratory that constituted the ‘partially known age’ group was estimated using Sharp et al. 2000, a study that assessed survival, growth and feeding in microwire tagged *P. argus* in the Florida Keys. Although this provided us with the best available estimate of juvenile age prior to rearing in captivity Sharp et al. 2000 notes significant variance in early size at age. For example, a 30 mm CL lobster had an age that ranged from 17-42 weeks. Thus it is possible, that some of the variance in counts for small lobsters might be explained by the variance in the original juvenile age estimate. In terms of the use of this technique in fisheries stock assessments, the precision would likely be better if sampling was restricted to larger individuals that are typically captured and of interest to the fishery. It is also of note, that of the five readers, only one had multiple years of experience with ageing. Therefore, with appropriate reader training and a robust reference sample collection, it should be possible to generate high quality age estimates for *P. argus*.

The bulk of the research was conducted in the Florida Keys, which is close to the species’ northern range limit, where environmental conditions are most variable. Average monthly sea surface temperatures in the Florida Keys can vary by 12°C over the course of a year (NOAA 2018) and photoperiod varies by 3 h between summer and winter (Time and Date 2018). Geographic location can significantly affect the readability of growth bands in fish otoliths and where growth is less seasonally driven, bands can be harder to discern (Green et al. 2009). Thus,
one might expect that this method may only be useful for *P. argus* in higher latitudes of the Caribbean (i.e., the Florida Keys, Bahamas, Cuba, Mexico) and less useful for *P. argus* at lower latitudes where environmental conditions are more constant (i.e., Venezuela, Brazil). However, the lobsters reared for 18 months under constant temperature (30°C) had ossicles with clear banding patterns. We are currently in the process of assessing differences in growth band deposition relative to latitude, habitat, and fishing intensity to further evaluate the utility of this method for *P. argus* throughout the Caribbean.

The ability to directly age *P. argus* has significant implications for fisheries management as well as our general understanding of the species’ population dynamics. With regional sampling it should be possible to develop size-at-age curves for different populations experiencing different environmental conditions, which would facilitate the development of more accurate age-based stock assessments and improve our ability to model growth. For a species that is of such economic and social importance to the Caribbean, this has the potential to fundamentally change the way the species is managed in the region.
CHAPTER 3

AN EXAMINATION OF REPRODUCTIVE SENESCENCE AND PARENTAL EFFECTS IN THE CARIBBEAN SPINY LOBSTER, *PANULIRUS ARGUS*

3.1 Introduction

Senescence is broadly defined as a gradual physiological deterioration that leads to a decrease in fitness relative to age (Williams 1957). Three mainstream theories to explain the physiological origin of senescence have emerged. ‘Antagonistic pleiotropy’ is the hypothesis that senescence is the result of a fixation of alleles that favor early life fitness traits that become costly later in life (Williams 1957). The accumulation of deleterious mutations that reduce fitness over time is another explanation for senescence coined ‘mutation accumulation’ by Medawar (1952) and the third mechanism proposed to explain senescence is the ‘disposable soma hypothesis’ in which senescence results from a limited amount of energy available for physiological processes and cellular repair (Kirkwood 1977).

Regardless of the physiological mechanisms that drive senescence, its evolutionary significance is puzzling. One would expect natural selection to favor traits that lengthen life or enhance fitness rather than shorten it. This line of reasoning led Medawar, to claim that animals in nature do not senescence because their environment was likely to kill them first (Medawar 1952). And yet senescence is clearly observed in nature – as an increase in mortality relative to age (actuarial senescence), a decline in reproductive fitness such as fecundity, sperm survival or

offspring survival (reproductive senescence) or cellular changes that reduce function (Ebert 2008, Nussey et al. 2013, Lemaître and Gaillard 2017).

In a review of senescence in wild animal populations, Nussey and colleagues (2013) documented 175 cases of senescence in 340 studies. The majority of these cases were in birds and placental mammals. Thus far, there has been little documented evidence of senescence in invertebrates – a reflection perhaps of their typically short lifespans and semelparity (Moya-Larano 2002), or a paucity of research in this area. Where studies of senescence in invertebrates do exist, evidence of senescence has been documented in ascidians (Chadwick-Furman and Weissman 1995), bryozoans (Bayer and Todd 1997), and marine copepods (Ceballos and Kiørboe 2011). However, senescence has not been observed in invertebrates with very long lifespans, such as: the ocean quahog *Arctica islandica*, the longest living mollusk with a lifespan of 374 years (Abele et al. 2008), or long-lived red sea urchin *Strongylocentrotus franciscanus* with a lifespan of over 100 years (Ebert 2008)

Furthermore, there are many examples in the marine realm where older or larger parents enhance the fitness or survival of their offspring because of differences in maternal investment or reproductive timing relative to age or size (Stafford et al. 2014). Parental effects on offspring fitness has gained considerable attention in recent decades as a consequence of the obvious age truncation effect on population structure observed in many exploited species (Berkeley et al. 2004, Venturelli et al. 2010). Evidence of positive parental effects on larval survival and growth offer credence to management strategies that call for protecting the larger and often older individuals in populations of exploited species (Berkeley et al. 2004, Birkeland and Dayton 2005).
The issue of senescence as it relates to a reduction in reproductive output, along with the possible parental size effects on offspring performance and survival are of particular relevance to commercially harvested marine species in which management tools such as maximum size limits and protected areas seek to conserve and protect mature spawning stocks (Conover and Munch 2002, Gwinn et al. 2013, Maxwell et al. 2013). But if there is a decline in reproductive capacity in large or old individuals, then it makes little sense to conserve these individuals. Management would be better directed at conserving smaller or younger age classes. On the other hand, if larger or older individuals confer a demonstrable survival advantage to their offspring, then the conservation of larger or older age classes ought to be encouraged (Steneck et al. 2009).

The spiny lobster, *Panulirus argus* (Latrielle 1804) is ubiquitous in the Caribbean and fisheries for the species are some of the largest and most economically valuable in the region with an estimated annual regional value in excess of $450 million USD (CRFM 2013). As a consequence, many regional populations are currently overcapitalized or overfished and regional landings have decreased by 55 percent since the early 1990s (Ehrhardt et al. 2010, CRFM 2011). Over the same time period there has also been a substantial decline in the average size of lobsters landed because the largest individuals are typically targeted first by fishers. At present there are no standardized management measures specific to *P. argus* across the Caribbean; nations where *P. argus* is fished have unilaterally developed a range of regulations that include minimum size limits, closed seasons, and gear restrictions. Regulations that explicitly aim to conserve spawning biomass do not exist with the exception of the widespread prohibition on the take of ovigerous females, which fails to provide lasting protection for those individuals. In recent years, however, greater attention has been focused on the implementation of maximum size limits specifically for the conservation of the largest individuals (Spiny Lobster Declaration 2015). Although a clear
positive relationship between fecundity and carapace length in female lobsters is well established (MacDiarmid and Sainte-Marie 2006, Butler et al. 2015a) the issue of reproductive senescence in the largest or oldest lobsters has not been investigated.

*Panulirus argus* are sexually dimorphic (Holthius 1991) with males reaching much larger sizes than females. Within a sex, lobster size is generally believed to scale positively with age although temperature and food availability can blur that relationship (Maxwell et al. 2009). Unfortunately, there are no proven methods for aging *P. argus*, although recent research on determination of age in decapod crustaceans is promising (Leland et al. 2011, Kilada et al. 2012, Sheridan et al. 2016, Chapter 2). Size at sexual maturity varies throughout the Caribbean and differences are generally attributed to differences in environmental conditions, lobster density, and fishing intensity (Chubb 2000). Although individuals as small as 57 mm carapace length (CL) have been observed with eggs (Maxwell et al. 2009) the average length at maturity for *P. argus* appears to be closer to 75-85 mm CL (Hunt and Lyons 1986). Individual fecundity is size related, however, with larger individuals producing exponentially more eggs (Bertelsen and Matthews 2001, MacDiarmid and Sainte-Marie 2006). Throughout much of the Caribbean spawning occurs year-round (Butler et al. 2010) though in more subtropical areas, where spawning is correlated to temperature and photoperiod, *P. argus* has a more defined breeding season: either spring-summer or summer-autumn (Chubb 2000). During this defined spawning period, lobsters may produce multiple clutches with larger females typically producing more clutches and spawning earlier in the season than smaller females (Briones-Fourzan and Lozano-Alvarez 1992). Larger males also have an advantage over smaller males with respect to mating success in terms of female mate selectivity, the clutch size that they can successfully fertilize,
and the number of females that they can sequentially mate with in a given reproductive season (MacDiarmid and Butler 1999, Butler et al. 2015a).

Thus all evidence to date indicates that large male and female *P. argus* enjoy a reproductive advantage over smaller individuals, supporting the notion that reproductive success of lobster populations (i.e., breeding stocks) would be enhanced if the largest individuals were more abundant. But we are unaware of any published studies that examines the reproductive success of the very largest/oldest *P. argus* or explicitly reproductive senescence in lobsters. In Butler et al. 2015a we investigated the effect of parental size on reproductive success using two primary experiments: (1) a spermatophore reduction experiment, in which the role of sperm availability on fertilization success was studied and (2) a sperm depletion and recovery experiment in which we examined whether large and small males differ in their production of spermatophores and their ability to recharge their sperm stores after multiple mating events. These two experiments also facilitated investigation of maternal size effects on egg production and larval attributes. That study, however, did not consider parental effects as they related to multiple clutches – as seen in larger lobsters. Building on the work of Butler et al. 2015a, we specifically sought to investigate the effects of parental size on gamete and clutch quality for gametes and clutches produced by the same individual in succession. We used a number of metrics, some similar to those used in Butler et al. 2015a and some new, and with a focus on the largest and smallest mature lobsters obtainable. Conducting such a study is complicated by the extent and intensity of fishing on *P. argus* populations in the Caribbean and the difficulty in obtaining the exceptionally large/old individuals that would ordinarily exist in an unfished population. Thus, the largest lobsters were collected from an old, well-enforced no-take marine reserve.
3.2 Methods

Lobsters were collected by divers from the Florida Keys, Florida USA (fished population) and the Dry Tortugas National Park, Florida USA (a 42-year-old no-take marine protected area), in February-March of 2015 and 2016. The absence of large lobsters from the Florida Keys fishery necessitated the collection of lobsters from the Dry Tortugas National Park and the absence of small, sexually mature individuals within the Dry Tortugas National Park meant that animals from the Florida Keys fishery had to be used as a comparison. Lobsters were collected prior to the onset of the breeding season and therefore should not have mated since the previous year. Large individuals in the Dry Tortugas start breeding earlier in the reproductive season so were collected in February or early March. The length of their breeding season meant that they remained in the experiment until late June-early July. Small adult lobsters in the Florida Keys were collected in May or June prior to the onset of their breeding activity and remained in the experiment until early August. Lobsters were transported in aerated live wells to the Fish and Wildlife Research Institute South Florida Regional Laboratory in Marathon, Florida (USA) where the experiments took place.

A single large (135-190 mm CL) or small male (67-84 mm CL) *P. argus* along with 3-4 females of equivalent size (large 102-149 mm CL; small 58-80 mm CL) were placed into tanks (1.75 m diameter, 1500L), representing typical lobster size and sex ratios found in the wild in unfished and fished areas (respectively) during the reproductive season (Bertelesen and Matthews 2001, Butler et al. 2015b). Tanks received aerated, filtered seawater on a flow through system thus ambient temperature conditions were maintained as was ambient photoperiod.
Lobsters were fed squid or shrimp *ad libitum* daily, supplemented with live mangrove oysters once a week.

Females were checked daily for the presence of spermatophores in situ with the use of an underwater endoscope so as to minimize disturbance to them. One haphazardly selected female per tank was selected and the spermatophores it received were removed while the remainder of the females were left undisturbed to extrude their eggs. Females who had spermatophores removed were returned to their original experimental tanks so they could remate. In the Florida Keys and Dry Tortugas large *P. argus* females mate and produce up to three and sometimes four clutches in a season, whereas small females produce only a single clutch per year (Fonseca-Larios and Briones-Fourzan 1998, Bertelsen and Matthews 2001, Butler et al. 2015a). As such we could rely on females remaining receptive to courtship and remating during the course of the experiment. The male mating frequency in the wild is unknown but the polygynous lek-style mating strategy of large males in unfished populations, in which large males defend a den from other large males and females chose among them indicates that at least large males mate repeatedly during the breeding season (MacDiarmid and Butler 1999, Butler et al. 2015b). Males repeatedly mated with the females in their experimental tanks and the order in which spermatophores were produced was recorded.

The smaller lobsters collected from the Florida Keys fishery did not mate in captivity as readily as the larger lobsters from the Dry Tortugas National Park. Additionally, even when mating did occur, females either did not extrude clutches, or lost clutches within the first few days of extrusion. To enable comparison between different sized lobsters, we therefore directly sampled egg clutches and spermatophores from smaller lobsters collected by divers from the Florida Keys fishery. We sampled females with eroded spermatophores and recently extruded
egg clutches (bright orange eggs). Individuals smaller than 75 mm CL were primarily targeted. As these individuals are typically the last to mate during the breeding season, we regularly checked reefs for breeding activity. Although we could not directly ascertain the size of the males that mated with each female, males in the fished areas we sampled average ~ 75-85mm CL and none exceed 110 mm CL. Furthermore, using published data (MacDiarmid and Butler 1999, Butler et al. 2015a) we calculated the predicted size of the male relative to the size of the spermatophore deposited and the size of the female. Such a relationship is likely because small males like those that dominate in the Florida Keys participate in few matings each year and cannot alter spermatophore size based on female size as can large males (MacDiarmid and Butler 1999). Based on previous data on size-specific mating patterns (MacDiarmid and Butler 1999, Butler et al. 2015a) we are confident that the females sampled in the wild in the Florida Keys had mated only once that season. Moreover, none had a previously deposited spermatophores beneath the new spermatophore, a clear indication that none had mated previously during the current reproductive season.

**Metrics of Male Quality**

Newly deposited spermatophores were left to harden for 24 hours and then removed from females with the use of flat forceps. Spermatophores were photographed and the area (cm$^2$) was calculated using Image J (Version 1.5lf, Rasband NIH, USA). Thickness was measured at the thickest point of the spermatophore on the left and right hand sides, typically in the middle towards the posterior end. A mean value was calculated to provide an overall thickness value. Spermatophores were then weighed to the nearest 100th of a gram and centrally divided. Sperm cells are evenly distributed between left and right halves of the spermatophore (Butler et al.}
so one half was frozen at -80°C for protein analysis while the other half was used for sperm counts conducted within 24 hours of sampling. Until sperm counts could be completed, spermatophores were kept refrigerated at 5°C in 10ml filtered seawater. We counted sperm using the methods of Butler et al. (2011), which consistently liberates > 70% of the sperm cells within the spermatophore matrix. The halved spermatophore was laterally sliced into thin sections (~0.5mm thick) with a scalpel into a petri dish and then washed into a 25ml centrifuge tube with 10ml sterile seawater. A drop of trypan blue was added to the tube and the tube was capped and mechanically shaken for 3 minutes to liberate sperm cells from the spermatophore matrix.

Trypan blue was used to assess cell viability: spermatozoa that have lost membrane integrity stain blue whereas spermatozoa that are intact remain clear on examination under a microscope (Cabrita et al. 2009). A hemocytometer was then used to count the number of sperm cells in 20 separate 10 µL aliquots of each sample. The total sperm number and sperm density (total sperm number/spermatophore weight) was then calculated.

**Metrics of Female Quality**

Female egg extrusion took anywhere between 1 and 30 days from the date of spermatophore deposition. On the 10th day after extrusion, when eggs were still bright orange in color, a haphazard sample (<10g) of the egg mass was removed. Removed eggs were rinsed in sterile seawater and the diameters of 20 eggs were measured with a compound microscope at x40 magnification. The remaining sample was frozen at -80°C for protein analysis. Before returning females to their holding tanks we also took measurements of egg mass length and egg segment height to estimate clutch size non-invasively (Currie et al. 2010, Gnanalingam and Butler 2018b). Traditional estimates of clutch size rely on stripping females of their entire clutch and
conducting a series of egg counts but as we wished to assess larval quality relative to female size and clutch number, stripping eggs was not an option.

**Metrics of Larval Quality**

Larval quality was measured in three ways following methods reported by Butler et al. (2015a): larval carapace length, survival, and swimming ability. Prior to spawning, females with late stage egg clutches (noticeably brown in color) were transferred to individual tanks. This way we could ensure the parentage of hatched larvae. Larvae were haphazardly collected within 12 hours of their release by dipping a bucket into the individual tank. Larval attributes were then immediately assessed (i.e., larval carapace length and swimming ability were measured), and starvation trials were set up within 24 hours of hatching. During the short processing time, larvae were held in multiple 11 L buckets with aeration. From every spawned clutch the carapace lengths of 20 first stage phyllosome larvae was measured with a compound microscope at x4 magnification. Larval survival was assessed by way of a starvation trial in which 20 first stage phyllosome larvae were individually housed in 15ml glass bowls filled with sterile seawater at ambient light and temperature and left unfed. The sterile seawater was replaced daily and the number of individuals alive each day was counted until all had died. Swimming ability of 10 individual phyllosomes per clutch was estimated in a seawater filled, black swimming chamber (25 cm long x 10 cm wide x 5 cm deep) with a 1cm grid scale on the bottom. Clear holes (0.5 cm diameter) at either end of the plastic chamber permitted light to enter the chamber. At the start of each trial the room was darkened and a single larva was added to one of the test chamber while the chamber was illuminated from the opposite end. First stage phyllosomes are positively phototactic so they swam towards the light at the opposite side of the chamber. We measured the
distance the larvae moved over a 10s period. Larvae were given a 30s respite in total darkness before we illuminated the opposite end of the chamber and repeated the procedure. The swimming distance of each larva was tested 10 times and a mean swimming distance was calculated.

**Protein Analysis**

The total protein content of a subsample of spermatophores (n = 20; 15 different males, spermatophore deposition # 1 & 3) and eggs (n = 20; 11 different females, clutch # 1-4) was determined by bicinchoninic acid assay (Smith et al. 1985) to assess differences relative to male/female size and spermatophore/clutch number. Proteins were chosen as the biochemical component of choice because it is typically the primary component of spermatophores in crustaceans (Subramoniam 1993), and forms a core component of crustacean egg yolks (Komatsu and Ando 1992). The bicinchoninic acid assay relies on the reduction of Cu^{2+} to Cu^{1+} in alkaline conditions and the level of reduction is proportional to the quantity of protein present (Smith et al. 1985). By comparing samples of an unknown quantity of protein to standards of a known quantity one can determine total protein content. Samples of spermatophore and eggs (5mg) were homogenized for 10 minutes in 1ml of phosphate buffered saline then centrifuged at 10,000 rpm at 4°C for 10 minutes. Once the homogenate was extracted, samples were run in triplicate against bovine serum albumin standards on a microplate reader and means and variance for each sample were calculated.

**Statistical Analyses**
Spermatophore attributes (area, weight, thickness, cell density) were assessed as functions of male size using general linear mixed models with male carapace length, female carapace length, days between spermatophore deposition, and spermatophore number as fixed factors, and individual as a random factor. An interaction term of male carapace length and spermatophore number was also included and kept in the model where significant. Both male and female carapace lengths were included as continuous variables. For cell viability, proportions of viable and non-viable sperm cells were categorized as high or low relative to whether samples contained greater than or less than 50 percent of each cell type. A generalized linear model with binomial distribution and logit link and the same fixed and random factors as the other spermatophore attributes was then used to assess differences in cell viability relative to male size. Female attributes (egg size) and larval characteristics (larval carapace length, days until 100% mortality and swimming speed) were analyzed with general linear models with female carapace length, male carapace length, and clutch number as fixed factors and individual as a random factor. An interaction term of female carapace length and clutch number was initially included but removed if no significant interaction was detected. Values for swimming speed were log + 1 transformed and values for 50% larval mortality were log transformed prior to analysis given their non-normality. Fecundity was similarly assessed but data were log transformed first, to linearize the exponential relationship. Effects from each model were tested with conditional F tests with Kenward Roger approximations. As protein analysis was conducted on only a few of the samples, statistical analyses were limited to a Wilcoxon rank test for the first spermatophores relative to male size and a t-test for the first clutches relative to female size. Statistics were run in R version 3.3.1 using the package lme4.
3.3 Results

*Male Quality*

Twelve small males (92 - 100 mm CL) and 12 large males (135 - 190 mm CL) were used to assess the relationship between male size and spermatophore attributes. Spermatophore thickness, and weight varied significantly relative to male carapace length and spermatophore number; larger males produced weightier and thicker spermatophores (Fig 7; Table 4). Larger males produced multiple spermatophores during the experiment, yet the weight, area, and thickness of those spermatophores did not decrease significantly over time (Fig 7; Table 4). Differences in sperm density per gram relative to male size was heavily influenced by the eighth spermatophore produced by one very large male (Fig 7; Table 4). It contained considerably more sperm cells per gram than the other spermatophores, thus the general linear model attributed significance to spermatophore number and apportioned a high amount of variance to the random factor ‘individual’ (Table 4). If this value was removed from the analysis significant differences in sperm cell density per gram were not apparent. The proportion of viable and non-viable sperm cells within the spermatophore also did not differ significantly relative to male size (Fig 7; Table 4). There were however differences in total protein content relative to male size for first spermatophores (Fig 8; Wilcoxon rank test W = 31, p < 0.001). And though it could not be statistically evaluated, third spermatophores appeared to contain a higher concentration of proteins than first spermatophores produced by small or large males (Fig 8).
Figure 7. The relationships between size of *P. argus* males and spermatophore attributes including: (A) spermatophore weight, (B) spermatophore area, (C) sperm density per gram, and (D) the proportion of non-viable sperm cells. Data for spermatophores deposited sequentially by males (1 - 8) are shown as separate colors and cross hatching of histograms. Note that small males only produced one spermatophore per annum whereas large males produced up to eight. Error bars represent standard error and numbers inside bars indicate sample size.
Table 4. Effect sizes and p-values for models testing spermatophore attributes. Values in bold indicate significant values where $P = 0.05$. **CL** = carapace length, $B$ = fixed effects coefficient estimates, **CI** = confidence intervals, **R2** = correlation between fitted and observed values, **Ω02** = 1- (residual variance / response variance).

<table>
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<tr>
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<th>Area</th>
<th>Density</th>
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<td>$CI$</td>
<td>$p$</td>
<td>$B$</td>
<td>$CI$</td>
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<td>0.157</td>
<td>-12.24</td>
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<td><strong>0.036</strong></td>
<td>0.03</td>
<td>0.00-0.06</td>
</tr>
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<td>-3.38-5.43</td>
<td>0.655</td>
<td>0.18</td>
<td>-0.36-0.73</td>
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<tr>
<td>Spermatophore No 4</td>
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<td>0.04</td>
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<td>Spermatophore No 8</td>
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<td>-0.06-0.05</td>
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<td>0.01-0.15</td>
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<td>-0.04-0.02</td>
<td>0.558</td>
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<td>-0.07-0.05</td>
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<tr>
<td>Male CL: Spermatophore No 4</td>
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<td>-0.09--0.03</td>
<td><strong>0.003</strong></td>
<td>-0.02</td>
<td>-0.09-0.04</td>
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<td>Male CL: Spermatophore No 5</td>
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<td>0.32</td>
<td>0.06-0.58</td>
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<td><strong>Random Effects</strong></td>
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<td>6.139</td>
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<td>33</td>
<td>33</td>
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<tr>
<td><strong>R2/Ω02</strong></td>
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<td>0.884/0.878</td>
<td>0.884/0.878</td>
<td>0.833/0.832</td>
<td>0.662/0.641</td>
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Figure 8. Mean protein content for spermatophores relative to male carapace length (mm) and spermatophore number (A); and protein content of eggs relative to female carapace length (mm) and clutch number (B). Error bars represent standard errors and numbers inside bars indicate sample size as number of individuals.
Female Quality

In total, 36 large females from the Dry Tortugas and 30 small females from the Florida Keys fishery were used in the experiments. Of the 36 large females, 36 mated in captivity and all but one extruded at least 1 clutch. In comparison, of the 30 small females only 4 mated in captivity and these 4 clutches were all dropped. Thus 100% of the small female data comes from field caught females. Egg production estimated by the non-invasive method produced the same exponential relationship observed in previous assessments of P. argus fecundity, with larger females producing clutches with significantly more eggs (Fig 9). Only the large females from the Dry Tortugas also produced a third (n = 9; 24%) or fourth clutch (n = 2; 0.05%) with relatively few females from the Florida Keys fishery producing even a second clutch (n = 4; 11%). Thus with clutches combined the exponential relationship between female carapace length and estimated annual egg production was clear (Fig 9). Egg estimates for the third and fourth clutches were not statistically different from first and second clutches suggesting that egg production by large females remained constant throughout the breeding season (Fig 9, Table 5). There were, however, differences in egg size relative to clutch number with egg size increasing in clutch numbers 2 and 3 (Fig 9, Table 5). Within a clutch, the coefficient of variation indicated minimal variation in egg size with a mean value of 4.8% (± 0.17) for large females and 4.3% (± 0.26) for small females. Male carapace length did not have a significant effect on either fecundity or egg size (Table 5). Statistical analysis of egg protein content was difficult given the small sample sizes involved but female size appeared not to significantly influence protein content of clutch 1 eggs (Fig 8, t test; t = 1.4709, df = 5, p = 0.198). Clutch number may also influence total protein content evidenced by the much higher protein values for clutch 2 eggs (Fig 8).
Figure 9. (A) Estimated fecundity relative to female carapace length (mm) per clutch (B) Estimated annual egg production for individuals in the experiment relative to female carapace length (mm). (C) Mean egg diameter by female carapace length (mm) from the Florida Keys (Small) and the Dry Tortugas National Park (Large). Error bars represent standard errors and numbers inside bars indicate sample size.
Table 5. Effect sizes and p-values for models testing female attributes. Values in bold indicate significant values where $P = 0.05$, CL = carapace length, $B$ = fixed effects coefficient estimates, CI = confidence intervals, R2 = correlation between fitted and observed values, $\Omega^2 = 1$ - (residual variance / response variance).

<table>
<thead>
<tr>
<th></th>
<th>Egg Diameter</th>
<th>Fecundity</th>
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<tbody>
<tr>
<td></td>
<td>B</td>
<td>CI</td>
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<tr>
<td>Fixed Effects</td>
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<tr>
<td>(Intercept)</td>
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<td>1.75-2.42</td>
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<tr>
<td>Female CL</td>
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<tr>
<td>Clutch No. 2</td>
<td>0.08</td>
<td>0.07-0.09</td>
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<tr>
<td>Clutch No 3</td>
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<td>0.09-0.13</td>
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<td>Clutch No. 4</td>
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<td>-0.00-0.00</td>
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<tr>
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<tr>
<td>Residual variance</td>
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<td>Observations</td>
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<tr>
<td>R2/\Omega^2</td>
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<td>0.541/0.530</td>
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</table>
**Larval Quality**

Mean larval carapace length (1.53mm) was the same irrespective of female size and clutch number but there was greater variability in larval carapace length for offspring from larger females (coefficient of variation: offspring from large females 6.2% ± 0.58; offspring from small females 4.1% ± 0.29). The larvae from second and third clutches from large females were also significantly larger than those of the first clutch (Fig 10, Table 6), whereas the larvae from the second clutch from the Florida Keys females were smaller (Fig 10, Table 6) and this observation is supported by a significant female carapace length and clutch number term in the model (Table 6). Male carapace length had no observable effect on larval size (Table 6). The increase in larval size from the second clutch produced by large females however did not translate to enhanced swimming ability. Those larvae swam significantly shorter distances than larvae from other clutches regardless of female carapace length (Fig 10, Table 6). Comparatively, larvae from third clutches swam significantly further (Fig 10, Table 6), and though larvae from fourth clutches appeared to be stronger swimmers, the sample size was extremely low (Fig 10, n = 1). Time to 50 percent larval mortality did not vary significantly relative to female or male size but did relative to clutch number (Fig 10, Table 6). This was driven by larvae from the third clutch of the large females living twice as long until 50 percent mortality as compared to first and second clutches (Fig 10). Time to 100 percent larval mortality, however, did not vary significantly relative to female size, male size, or clutch number. Larvae from Keys individuals had a mean time to 100 percent mortality of 7.21 days (± 2.78) whereas larvae from Dry Tortugas individuals had a mean time of 8.62 (± 3.24) days (Fig 10).
Figure 10. The relationships between female carapace length (mm) and larval attributes per clutch: (A) larval carapace length (mm), (B) swimming distance in cm/10 s, (C) days to 50% mortality, and (D) days to 100% mortality. Error bars represent standard errors and numbers inside bars indicate sample size.
Table 6. Effect sizes and p-values for models testing larval attributes. Values in bold indicate significant values where \( P = 0.05 \). CL = carapace length, \( B \) = fixed effects coefficient estimates, CI = confidence intervals, R2 = correlation between fitted and observed values, \( \Omega^2 = 1 - (\text{residual variance} / \text{response variance}) \).

<table>
<thead>
<tr>
<th></th>
<th>Days to 50% Mortality</th>
<th>Days to 100% Mortality</th>
<th>Distance Swum</th>
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<tr>
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<td>CI</td>
<td>( p )</td>
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<tr>
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<td>0.356/0.334</td>
<td>0.062/0.059</td>
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</table>
3.4 Discussion

We found no evidence of reproductive senescence in the Caribbean spiny lobster based on lobster size. Instead, several metrics suggested that parental effects could confer an advantage to resultant offspring. A positive relationship between female size and egg characteristics was apparent with exponentially higher fecundity for large females, larger egg sizes in clutches two and three, and greater protein content in eggs produced by large females. The exponential relationship between female size and fecundity has been well referenced in past research (MacDiarmid and Sainte-Marie 2006) and this study supports those findings but with the use of a non-invasive method for estimating clutch size. That increasing clutch number did not have any observable effect on fecundity is perhaps unexpected, as one would have hypothesized that egg production would decrease as multiple clutches are produced in a given season, particularly as there was no observed trade-off in egg quality (e.g. Beyer et al. 2015).

Taken as a whole, spermatophore attributes did not show a clear positive or negative association with male size. None of the metrics that we assessed showed declines in spermatophore quality relative to male size that would be suggestive of reproductive senescence and though the spermatophores of large males were substantially heavier and thicker, the sperm cell density and the viability of the cells did not differ significantly with male size. These findings mirror the results of Butler and colleagues (2015a), and suggest that the accessory fluid component of the spermatophore is greater for large males than small males – a trend also seen in the blue crab *Callinectes sapidus*, American lobster *Homarus americanus*, and the snow crab *Chionoecetes opilio* (Sainte-Marie and Lovrich 1994, Kendall et al. 2001, Kendall et al. 2002, Pugh et al. 2015). Spermatophore accessory fluid has several functions in decapod crustaceans (Subramoniam 1991). It acts as a sperm plug in species with internal spermathecae (Christy...
1987, Carver et al. 2005), as an antibacterial agent or nutritive source for sperm (Subramoniam 1991, Bissoondath and Wiklund 1995), and as a way to store and retain sperm (see review in Mann 2012). Previous experiments with *P. argus* indicate that spermatophores provide both physical and chemical cues to males and females that prevent further mating (Butler et al. 2011) but the precise role of the spermatophore matrix in *P. argus* and why it is greater in large males is not clear. In crustaceans, spermatophores are typically rich in proteins and include carbohydrates and lipids in lesser quantities (Jeyalectumie and Subramoniam 1991, Hinsch 1991). For males that produced multiple spermatophores, spermatophores deposited later appeared to have a higher total protein content (Fig 8). This result was unexpected given that spermatophore weight declined with the deposition of consecutive spermatophores. A similar result was reported by Butler et al. 2015a in which sperm depletion was explicitly tested and in which males were found to require approximately a week to restore spermatophore size (Butler et al. 2015a). Large males in this study typically re-mated within 2-3 days of spermatophore removal – hence the unexpected protein content results. Given the costs associated with sperm production (Dewsbury 1982) one would expect that males would either expend a greater effort earlier in the mating season, or economize sperm reserves so as to maintain consistent fertilization success through successive matings (Sato et al. 2006) However, given the small sample size of varying spermatophore number tested for protein content and our lack of understanding of the specific protein components in the *P. argus* spermatophore, it is difficult to draw strong conclusions. The results indicate, however, that further biochemical analysis including investigation of the chemical interactions between the spermatophore matrix and sperm cells may be warranted (i.e., Hou et al. 2010)
The major difference that we observed in larval attributes relative to parental size was in larval carapace length with the second and third clutches of large females producing larger larvae on average. There was also greater variability in the size of larvae produced by large females irrespective of clutch number. Differences in larval size between individuals of the same population is not unusual in marine invertebrates but the consequences of this variation is not often known (Marshall et al. 2003). There is evidence from several taxa of enhanced survival, growth, and earlier time to reproduction for larger larvae (Berkeley et al. 2004, Birkeland and Dayton 2005, Cabrera-Guzmán et al. 2013). For *P. argus*, the differences we observed in larval size relative to clutch number were not correspondingly observed in enhanced swimming ability or survival under starvation conditions. However, other larval qualities associated with size that we did not measure (e.g., prey capture efficiency, vertical migratory behavior, etc.) may confer a fitness advantage that was not revealed by these assays (Marshall et al. 2003). Importantly, as with the other metrics that we assessed, we found no detrimental effects of parental size on offspring fitness that would suggest that senescence is an issue for the largest lobsters, in fact, some measures suggest just the opposite.

Egg diameter and larval size were consistently larger when produced in the second and third annual clutches of *P. argus* and while swimming distance and time to 50% mortality produced mixed results; there were no negative associations. Only large females produce multiple clutches in a single reproductive season, so these results portend an unexpected advantage of large size. From an evolutionary standpoint it makes more sense for individuals to invest resources in the first reproductive event of a breeding season rather than saving resources for future events given the risk of mortality, or loss of potential mating opportunities. Alternatively, given that the predominately large lobsters from the Dry Tortugas typically start
reproduction earlier than the small lobsters present in the heavily fished Florida Keys, suggests that multiple clutches with better quality later clutches represents bet-hedging (Marshall et al. 2008; Olofsson et al. 2009). That is, large females capable of producing multiple clutches may spawn an early, less well provisioned first clutch when environmental conditions are more variable and risky for larval survival. Yet, any early-release larvae that do survive gain a subsequent size advantage over later spawned larvae of small females. The later, better-provisioned larvae of subsequent clutches of large females also have an advantage over the smaller first clutch larvae that are simultaneously spawned by small females.

It is also possible that the differences we observed between large and small adult *P. argus* in larval and egg attributes are a laboratory artifact. Because large females produce multiple clutches over a longer period of time, they were held longer in captivity than were small females whose reproductive season is shorter. If the food we provisioned lobsters with in the laboratory was more abundant or nutritious than what they generally obtain in nature, then large females held in the laboratory for a longer period of time - as well as their offspring - may have benefitted (cf Shin et al. 2003). In the same manner, it is also possible that lobsters held in the laboratory expend less energy foraging, avoiding predators, and finding mates than those in the wild. The same conclusion could perhaps be drawn for the differences in egg protein content relative to female size and spermatophore protein content relative to spermatophore number.

Similarly, while it is indeed possible that the source of the lobsters (Dry Tortugas National Park v Florida Keys fishery) may have influenced reproductive output, the geographical mismatch was unavoidable. Given the extent of fishing within the Florida Keys, it is nigh on impossible to find reproductively active individuals greater than 130 mm CL (Bertelsen and Matthews 2001, Butler et al. 2015a). Likewise, individuals in the Dry Tortugas National Park do
not seem to mature until they are above 100mm CL (Bertelsen and Matthews 2001, Maxwell et al. 2009) thus it was not possible to source individuals for the comparison from one location. However, even with the different source locations, it does not seem likely that diet could have contributed to differences in reproductive output. *Panulirus argus* are opportunistic foragers, feeding in a range of habitats including algal flats, rubble zones, and sea grass beds (Cox et al. 1997, Briones-Fourzán and Lozano-Álvarez 2013). The study by Cox and colleagues (1997) compared gut contents between two disparate locations (Dry Tortugas National Park and Biscayne National Park) in Florida. They documented *P. argus* feeding on a wide variety of prey, 115 taxa in all and found that there were no differences between sites in the prey consumed. Thus, while it is possible that the lobsters original diet may have played some role in their reproductive output, their non-discriminant feeding would suggest otherwise.

The absence of reproductive senescence may not be entirely surprising for a species that is predicted to have a maximum lifespan of approximately 20 years (Maxwell et al. 2009, 2013) and does not engage in parental investment once larvae hatch. Two of the leading hypotheses for female senescence - the ‘attentive mother hypothesis’ and the ‘helpful grandmother hypothesis’ - both argue that such behaviors are advantageous for females that provide greater care to offspring of their own or belonging to closely associated kin (Ward et al. 2009). Evidence to support these hypotheses come largely from longer lived mammalian species such as humans (Sear et al. 2008, Kachel et al. 2011), chimpanzees (Hawkes and Smith 2010), and killer whales (Ward et al. 2009). That said, there is evidence of reproductive senescence in a number of short-lived taxa that invest little to nothing in parental care such as guppies (Reznick et al. 2006), beetles (Omkar et al. 2006), cockroaches (Moore and Moore 2001), and antler flies (Bonduriansky and Brassil 2002).
One factor, however, that complicates our ability to accurately assess senescence in lobsters, and indeed crustaceans in general, is the poor relationship between lobster size and age. Lobster growth is influenced by a number of abiotic and biotic factors including temperature, salinity (Field and Butler 1994, Kearney et al. 2015), food availability (Behringer and Butler 2006), and disease (Behringer et al. 2011). Furthermore, intensive commercial and recreational fisheries can also reduce lobster survival and growth (Parsons and Eggleston 2005). Thus, given existing growth models we can assume that the large lobsters from the Dry Tortugas National Park are older, but we cannot definitively conclude that this is the case without an accurate means of assessing age independent of size. Until a direct measure of age exists, age dependent senescence will be difficult to assess.

Although we found no evidence of physiologically mediated reproductive senescence in *P. argus* one avenue not explored by this study is the possibility of behaviorally driven senescence. For example, three large males initially used in the experiments showed no inclination to mate and were thus replaced by other large males to ensure a balanced design was maintained. The substituted males successfully mated with all the females in their tank within days, an indication that the lack of reproductive interest of those few males was not because females were not receptive to mating. In addition, while molt stage is known to influence mating behavior in lobsters (Lipcius and Herrnkind 1987, Waddy et al. 2017), the males that did not mate, also did not molt during their time in captivity (between 4-5 months) which suggests that molting was not the problem. While the laboratory environment could have influenced the reproductive behavior of those lobsters, there is increasing evidence in other species that individuals differ in their rates or occurrence of senescence. In the last decade, evolutionary ecologists have included individual personality as one driver of senescence (Bouwhuis et al.
‘Shy’ male wandering albatross, for example, decline sharply in reproductive performance with age that is also closely associated with shorter foraging times (Patrick and Weimerskirch 2015). In many polygynous species males defend territories or harems of reproductive females and there is evidence from some species of deer that older males are unable to do so (Nussey et al. 2009, Vanpé et al. 2009). In intact, unfished lobster populations replete with large adults, lobsters display a polygynous mating system where large males defend a den of females from other large males (MacDiarmid 1991, Bertelsen and Cox 2001, Butler et al. 2015b). Whether reproductive senescence manifests as an inability for the largest males to maintain and defend such a den is unknown but for most of the heavily fished populations of *P. argus* the point is moot because populations containing these large individuals no longer exist and neither does this mating system, replaced instead by scramble competition for mates from similar-sized adults.

The implications of this study for the long-term management of *P. argus* fisheries are significant. The results suggest that management ought to favor the conservation of the largest lobsters given both the quantity and quality of gametes and offspring produced by these individuals. The most common harvest regulation for *P. argus* throughout the Caribbean is a minimum harvest size to protect juveniles and sub-adult lobsters (CRFM 2011) and although many fisheries also prohibit the take of ovigerous (‘berried’) females, there are no regulations at present designed to provide lasting protection for large individuals. Maximum size limits such as those used throughout US fisheries for the American clawed lobster (NOAA 2016) would, for example, provide some measure of protection for these largest sizes. Indeed, in the last few years there have been calls from fisheries managers in the Caribbean to investigate the possible use of maximum size limits or slot limits for *P. argus* (Spiny Lobster Declaration 2015). With the lack
of apparent senescence in the largest individuals, managers looking to increase reproductive potential in *P. argus* populations ought not to be concerned about large individuals producing offspring of lesser quality. With the sustained and excessive removal of large lobsters throughout the Caribbean, in addition to disease (Behringer et al. 2012), and environmental degradation (i.e. Butler et al. 1995) it is little wonder that catches in the last decade have been declining with a reduction in total stock fecundity and the possibility of recruitment overfishing in some populations (Ehrhardt and Fitchett 2010). Fisheries for the Caribbean spiny lobster are of considerable value to the region – thus it is imperative that we incorporate knowledge of mating systems in stock assessments and fisheries management. Like previous studies of *P. argus* mating dynamics and reproduction, this study highlights the importance of maintaining large individuals in fished populations if the persistence of healthy *P. argus* populations is a long term objective.
CHAPTER 4

MODELING HARVEST SLOT LIMITS AND MPAS FOR THE
RESTORATION OF SPAWNING STOCKS

4.1 Introduction

After decades of intense fishing pressure, 31% of global marine fisheries are considered overfished with another 58% fully exploited (FAO 2016). Moreover, the sustained and targeted fishing of the largest or oldest spawning individuals has undermined population stability and reproductive potential (Barnett et al. 2017). Although conventional catch and effort controls such as vessel licensing, trip limits, and quotas can control the total biomass that is harvested, they typically fail to conserve population structure and spawning stock. For many fished species it is the largest or oldest individuals that have the highest fecundities; often an order of magnitude higher than smaller conspecifics (Hixon et al. 2014). Older parents may also produce offspring of a higher quality (Berkeley et al. 2004a, Birkeland and Dayton 2005). Over-fishing of these individuals therefore results in the burden of reproduction, hence population survival, shifting to smaller/younger individuals. In extreme cases, intense fishing of the largest individuals has driven the selection of life history characteristics favoring earlier size (or age) at reproduction (Conover and Munch 2002, Hutchings and Rowe 2008), whereas in other situations over-exploitation has simply led to population collapse (Myers et al. 1997, Hutchinson 2008). Several management tools however are explicitly designed to protect older/larger individuals from harvest including: maximum size limits, gear restrictions (i.e., minimum net sizes, trap sizes),
prohibitions on the take of breeding individuals, and temporal or spatial closures (Berkeley et al. 2004b, Hixon et al. 2014, Barnett et al. 2017).

Regulations that restrict catch based on size are common in fisheries management, particularly minimum size limits that are designed primarily to protect juveniles and avoid recruitment overfishing (Allen et al. 2013). Maximum size limits where only individuals below a given size are harvested are common in recreational freshwater finfish fisheries (e.g. for pike, *Esox lucius*, and walleye, *Sander vitreus*; Minnesota Fishing Regulations 2018), but are less so in marine fisheries. Maximum size limit regulations are particularly well suited to species that exhibit high recruitment, slow growth, and moderate natural mortality (FAO 2012).

Combinations of minimum and maximum size limits result in ‘slot limits’, where individuals of an intermediate range may be harvested (harvest slot limits, open slot) or protected (protected slot limit, closed slot) (Gwinn et al. 2013). Harvest slot limits that are designed to protect both young recruits and spawning stocks are particularly useful when maternal reproductive output or the provisioning of young increases with size, or when fishing depletes spawning biomass (McPhee 2008, Arlinghaus et al. 2010).

Although size limits are common in fisheries management, no-take marine protected areas (MPAs) closed to fishing are not. The genesis of MPAs was in the conservation of natural and cultural resources, but their utility has expanded as a means of explicitly controlling harvests and conserving marine resources for sustainable fisheries (Roberts and Polunin 1991, Dayton et al. 2000, Pande et al. 2008). They have been credited with increasing density, biomass, organism size, and species diversity (Halpern 2003, Gill et al. 2017) and have been used to protect and rebuild large, mature individuals and spawning biomass for a number of species including: Atlantic cod (*Gadhus morhua*) (Moland et al. 2013), blacktip sharps (*Carcharhinus tilstoni*, *C.*
limatus) (Yates et al. 2016), and spiny lobsters (*Jasus edwardsii*) (Shears et al. 2006, Jack and Wing 2013). Additionally, MPAs can enhance the supply of larvae and the movement of adults beyond their boundaries - termed ‘spill-over’ (Harrison et al. 2012, Di Lorenzo et al. 2016).

Combined, harvest slot limits and MPAs potentially offer a means of protecting the most fecund individuals in a fished population, while allowing fishers to continue fishing (Steneck et al. 2009), but the use of these two mechanisms together for the explicit management of a target species has rarely been assessed. Here we investigate the potential of slot limits combined with no-take MPAs to enhance spawning stocks in one of the Caribbean’s largest and most valuable species: the Caribbean spiny lobster, *Panulirus argus*.

*The Caribbean spiny lobster*

The Caribbean spiny lobster, *Panulirus argus* is abundant and ubiquitous throughout much of the southern Western Atlantic, Gulf of Mexico, and Caribbean Sea (Holthius 1991). The wide distribution of the species is due to the planktonic phyllosoma larval phase during which larvae spend 5-9 months in the water column, transported by oceanic currents (Goldstein et al. 2008, Kough et al. 2013). Recent genomic analyses and biophysical modeling indicates some spatial genetic patchiness in retentive regions of the Caribbean (e.g., Gulf of Honduras, Bahamas) but also significant demographic connectivity among Caribbean nations - making it a true transboundary species (Kough et al. 2013, Truelove et al. 2015, 2017). Fisheries for *P. argus* are some of the largest and most economically valuable in the Caribbean (CRFM 2011) but decades of intense fishing pressure have left many regional populations fully capitalized or overfished (Ehrhardt 2010). As regional landings decline, population size frequency distributions have also become truncated because of the sustained fishing of the largest sizes (e.g., Moreno
and Fernandez 2003, Gongora 2009, SEDAR 2010). Despite strong evidence that large females are exponentially more fecund and produce higher quality offspring, and large males are more prolific mates that produce more and perhaps better quality sperm (MacDiarmid and Butler 1999, Butler et al. 2015a, Gnanalingam and Butler 2018a), there is only one management measure in place in the Caribbean that protects large individuals - a prohibition on the fishing of ovigerous females. But even this protection is temporary, because females become vulnerable to fishing as soon as they spawn. Few lobster fisheries in the world have instituted slot limits as a management measure. One example is the New England (USA) fisheries for the American lobster (Homarus americanus) in which state and federal slot limits have been instituted (NOAA 2018). These fisheries also protect breeding females via v-notching of the telson: a ‘tag’ that persists through several molts (DeAngelis et al. 2010). The New England lobster fishery is experiencing some of its highest landings in decades (Atlantic States Marine Fisheries Commission 2018), ascribed in part to these long-term management measures.

We used a two-sex, stage-based matrix meta-population model to assess the potential use of harvest slot limits and marine protected areas - separately and combined - for rebuilding spawning biomass for P. argus in the Caribbean. The complexity of P. argus as a transboundary species, in addition to limited data in the Caribbean means demographic analysis is an ideal method for this (Simpfendorfer 2005). Stage-structured models have been used to assess reserve design for the management of black bears (Salinas et al. 2005), as well as the harvesting of Atlantic horseshoe crabs (Grady and Valiela 2006), and mako sharks (Tsai et al. 2014). They have not been specifically applied to this kind of assessment with the exception of a preliminary study by Steneck et al. 2009 that used a single population of P. argus as its focus. The model we developed links P. argus populations from the 10 regions with the largest P. argus fisheries in
the Caribbean that together constitute approximately 97% of the landings for this species. We considered four management scenarios: (i) the current fishery without MPAs or harvest slot limits, (ii) MPAs + fishing, (iii) harvest slot limits, and (iv) MPAs + harvest slot limits. Each of these four management scenarios were tested at three fishing intensities ranging from 1/2 the current level of fishing to twice the estimated current level of fishing intensity.

4.2 Methods

Matrix modeling

Demographic models are typically single sex, based solely on female dynamics (Caswell 2001). Single sex models, however, ignore sexual differentiation in vital rates by assuming that growth and mortality are the same for males and females. They also fail to consider the role of males in reproduction that for species like *P. argus*, in which size selective mating and sperm limitation has been documented, can be consequential (MacDiarmid and Butler 1999, Butler et al. 2015a). We therefore opted for a two-sex model that allowed incorporation of differential growth, mortality, and reproduction based on observed data. A stage-based/size-based model was selected rather than an age-based model for two primary reasons: (1) until recently an accurate method for directly aging lobster did not exist (though see Chapter 2, and Leland et al. 2011, Kilada et al. 2012), and (2) harvest slot limits are more easily applied to size classes than age classes.

Data sources and biological parameters

The meta-population model links the 10 countries in the Caribbean and Western Atlantic with the highest landings of *P. argus* (The Bahamas, Cuba, Nicaragua, USA, Dominican
Republic, Honduras, Mexico, Haiti, Venezuela, Belize) via larval connectivity (Fig 11).

Landings from these countries account for 97% of total production in the Caribbean (FAO 2014). Given that the fisheries for *P. argus* in the Caribbean region are considered over- or fully-exploited (Erhardt 2010), we considered the magnitude of these landings to be a reasonable reflection of lobster population abundance. In other words, the 10 countries that dominate *P. argus* landings are presumably where the largest stocks reside. Although Brazil also ranks high in *P. argus* landings it was not included here because Brazilian landings often include catches of *P. laevicauda* (FAO 2015). Additionally, the species traditionally considered *P. argus* in Brazil has recently been re-described as a separate species, *P. meripurpuratus* (Giraldes and Smyth 2016). Data for model inputs came primarily from published literature (Table 7), with the exception of fecundity estimates that were empirically derived (see Chapter 3). Given the absence of published data for many Caribbean populations, however, we relied heavily on stock assessment data from the United States, particularly Florida, for model parameterization (SEDAR 2010).
Figure 11. Stage based matrix model of reproductive and non-reproductive cycles used for ten top *P. argus* harvesting countries in the Western Atlantic. On the left, is a diagram of the larval connectivity matrix among the ten countries. Country codes: HAI = Haiti, BEL = Belize, DR = Dominican Republic, HON = Honduras, MEX = Mexico, BAH = Bahamas, CUB = Cuba, VEN = Venezuela, NIC = Nicaragua, USA = United States of America. The graphic on the right depicts the sex-and stage-based structure of the model depicted for a single country (e.g., Belize), which are modelled separately for reproductive (top) and non-reproductive (bottom) portions of the population before summing to obtain results for the entire population. Arrows indicate individuals surviving and growing to the next stage ($G_x$); or the probability of an individual surviving and remaining in its current stage ($P_x$), and $F_x$ represents stage-specific fertility. Codes for life history stages are described in Table 8.
Table 7. Data sources and biological parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural mortality ((M))</td>
<td>Decay function 0.51 for a 1 year old</td>
</tr>
<tr>
<td>Fished mortality ((F))</td>
<td>0.34</td>
</tr>
<tr>
<td>Size specific fecundity ((f_i))</td>
<td>(0.4527CL^{2.8669})</td>
</tr>
<tr>
<td>Stock structure and landings</td>
<td></td>
</tr>
<tr>
<td>Larval connectivity probabilities</td>
<td>Larval probability matrix</td>
</tr>
<tr>
<td>von Bertalanffy Growth Equation</td>
<td></td>
</tr>
<tr>
<td>(L_x)</td>
<td>Female 151 \hspace{1cm} Male 210</td>
</tr>
<tr>
<td>(K) (year(^{-1}))</td>
<td>0.23 \hspace{1cm} 0.2</td>
</tr>
<tr>
<td>Sex Ratio</td>
<td>1.0 \hspace{1cm} 1.0</td>
</tr>
</tbody>
</table>
Landings data from the Caribbean Regional Fisheries Mechanism (CRFM), Western Central Atlantic Fishery Commission (WECAFC) and Food and Agriculture Organization of the United Nations (FAO) were used to calculate starting population sizes and stock structure. Where landings were recorded in weight (metric tons), values were converted to individuals using $TWt = 0.0422CL^{2.64091}$ where $TWt =$ Total weight (g), and $CL =$ carapace length (mm) (Lyons et al. 1981).

Fecundity in *P. argus* scales positively to carapace length with larger females producing exponentially more eggs per clutch (MacDiarmid and Sainte-Marie 2006). Large females (> 80mm CL) also often produce multiple clutches in a single spawning season (MacDiarmid and Butler 1999, Bertelsen and Matthews 2001). The equation for size specific fecundity (egg production per clutch x number of clutches per year) was based on the empirical study in Chapter 3 (Gnanalingam and Butler 2018a, b). Growth was modeled using the von Bertalanffy growth equation and parameter estimates for males and females from Erhardt (2008). That study, which was based on mark recapture and growth per molt data from South Florida, provided growth estimates that recognized that females typically grow slower and reach a smaller maximum size than males. A sex ratio of 1:1 was used in the model. Although sex ratios of *P. argus* can vary relative to season and habitat with more females observed during reproductive period (Gregory et al 1982, Cox et al. 1997), the sex ratio is typically 1:1 (Kanciruk and Herrnkind 1976). To account for demographic connectivity among the 10 subpopulations, offspring produced every year were split among the 10 subpopulations according to a normalized larval probability matrix derived from a multi-scale biophysical model coupled with empirical estimates of larval behavior and gamete production (Kough et al. 2013).
**Model development**

The model takes the basic form:

\[ N_{t+1} = A_t N_t \]

where \( N_t \) is a vector of numbers of lobsters in each stage class at time \( t \), and \( A_t \) is the life history projection matrix composed of survival and fecundities for each stage at time \( t \) (Caswell 2001). Model stages reflect the four main life stages for *P. argus*: larvae, juvenile, subadult and adult (Table 8). The adult life stage is further divided into 10 mm CL size classes (A1-A15 for males; A1-A10 for females) to account for differences in reproductive output, growth, and mortality and to enable assessment of different harvest size limits (Fig 11). A 6-month time step was selected given the estimated growth rates and the 10mm CL size classes. This necessitated the creation of reproductive and non-reproductive matrices so as to avoid overestimating reproduction (Fig 11). Annual survival probabilities were also adjusted accordingly. The two-sex stage-structured model with reproduction took the form illustrated in figure 12, where \( \rho \) is the sex ratio at birth (set to 0.5 for a 1:1 sex ratio), \( P_{i,s} \) is the probability of an individual surviving and remaining in its current stage, \( G_{i,s} \) is the probability of an individual surviving and moving to the next stage and \( f_{i,s} \) represents stage specific fertility (given by size specific fecundity \( \times \sigma_{i,s} \), the probability of an individual in stage \( i \) surviving). The matrix without reproduction was identical except for the substitution of zeros in place of the fertility coefficients \( f_i \). Models ran for 30 years.

Natural mortality (\( M \)) was estimated using an exponential decay function centered around 0.51 for a 1-year-old *P. argus* (Forcucci et al. 1994). Values were applied uniformly to all 10 populations. Fishing mortalities (\( F \)) were based on the mean estimated value for all size classes
in the Florida *P. argus* fishery (SEDAR 2010) and were again, applied uniformly to all 10 populations. Total mortality was thus $M + F$ and annual survival $1 - (M + F)$.

Table 8. Life history stages and codes for *Panulirus argus* used in the matrix model

<table>
<thead>
<tr>
<th>Sex</th>
<th>Stage-Class</th>
<th>Code</th>
<th>Carapace Length (mm)</th>
<th>Approximate age (years)</th>
<th>Expected stage duration (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Larvae</td>
<td>L</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Juveniles</td>
<td>J</td>
<td>0-50</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Subadults</td>
<td>SA</td>
<td>50-65</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 1</td>
<td>A1</td>
<td>65-75</td>
<td>2.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 2</td>
<td>A2</td>
<td>75-85</td>
<td>3</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 3</td>
<td>A3</td>
<td>85-95</td>
<td>3.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 4</td>
<td>A4</td>
<td>95-105</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 5</td>
<td>A5</td>
<td>105-115</td>
<td>4.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 6</td>
<td>A6</td>
<td>115-125</td>
<td>5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 7</td>
<td>A7</td>
<td>125-135</td>
<td>5.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 8</td>
<td>A8</td>
<td>135-145</td>
<td>6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 9</td>
<td>A9</td>
<td>145-155</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adult 10</td>
<td>A10</td>
<td>155-165</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adult 11</td>
<td>A11</td>
<td>165-175</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adult 12</td>
<td>A12</td>
<td>175-185</td>
<td>10.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Adult 13</td>
<td>A13</td>
<td>185-195</td>
<td>12.5</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>Adult 14</td>
<td>A14</td>
<td>195-205</td>
<td>14</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>Adult 15</td>
<td>A15</td>
<td>205+</td>
<td>14+</td>
<td>-</td>
</tr>
<tr>
<td>Females</td>
<td>Larvae</td>
<td>L</td>
<td>0.5</td>
<td>0</td>
<td>0.5</td>
</tr>
<tr>
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<td>Juveniles</td>
<td>J</td>
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<td></td>
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<td>65-75</td>
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<tr>
<td></td>
<td>Adult 2</td>
<td>A2</td>
<td>75-85</td>
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<td>1</td>
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<tr>
<td></td>
<td>Adult 3</td>
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<td>85-95</td>
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<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Adult 4</td>
<td>A4</td>
<td>95-105</td>
<td>4.5</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Adult 5</td>
<td>A5</td>
<td>105-115</td>
<td>5.5</td>
<td>1</td>
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<tr>
<td></td>
<td>Adult 6</td>
<td>A6</td>
<td>115-125</td>
<td>7</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Adult 7</td>
<td>A7</td>
<td>125-135</td>
<td>8.5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Adult 8</td>
<td>A8</td>
<td>135-145</td>
<td>11.5</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>Adult 9</td>
<td>A9</td>
<td>145-155</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Adult 10</td>
<td>A10</td>
<td>155-165</td>
<td>16+</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 12. Reproductive matrix used in stage based model.

<table>
<thead>
<tr>
<th>( G_{i,j} )</th>
<th>( P_{j,j} )</th>
<th>( 0 )</th>
<th>( 0 )</th>
<th>( \rho F_m(A1) )</th>
<th>( \vdots )</th>
<th>( \rho F_m(A15) )</th>
<th>( 0 )</th>
<th>( 0 )</th>
<th>( 0 )</th>
<th>( \rho F_r(A1) )</th>
<th>( \vdots )</th>
<th>( \rho F_r(A10) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( G_{j,sa} )</td>
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<td>( 0 )</td>
<td>( 0 )</td>
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</tr>
<tr>
<td>( (1-\rho)F_m(A1) )</td>
<td>( \vdots )</td>
<td>( (1-\rho)F_m(A15) )</td>
<td>( \rho F_m(A15) )</td>
<td>( \rho F_r(A1) )</td>
<td>( \vdots )</td>
<td>( (1-\rho)F_r(A15) )</td>
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</tr>
<tr>
<td>( G_{A1,A2} )</td>
<td>( 0 )</td>
<td>( 0 )</td>
<td>( 0 )</td>
<td>( 0 )</td>
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</tr>
<tr>
<td>( P_{A10,A10} )</td>
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<td>( 0 )</td>
<td>( 0 )</td>
<td>( 0 )</td>
<td>( 0 )</td>
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</tr>
</tbody>
</table>
Female lobsters typically reach sexual maturity at a smaller size than males (Maxwell et al. 2009), however, sexual maturity in males can be difficult to determine (Chubb 2000). Size at sexual maturity varies geographically - attributed to differences in environmental conditions, densities, and fishing intensities (Chubb 2000). For consistency, size at sexual maturity was therefore set at stage A1 (65-75 mm CL) for both sexes across all populations. Mating was then modeled using a modified harmonic mean birth function (Caswell 2001).

\[
\begin{align*}
    f_{\text{male}}(n) &= \frac{kn_f}{n_f+n_m} \\
    f_{\text{female}}(n) &= \frac{kn_m}{n_f+n_m}
\end{align*}
\]

where \( k \) is clutch size and \( n_m \) and \( n_f \) are the densities of reproductive males and females, respectively. Female *P. argus* preferentially mate with male *P. argus* that are of equivalent or larger size, whereas males are less selective (Butler et al. 2015b). The birth function accounted for this selectivity by restricting mating for females to males of equivalent or larger size (i.e., an A1 female could mate with males A1-A15 and A8 female could only mate with males A8-A15).

The model ignores density dependent effects on survival, fecundity, and growth. Given that little is known of the effects of density dependence on these parameters for *P. argus* it was beyond the scope of this study to investigate those effects.

**The management scenarios**

Four management scenarios were applied uniformly across all 10 subpopulations (Table 9) and each were assessed at three different fishing mortalities (i.e., the inverse of fishing effort):
low fishing mortality \((F = 0.17)\), intermediate fishing mortality or ‘status quo’ \((F = 0.34)\), and high fishing mortality \((F = 0.68)\). The value for the ‘status quo’ \((0.34)\) was based on the mean \(F\) estimated for all size classes in the Florida \(P.\ argus\) fishery (SEDAR 2010).

Table 9. Management scenarios and fishing restrictions applied to each model run for the 10 \(P.\ argus\) populations.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Fishing Restrictions</th>
<th>Justification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Fishing Status Quo</td>
<td>Min Size Limit 75 mm</td>
<td></td>
</tr>
<tr>
<td>2. Slot Limit</td>
<td>Slot Limit 75 – 105 mm</td>
<td>105 mm CL is the size at which fisheries landings sharply decline in the Florida trap fishery</td>
</tr>
<tr>
<td></td>
<td>Slot Limit 75 - 135 mm</td>
<td>135 mm CL is the point at which a significant number of female lobsters produce a 3rd clutch</td>
</tr>
<tr>
<td>3. MPA</td>
<td>Min Size Limit 75 mm + 2% no take protection</td>
<td>2% = current area protected by MPA in the Caribbean (Knowles et al. 2015)</td>
</tr>
<tr>
<td></td>
<td>Min Size Limit 75 mm + 10% no take protection</td>
<td>10% = target for MPA protection under the Convention on Biological Diversity</td>
</tr>
<tr>
<td></td>
<td>Min Size Limit 75 mm + 30% no take protection</td>
<td>30% = target for MPA protection from the World Park’s Congress</td>
</tr>
<tr>
<td>4. MPA + Slot Limit</td>
<td>2% no take protection + Slot Limit 75 – 105 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2% no take protection + Slot Limit 75 – 135 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30% no take protection + Slot Limit 75 – 105 mm</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30% no take protection + Slot Limit 75 – 135 mm</td>
<td></td>
</tr>
</tbody>
</table>
A minimum size limit of 75 mm was applied to all four scenarios, which is close to several of minimum legal sizes currently applied in the Caribbean. A fishing mortality of 0.13 (SEDAR 2010) for subadult lobsters was included despite the minimum size limit to account for the take of undersized lobster as inadvertent bycatch, poaching, and the use of subadults as live social attractants (‘bait’) within traps in Florida (SEDAR 2010). The response variables produced by the model were: total abundance \( N \), spawning stock abundance (the number of breeding individuals only), total egg production (reproductive output - the number of breeding females multiplied by their fecundity), fishery biomass, the number of migrants moving from MPAs to fished areas, and the population growth rate \( \lambda \).

In scenarios with MPAs, the model accounted for the movement of adults between protected and fished areas. A carrying capacity for the fishery \( K_f \) was set based on the estimated area occupied by \( P. \ argus \) in the Caribbean multiplied by the highest densities of lobsters observed in a Florida MPA (0.031 per m\(^2\)) (Eggleston and Parsons 2008). The carrying capacity of MPAs \( K_p \) was then the percent of the population being protected \( \times K_f \). Emigrant mortality was set at 0.8 as lobsters are often targeted at MPA boundaries as they move from protected areas to fished areas (‘spillover’) (Goñi et al. 2010). Probability of movement decreased linearly with increasing lobster size; thus smaller adults (< 75 mm CL) were more likely to move than very large adults (> 110 mm CL) that tend to become more philopatric with size (Bertelesen and Hornbeck 2009, Kelly and MacDiarmid 2003).

**Evaluation of parameter uncertainty**

Using management scenario 3 (MPA with 30% no take protection), the stochasticity in four life history parameters \( M, \) larval mortality, fecundity, and emigrant mortality) (Table 10)
was evaluated. The purpose was to examine the effect of uncertainty in the chosen life history parameters on population growth rate ($\lambda$), total abundance, spawning stock biomass, reproductive output, and adult spillover into the fishery from the MPA. Stochasticity in larval mortality was based on long term variability (30%) in fisheries landings (FAO 2018) and emigrant mortality was 0.8-1.0 based on the Goñi et al. (2010) estimate of lobster mortality from an MPA in the Mediterranean. Natural mortality and fecundity varied by 10%. A uniform probability function was used for all four parameters to select values within the chosen bounds. A fifth scenario was also run that combined uncertainty in all four parameters. Each scenario was run 10 times at a low fishing effort (0.17), with the other parameters universally applied.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Source of Uncertainty</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Larval survival</td>
<td>$\pm$ 0.15</td>
</tr>
<tr>
<td>B</td>
<td>Natural mortality</td>
<td>$\pm$ 0.05</td>
</tr>
<tr>
<td>C</td>
<td>Emigrant survival</td>
<td>0.8-1.00</td>
</tr>
<tr>
<td>D</td>
<td>Fecundity</td>
<td>$\pm$ 0.05</td>
</tr>
<tr>
<td>E</td>
<td>Scenarios A-D combined</td>
<td></td>
</tr>
</tbody>
</table>

4.3 Results

*Deterministic estimates*

Fishing effort and management scenario had obvious effects on total abundance ($N$), spawning stock abundance, egg production, fishery biomass, and estimates of $\lambda$ (the finite rate of population increase) for the 10 modeled populations. Fishing effort was particularly influential (Fig 13). When fishing effort was low ($F = 0.17$), populations in all 10 management scenarios
grew exponentially; but when fishing effort was high \((F = 0.68)\) both scenario 1 \((fishing\ status quo)\) and scenario 2 \((slot\ limits\ only)\) populations collapsed. In comparison, in the other two scenarios: scenario 3 \((MPAs\ only)\) and scenario 4 \((MPA + slot\ limit)\) at high fishing effort, populations grew after 20-27 years depending on the specific model being run. The effect of fishing effort was reflected in values of \(\lambda\) where populations stabilized (Scenarios 1 & 2 only, Table 11). At low fishing effort, \(\lambda\) was > 1.3 (indicating exponential growth in these populations), at mid fishing effort \(\lambda\) was centered around 1.0, whereas at high fishing effort \(\lambda\) was < 1 (indicating population decline).
Figure 13. Example of how fishing effort influenced total abundance ($N$) using Scenario 1 – Fishing, for the 10 populations combined. Note the differences in the scale of the y-axes, which were necessary to compare the temporal patterns in population change despite large differences in the magnitude of the effects of different levels of fishing.
The management option that yielded the highest $N$, fishery biomass, spawning stock abundance, and total egg production for the 10 populations combined after 30 years, was scenario 4 ($MPAs + harvest slot limits$) (Fig 14-16, Fig 17, Table 11). Regardless of fishing effort, the most conservative management scenario (30% of area designated as a MPA + maximum slot limit (MSL) of 105 mm CL) performed the best, followed by the same MPA designation coupled with a larger MSL (30% MPA + MSL 135 model; Fig 14-16, Fig 17, Table 11). The other scenarios varied in their performance relative to fishing effort. For example, when 30% of the area was designated a MPA the model predicted very high total lobster abundance and spawning stock abundance at a low fishing effort but failed to perform as well at a high fishing effort (Fig 14-16, Table 11). When differences are viewed as relative change (Year 30/Year 0) rather than temporally the results were the same (Fig 18-20). The various models took between 5 and 27 years for the population size ($N$) to double, depending on the scenario modeled. The shortest doubling time (between 5-7 years) was achieved with a MSL of 105 mm CL (either alone or combined with a 30% MPA) and the longest with a MSL of 135 mm CL alone.

After 30 years, spawning individuals represented a very small percentage of the total $N$ across the 30 management combinations. However, both the 2% MPA + MSL 105 and 30% MPA + MSL 105 scenarios offered greater protection for larger size classes (Fig 21) than the other scenarios. When fishing effort was high, there was little protection for large individuals in scenarios 1 ($Status quo$) and 2 ($harvest slot limits$) and by Year 30 these largest size classes crashed. In scenarios 3 ($MPAs$) and 4 ($MPAs + harvest slot limits$) adult populations either stabilized or increased exponentially after 10 years.
Figure 14. Effect of management scenario and fishing effort on total abundance ($N$) for the 10 countries combined over 30 years. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
Figure 15. Effect of management scenario and fishing effort on spawning stock abundance for the 10 countries combined over 30 years. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
Figure 16. Effect of management scenario and fishing effort on total egg production (reproductive output) for the 10 populations combined over 30 years. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
Figure 17. Biomass of legal sized lobsters (> 75 mm carapace length) at Year 30 for each management scenario and the 10 populations combined. Models were run at mid fishing effort ($F = 0.34$).
Table 11. Summary table from deterministic model runs incorporating management scenario and fishing effort on total abundance (N), spawning stock abundance, reproductive output, number of migrants, and \( \lambda \) for the 10 populations combined over 30 years. ‘Migrants’ represents the average number of lobsters that move into the fishery from MPAs for the 10 populations over the 30-year period.

### Low Fishing Effort

<table>
<thead>
<tr>
<th>Management Scenario</th>
<th>Scenario Particulars</th>
<th>N</th>
<th>Spawning Stock</th>
<th>Reproductive Output</th>
<th>Migrants</th>
<th>( \lambda )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status quo</td>
<td></td>
<td>1.87E+13</td>
<td>1.02E+11</td>
<td>9.16E+15</td>
<td>NA</td>
<td>1.34</td>
</tr>
<tr>
<td>Harvest Slot Limits</td>
<td>MSL 105</td>
<td>2.35E+14</td>
<td>1.14E+12</td>
<td>1.08E+17</td>
<td>NA</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>MSL 135</td>
<td>3.14E+13</td>
<td>1.67E+11</td>
<td>1.49+16</td>
<td>NA</td>
<td>1.36</td>
</tr>
<tr>
<td>MPA</td>
<td>2% coverage</td>
<td>3.63E+13</td>
<td>1.84E+11</td>
<td>1.62E+16</td>
<td>3.36E+08</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>10% coverage</td>
<td>1.64E+14</td>
<td>8.14E+11</td>
<td>7.27E+16</td>
<td>1.06E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>30% coverage</td>
<td>4.31E+14</td>
<td>2.38E+12</td>
<td>2.11E+17</td>
<td>1.72E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td>MPA + Harvest Slot Limit</td>
<td>MSL 105 + MSL 2%</td>
<td>1.34E+14</td>
<td>5.66E+11</td>
<td>5.34E+16</td>
<td>1.13E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 135 + MPA 2%</td>
<td>5.29E+13</td>
<td>2.49E+11</td>
<td>2.04E+16</td>
<td>1.50E+09</td>
<td>Not stable</td>
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<tr>
<td></td>
<td>MSL 105 + MPA 30%</td>
<td>1.08E+15</td>
<td>4.99E+12</td>
<td>4.70E+17</td>
<td>4.05E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 135 + MPA 30%</td>
<td>4.87E+14</td>
<td>2.47E+12</td>
<td>2.2E+17</td>
<td>5.50E+09</td>
<td>Not stable</td>
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### Medium Fishing Effort

<table>
<thead>
<tr>
<th>Management Scenario</th>
<th>Scenario Particulars</th>
<th>N</th>
<th>Spawning Stock</th>
<th>Reproductive Output</th>
<th>Migrants</th>
<th>( \lambda )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status quo</td>
<td></td>
<td>1.32E+09</td>
<td>8.89E+06</td>
<td>5.53E+11</td>
<td>NA</td>
<td>0.98</td>
</tr>
<tr>
<td>Harvest Slot Limits</td>
<td>MSL 105</td>
<td>1.45E+11</td>
<td>7.79E+08</td>
<td>5.38E+13</td>
<td>NA</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>MSL 135</td>
<td>3.12E+09</td>
<td>2.01E+07</td>
<td>1.25E+12</td>
<td>NA</td>
<td>1.01</td>
</tr>
<tr>
<td>MPA</td>
<td>2% coverage</td>
<td>6.12E+12</td>
<td>4.33E+10</td>
<td>2.36E+15</td>
<td>3.51E+08</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>10% coverage</td>
<td>3.03E+13</td>
<td>2.12E+11</td>
<td>1.16E+16</td>
<td>1.78E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>30% coverage</td>
<td>8.02E+13</td>
<td>3.48E+11</td>
<td>3.09E+16</td>
<td>5.02E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td>MPA + Harvest Slot Limit</td>
<td>MSL 105 + MPA 2%</td>
<td>6.99E+13</td>
<td>3.52E+11</td>
<td>2.45E+16</td>
<td>1.63E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 135 + MPA 2%</td>
<td>2.21E+13</td>
<td>9.72E+10</td>
<td>4.60E+15</td>
<td>1.68E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 105 + MPA 30%</td>
<td>5.91E+14</td>
<td>2.67E+12</td>
<td>1.84E+17</td>
<td>7.41E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 135 + MPA 30%</td>
<td>2.14E+14</td>
<td>1.02E+12</td>
<td>5.74E+16</td>
<td>1.38E+10</td>
<td>Not stable</td>
</tr>
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</table>
## High Fishing Effort

<table>
<thead>
<tr>
<th>Management Scenario</th>
<th>Scenario Particulars</th>
<th>N</th>
<th>Spawning Stock</th>
<th>Reproductive Output</th>
<th>Migrants</th>
<th>λ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status quo</td>
<td></td>
<td>0.00</td>
<td>0.00</td>
<td>0.73</td>
<td>NA</td>
<td>0.41</td>
</tr>
<tr>
<td>Harvest Slot Limits</td>
<td>MSL 105</td>
<td>9.13E+06</td>
<td>49394</td>
<td>3.87E+09</td>
<td>NA</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>MSL 135</td>
<td>2.14+05</td>
<td>1175</td>
<td>5.84E+07</td>
<td>NA</td>
<td>0.81</td>
</tr>
<tr>
<td>MPA</td>
<td>2% coverage</td>
<td>6.59E+11</td>
<td>1.49E+10</td>
<td>1.53+14</td>
<td>3.43+08</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>10% coverage</td>
<td>3.26E+12</td>
<td>7.42E+10</td>
<td>7.47E+14</td>
<td>1.93E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>30% coverage</td>
<td>9.14E+12</td>
<td>2.03E+11</td>
<td>1.96E+15</td>
<td>7.24E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td>MPA + Harvest Slot Limit</td>
<td>MSL 105 + MPA 2%</td>
<td>9.6E+13</td>
<td>2.34E+11</td>
<td>1.72E+16</td>
<td>1.59E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 135 + MPA 2%</td>
<td>4.07E+13</td>
<td>1.11E+11</td>
<td>9.77E+15</td>
<td>1.65E+09</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 105 + MPA 30%</td>
<td>7.99E+14</td>
<td>2.36E+12</td>
<td>1.09E+17</td>
<td>1.02E+10</td>
<td>Not stable</td>
</tr>
<tr>
<td></td>
<td>MSL 135 + MPA 30%</td>
<td>4.57E+14</td>
<td>1.21E+12</td>
<td>3.20E+16</td>
<td>2.10E+10</td>
<td>Not stable</td>
</tr>
</tbody>
</table>
Figure 18. Relative change in total abundance (N) as Year 30/Year 0 for each of the management scenarios at low, mid, and high fishing efforts. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
Figure 19. Relative change in spawning stock abundance as Year 30/Year 0 for each of the management scenarios at low, mid, and high fishing efforts. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
Figure 20. Relative change in total egg production (reproductive output) as Year 30/Year 0 for each of the management scenarios and low, mid, and high fishing efforts. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
Figure 21. Stock structure of fisheries sized lobsters (> 85mm CL) at Year 30 for each of the management scenarios run at mid fishing effort ($F = 0.34$). Fisheries sized lobsters 75-85 mm CL were removed from the figure given their much higher abundances. With their inclusion, the detail in stock structure was not clear. Note the differences in the y-axis scales, which permits easier visualization of the relative results among scenarios.
In model scenarios that included MPAs (scenarios 3 and 4), protected populations reached their carrying capacities by year 30 regardless of the fishing pressure outside of the MPA. Populations within MPAs, typically consisted of a high proportion of spawning individuals thus reproductive output within MPAs remained high particularly when fishing effort was high. Given the varying carrying capacities ($K_p$) for the MPAs, the number of migrants between protected and fished areas also varied. What was highlighted however was that without the additional protection of harvest slot limits provided for by Scenario 4, up to 41% of lobster emigrating from MPAs would be lost to fishing mortality.

Estimates with uncertainty

For the five stochastic scenarios tested, population growth ($\lambda$) failed to stabilize within the 30 year run, as was the case for the deterministic model, suggesting that a longer model run was required. Of the four life history parameters tested, stochasticity in natural mortality had the greatest effect on response variables (Fig 22). Stochasticity in emigrant mortality also had a large effect on the number of migrants (Fig 22). Model runs that included a combined stochasticity for all four parameters (larval mortality, natural mortality of other life stages, fecundity, and MPA emigrant mortality) had the next greatest effect on the model outcomes, followed by the scenario in which only fecundity varied stochastically (Fig 22).
Figure 22. Box plot of variation in (A) total abundance (N), (B) spawning stock abundance, (C) reproductive output and (D) emigrant mortality as a result of stochasticity (natural mortality (M), larval mortality, fecundity, emigrant mortality, combination of the previous four). Model run was 30% MPA + fishery. Dotted red line indicates the median value from the model run without stochasticity.
4.4 Discussion

The modeling results indicate that combining MPAs and harvest slot limits offers the best protection for the maintenance of *P. argus* populations and allows for the rebuilding of spawning stocks regardless of the level of fishing effort simulated. At levels of fishing effort currently estimated for *P. argus* in the Florida Keys or levels twice or half that, implementation of a maximum size limit (MSL) of 105 mm CL along with the setting aside of 30% of the fished area as no-take MPA produced the largest lobster population (*N*), spawning stock abundance, fishery biomass, and reproductive output. However, strong positive effects on lobster stock sustainability was also predicted by the model if only 2% of the total fishing area is protected within MPAs and combined with the same MSL of 105 mm CL. Thirty years after implementation of the MPA 30% + MSL 105 and MPA 30% + MSL 135 model scenarios, total lobster population size and spawning stock abundance were projected to increase 5-fold as compared to current levels of fishing and management (i.e., the status quo scenario). Compared to the increase in lobster stocks (2-109%) predicted if various slot limits were implemented, the effect of combining MPAs and harvest slot limits is an order of magnitude higher. Three management scenarios (MSL 105, 2% MPA + MSL 105, and 30% MPA + MSL 105 models) predicted a doubling in lobster stocks in the least amount of time - only 5-7 years, although over a longer time frame (i.e., 30 years), although those management measures did not necessarily produce the highest total or spawning stock abundances.

The model results also confirm the pronounced impact of fishing effort on lobster stocks in the absence of other management tools, such as MPAs and slot limits. Cutting fishing effort in half from present day levels, had the drastic effect of increasing the predicted total abundance, spawning stock, and reproductive output of lobsters 100 fold over three decades. Although a
large reduction in fishing effort is unlikely to be economically feasible or desirable to fishers, if lobster populations are indeed severely depleted, as most empirical and modeling studies suggests, a reduction in fishing mortality may be the only way to rebuild lobster populations. Though extreme, there are examples of such dramatic management measures. A case in point is the Atlantic striped bass (*Morone saxatilis*) fishery in which the population rebounded after what was essentially a 5-year moratorium on fishing (Richards and Rago 1999, Secor 2000).

The imposition of slot limits and MPAs independently (Scenarios 2 and 3) also had positive effects on fishery biomass and spawning stock abundance, but only when the fishing effort was low. At current levels of fishing or even higher levels, slot limits alone were ineffective because fishing removed individuals from the population before they could grow into the larger protected size classes, which in turn translated into poor reproductive output. Based on estimated rates of growth, lobsters that reach legal size in Florida (76 mm CL) would have to avoid capture for 4 - 5 years (males) or 5 - 8 years (females) years to reach the slot-limit size sanctuary of 105 or 135 mm CL, respectively.

Traditionally, minimum size limits for the harvest of marine fisheries are often set at the size where 50% of the individuals of that size become reproductive (Hill 1990). But part of the difficulty associated with the use of slot-limits is designating an appropriate maximum size limit. Ideally, designation of a MSL is one that balances economic and industry needs with what is biologically necessary for long-term stock sustainability. In the Florida Keys, for example, individuals > 100 mm CL made up only 3% of the total trap fishery catch in 2009-2010 (SEDAR 2010). Thus, a MSL of 135 mm CL alone without the complementary advantage of no-take MPAs makes little sense; few if any lobsters would avoid capture and survive to that size. A MSL of 105 mm CL would protect considerably more individuals and, if implemented
Caribbean-wide, would increase lobster stocks nearly 100-fold compared to current levels of fishing (i.e., status quo) by the end of 30 years. But a MSL that small is less likely to be supported by fishers particularly in areas of the Caribbean where a greater fraction of the fishery landings are lobsters greater than 100 mm CL.

The buildup of biomass and stocks within MPAs has been demonstrated for a number of species including Queen conch (*Lobatus gigas*) (Kough et al. 2017), mutton snapper (*Lutjanus analis*) (Ault et al. 2006) and several species of spiny lobsters: *Jasus edwardsii* in New Zealand (Jack and Wing 2010), *P. elephas* in Spain (Diaz et al. 2011), and *P. argus* in Florida (Cox and Hunt 2005). Furthermore, a number of empirical studies demonstrate the potential benefit to fisheries of marine protected areas both in terms of exporting larvae (Diaz et al. 2011) and fishery-sized individuals (Ley-Cooper et al. 2014). Similarly, the model simulations that included no-take MPAs alone with no slot limits predict the accumulation of spawning stock biomass within MPAs and the export of larvae and adult lobsters into the adjoining fishery areas. At moderate to high levels of fishing, those inputs to the fishery did not compensate for high fishing mortality unless the total area protected was high (i.e., 30%), probably unrealistically high. Without the additional protection (e.g., harvest slot limits) for individuals that move from protected areas into the fishery, high emigrant mortality removes these individuals from the fished population (Goñi et al. 2010). Indeed, fishers often capitalize on the existence of MPAs by fishing along those boundaries, increasing mortality on MPA emigrants (see examples in Gell and Roberts 2003, Kelly and MacDiarmid 2003). We captured this phenomenon in the model's dynamics. Therefore, despite the various benefits of no-take MPAs, they alone are likely to be too few and too small to substantially increase population densities and conserve spawning stocks of exploited species (Gaines et al. 2010). It is imperative that MPAs be paired with other
management measures to ensure the sustainability of fisheries, and the model results for lobsters indicate that harvest slot limits are a logical and effective means of expanding the ecological footprint of MPAs (Steneck et al. 2009).

The effectiveness of demographic models to describe population dynamics is constrained by the quality of the data inputs and the model’s assumptions. Several key assumptions were made here. The use of the same values for mortality, growth, and fecundity for all 10 Caribbean populations, for example, is not reflective of reality. However, good estimates of the spatio-temporal variation in those parameter rates is lacking for many species including \textit{P. argus}. The model preserves the option to include those parameters should adequate data become available. The growth function, derived from Ehrhardt (2008) was based on tag recapture and molt increment data from south Florida. The south Florida population is at the northern extent of the \textit{P. argus} distribution and subject to considerably greater seasonal variations in temperature and photoperiod, thus growth is probably slower than it is for \textit{P. argus} closer to the equator (Lellis and Russell 1990). The model therefore uses estimates of growth that are likely to be conservative for several of the populations modeled. Likewise, estimates of fecundity were based on a non-invasive technique (Gnanalingam and Butler 2018b) that yields more conservative estimates of size-specific fecundity than those based on gravimetric methods (Cox and Bertelsen 1997, Fonseca and Briones 1998). Other assumptions include: equal fishing mortality for both sexes, equal emigrant mortality regardless of size, constant fishing mortality relative to size class (with the exception of subadults), and patterns of larval connectivity that do not change from year to year. In sum, we believe that the modeling results are likely to be conservative in terms of the response variables measured and the population effects achievable with new management measures.
Our assessment of uncertainty in four important population parameters used in the model demonstrated the sensitivity of the response variables to stochasticity. Natural mortality, a parameter that is often difficult to estimate for various size-classes, produced the largest change in my model's response variables when it was varied. The estimate of natural mortality ($M$) used in the model was based on an exponential decay function anchored on an estimate of 0.51 for juvenile lobsters (< 45 mm CL) from a mark-recapture study conducted in the Florida Keys and based on monthly recaptures over 14 months (Forcucci et al. 1994). In other areas of the Caribbean, juvenile lobsters are found in seagrass beds and mangrove areas where mortality from predators differs (Acosta and Butler 1997, Lipcius et al. 1998). The 10% variability in $M$ used in the uncertainty simulations resulted in estimates of $N$ that were typically higher than the model run without stochasticity, suggesting again that model predictions probably yield conservative estimates of population size.

We did not take into account density-dependent effects on lobster growth or survival in the model, which if present and unaccounted for would probably contribute to some overestimation of response variables (Beverton and Holt 1957). Density dependence has been documented as influencing size at sexual maturity, growth, reproductive output, and mating success in a number of species (e.g., in silver hake, Helser and Almeida 2005; green turtles, Bjorndal et al. 2000, and sardines, Kim et al. 2006). Unfortunately, density dependent effects are often difficult to test. However, in a study of juvenile and subadult $P. \text{argus}$ in Florida, density had little effect on lobster growth, mortality, or susceptibility to disease (Behringer and Butler 2006). The only evidence of density-dependent effects on population dynamics in spiny lobsters of which we are aware is a study conducted in Hawaii where high densities of adult $P. \text{marginatus}$ appear to have experienced increased natural mortality (Polovina 1989).
Although the design and implementation of regulatory mechanisms that restrict fishing are likely to be contentious, the long-term benefits of protecting mature spawning stocks are undeniable. As is true for many harvested species, the largest individuals are more fecund (Hixon et al. 2014) and often produce larvae of a higher fitness (Berkeley et al. 2004). A mature population structure in long-lived species also provides resilience (‘the storage effect’) when environmental conditions are unfavorable (Chesson and Warner 1981, Roberts et al. 2005, Anderson et al. 2008). In the Caribbean, the idea of using harvest slot limits and MPAs for the enhancement of *P. argus* fisheries is gaining traction (St Georges Declaration 2015). In a preliminary survey of lobster fishermen in the Florida Keys and The Bahamas, the majority of respondents (64%) were in favor of using these tools in their own fisheries and 59% were supportive of their use throughout the Caribbean even if there was little or no obvious benefit to their own fishery (G. Gnanalingam, unpub. data). If *P. argus* fisheries are to be sustainable in the long term, it is imperative that more is done to conserve spawning stocks. As the modeling results presented here illustrate, the combined use of harvest slot limits and MPAs is a promising way to achieve this goal in the face of increasing fishing pressure.
CHAPTER 5

EXAMINING THE RELATIONSHIP BETWEEN SIZE AND FEEDING PREFERENCES IN THE CARIBBEAN SPINY LOBSTER _PANULIRUS ARGUS_ (LATREILLE 1804)

5.1 Introduction

No-take marine protected areas (MPAs) are becoming increasingly more prevalent as a means of conserving and protecting species and ecosystems vulnerable to human impacts, particularly fishing (Halpern and Warner 2002, Halpern 2003; Lester et al. 2009). Given the complex linkages between and among species in marine ecosystems, imbalances created by intensive fishing and management tools such as MPAs, that aim to restore these imbalances can have unintended effects for non-target species in the same complex food web (Pinnegar et al. 2000). There are a number of MPAs, for example, where unequal conservation benefits and unintended negative effects on lower trophic level prey species occur because of an increased abundance of higher trophic level predators or competitors released from fishing pressure (Micheli et al. 2004). Over time, MPAs can substantially increase the abundance and density of top predators resulting in trophic cascades and major changes in community structure. Well-documented examples include predator-urchin-algae trophic cascades in which increasing predator (i.e., lobster) biomass severely reduces sea urchin abundance, which in turn results in positive effects for kelp (Babcock et al. 1999, Guidetti 2006, Shears et al. 2012).

Lobsters are ecologically dominant benthic predators in a number of habitats (Robles 1987, Pedersen and Johnson 2006, Butler and Kintzing 2016). Typically considered opportunistic omnivores, their diet and the extent of their foraging relies heavily on the habitat around them (Briones-Fourzán and Lozano-Álvarez 2013). Their selective predation, however, may significantly affect species composition and benthic community structure (Tegner and Levin 1983, Butler and Kintzing 2016). Like other Palinurids, *Panulirus argus* (Latreille 1804) feed nocturnally on a variety of benthic and infaunal species including mollusks, echinoderms, and crustaceans (Cox et al. 1997, Briones et al. 2003, Nizinski 2007). Their prey may also include species of conservation and fisheries value such as the long spined sea urchin, *Diadema antillarum* (Philippi 1845), the Queen conch, *Lobatus gigas* (Linnaeus 1758), and the donkey dung sea cucumber, *Holothuria Mexicana* (Ludwig Diels 1875) (Davis 1992, Cox et al. 1997, Kintzing and Butler 2014).

With decades of intense harvest of *P. argus* throughout much of the Caribbean, population size structure has clearly changed. Most populations are truncated at the largest sizes because fishing differentially targets large individuals, which negatively impacts the reproductive potential of the population (MacDiarmid and Butler 1999, Bertelsen and Matthews 2001, Butler et al. 2015). Recognizing the importance of maintaining a mature population structure and spawning stock, fisheries managers in the Caribbean have started arguing in favor of regulations that specifically protect larger individuals. The St. George’s Declaration on Conservation, Management, and Sustainable use of the Caribbean Spiny Lobster 2015, for example, calls on signatories to adopt maximum size limits that protect the largest individuals (Article 5[4]; Article 6[4]). While this is likely to be beneficial for the sustainability of *P. argus*, it may have unintended effects on their prey particularly if larger lobsters have prey preferences
that differ from smaller lobsters, consume significantly more biomass, or are not constrained in the size of prey they can consume. Through a series of cafeteria experiments, we sought to discern differences in prey preferences and size selectivity for *P. argus* of different sizes using common prey or suitable proxies for prey.

5.2 Methods

Lobsters were hand caught by divers in 2015 and 2016. Small sexually mature lobsters (Carapace Length (CL) 58.2–83.8 mm) were collected from reefs around Long Key, Florida – a population subject to intensive fishing. Given their absence from the Florida Keys fishery, large mature lobsters (CL 107–164 mm) were collected from the Dry Tortugas National Park, which has been closed to lobster fishing since 1973. Only intermolt individuals that had finished reproductive activity for the year were used in these experiments, because lobster feeding habits differ with molt stage and reproductive condition (Lipcius & Herrnkind, 1982). Lobsters were transported in aerated live wells to the Florida Wildlife Conservation Commission laboratory in Marathon, Florida and placed into experimental tanks (1.75 m diameter, 1500 L) that received aerated, filtered flow-through seawater. Ambient seawater temperature and photoperiod were maintained while animals were held in captivity, and individuals were fed frozen shrimp or squid, or live mangrove oysters (*Crassostrea gasar*; Lamarck, 1819) *ad libitum*. Prior to the cafeteria experiments, lobsters were isolated in individual tanks with a shelter made from concrete blocks and starved for 48 hours to standardize levels of satiation.

Prey items used in the cafeteria experiments were based on known lobster prey or suitable proxies (Cox et al. 1997, Briones et al. 2003), or based on preliminary trials conducted in 2014. Six different prey items were offered to individuals: long spined sea urchins (*Diadema*
antillarum), pencil urchins (*Eucidaris tribuloides*; Lamarck 1816), mangrove oysters (*Crassostrea gasar*); little neck clams (*Mercenaria mercenaria*; Linnaeus 1758); West Indian star snails (*Lithopoma tectum*; Lightfoot 1786); and murex snails (*Thais deltoidea*; Lamarck 1822). These prey species were sourced from reefs around the Florida Keys, with the exception of the mangrove oysters, collected off mangroves, and the little neck clams which were store bought and used as a generic bivalve prey proxy. Similarly, field-caught murex snails were representative of lobster gastropod prey such as moon snails (Naticidae), top snails (Trochidae), and the economically valuable Queen conch (*Lobatus gigas*) (Davis 1992, Cox et al. 1997). After preliminary trials in 2014, sea cucumbers, (*Holothuria* sp.) were not offered to lobsters, because lobsters did not demonstrate any penchant for them and because the sea cucumber's chemical defenses were an obstacle to maintaining them and other prey species in captivity.

After the 48-hour starvation period lobsters were offered six of each prey item of different sizes. The tank water level was lowered to ensure that prey items could not climb beyond the reach of lobsters. Lobsters were left for 24 hours, and at the end of each trial period the type of prey that survived and their size were recorded.

Following cafeteria experiments, the organic and inorganic content of different sizes of the offered prey species were quantified, as a possible explanatory factor of any observed trends in prey preference and consumption. Prey were measured and weighed to obtain wet weight (g). They were then dried in a drying oven at 70°C for at least 24 h (until a constant weight was reached) and re-weighed to obtain their dry weight (g). Samples were then ashed for 12 h at 800°C to enable calculation of prey organic content (Ash free dry weight (g)) relative to prey size (Wacasey and Atkinson 1987, Eklöf et al. 2017). Differences in prey organic content were tested with a general linear model with prey species and prey size as independent variables and
organic content (g) as the dependent variable. Because organic content values were non-normal values were log transformed prior to analysis.

Three measures of lobster feeding were examined relative to lobster size: (a) prey preference, (b) total consumption, and (c) prey size selectivity. Rodgers’ Index for cafeteria experiments was used to assess prey preference (Krebs 1998). The index takes into account the inability to replenish food during cafeteria experiments and allows for one or more food types to be completely consumed during the experiment (Krebs 1998). The most preferred food has a preference score of 1.0, whereas smaller values indicate a less preferred food. Total consumption relative to lobster size was tested by way of a general linear model with lobster carapace length and sex as independent variables and total organic content consumed as the dependent variable. Total organic content consumed was calculated by converting the type and size of prey species consumed into organic contents (g) using regression equations derived from the ash free dry weights. Prey size selectivity was assessed as a function of lobster size with a generalized linear model (GLM) with a binomial distribution and logit link. Lobster carapace length, prey type, and prey size were fixed factors with consumption (yes/no) the dependent variable.

5.3 Results

The ash free dry weights (organic content) of each of the prey species typically increased linearly with the size of the individual (i.e., larger prey items had a higher organic content; Fig. 23). The highest values belonged to the oyster *C. gasar*, followed by the long spined urchin *D. antillarum*, and the clam *M. mercenaria*. When organic content was assessed relative to prey species and size by general linear model two species, the snail *L. tectum* and urchin *E.*
tribuloides were found to have significantly lower organic content (L. tectum: estimate = 0.135, se = 0.031, t = 4.347, \( P < 0.001 \); E. tribuloides: estimate 0.066 se = 0.023, t = 2.836, \( P = 0.005 \)).

Rogers Index values highlighted clear prey preferences relative to lobster size (Fig. 24). Lobsters of both size classes preferred three of the six species offered: the pencil urchin E. tribuloides, little neck clams M. mercenaria, and mangrove oysters C. gasar (Fig. 24). The three remaining prey species had comparatively lower Rogers Index values. Rogers Index values also differed with lobster size. Large lobsters preferentially selected the pencil urchin E. tribuloides, the West Indian star snail L. tectum, and the murex snail T. deltoidea (Fig. 24).

Despite larger lobsters typically eating a greater number of prey, the general linear model testing total organic content consumed relative to lobster size and sex suggested no significant relationship between total organic content consumed and lobster size or sex (Lobster CL: estimate = 0.362, se = 0.391, t = 0.925, \( P = 0.360 \); Sex: estimate = 48.22, se = 48.047, t = 1.004, \( P = 0.321 \)).
Figure 23. Organic content of prey species (in grams; g) relative to prey size: panel A: Mangrove oyster, *Crassostrea gasar*; panel B: Long spined sea urchin *Diadema antillarum*; panel C: Pencil urchin *Eucidaris tribuloides*; panel D: Clams *Mercenaria mercenaria*; panel E: West Indian Star Snail *Lithopoma tectum*.
The generalized linear models revealed some prey size selectivity. Both the size of the prey and the size of the lobster played a significant role in determining whether the urchins *E. tribuloides* and *D. antillarum* were eaten (Table 12). For both urchin species, large lobsters were not constrained by the size of the urchins and ate the full range of sizes offered to them. Smaller lobsters, however, did not eat the largest urchins offered. For all other prey species, the size of the prey or lobster did not influence which prey were consumed.
5.4 Discussion

This study indicates that predation by lobsters in populations replete with a full range of lobster sizes (e.g., unfished natural populations) are likely to result in prey abundance and size structures that differ from those in heavily fished areas - including effects on some prey that themselves are of conservation and fisheries interest such as the long spined urchin *D. antillarum* (Maci et al. 2007) and queen conch (Theile 2001, NOAA Fisheries 2005). Although the largest lobsters were capable of consuming all sizes of prey that we offered them, their preference for certain prey may play a role in determining whether they do. *Diadema antillarum*, for example, ranked low on the Rogers’ Preference Index; the largest lobsters preferred every other species over *D. antillarum*. This may perhaps explain why despite the presence of very large lobsters at the Dry Tortugas National Park there is also a conspicuously large number of *D. antillarum* on the same patch reefs (G. Gnanalingam, pers. obs.). Increasing the abundance and densities of the largest sized lobsters through management practices meant to rebuild spawning stocks may thus have negative consequences for some prey species, including some (e.g., conch) classified as ecological important, with the intensity of the effect dependent upon relative prey availabilities.

This study also suggests that the total organic content consumed by individual lobsters does not vary relative to lobster size, even though the mean number of prey consumed did.
Table 12. Results of GLMs testing relationship between lobster carapace length (CL; mm) and prey size (mm) relative to consumption. Bold typeface indicates significant result ($P = 0.05$)

<table>
<thead>
<tr>
<th>Prey</th>
<th>Coefficient</th>
<th>Estimate</th>
<th>Std Error</th>
<th>Z value</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diadema antillarum</td>
<td>Intercept</td>
<td>5.123</td>
<td>1.471</td>
<td>3.483</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Lobster CL</td>
<td>-0.047</td>
<td>0.014</td>
<td>-3.325</td>
<td>$&lt; 0.001$</td>
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<tr>
<td></td>
<td>Urchin test size</td>
<td>-0.180</td>
<td>0.044</td>
<td>-4.093</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td></td>
<td>Lobster CL $\times$ Urchin test size</td>
<td>0.001</td>
<td>0.000</td>
<td>3.369</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>Mercenaria mercenaria</td>
<td>Intercept</td>
<td>-1.782</td>
<td>3.395</td>
<td>-0.525</td>
<td>0.600</td>
</tr>
<tr>
<td></td>
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<td>0.052</td>
<td>0.0327</td>
<td>1.580</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>Clam size</td>
<td>0.055</td>
<td>0.068</td>
<td>0.811</td>
<td>0.417</td>
</tr>
<tr>
<td></td>
<td>Lobster CL $\times$ Clam size</td>
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<td>0.001</td>
<td>-1.739</td>
<td>0.082</td>
</tr>
<tr>
<td>Crassostrea gasar</td>
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<td>2.028</td>
<td>1.984</td>
<td>0.0473</td>
</tr>
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<td>0.019</td>
<td>-1.387</td>
<td>0.1655</td>
</tr>
<tr>
<td></td>
<td>Oyster size</td>
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<td>0.035</td>
<td>-1.897</td>
<td>0.0578</td>
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<td></td>
<td>Lobster CL $\times$ Oyster size</td>
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<td>0.000</td>
<td>1.343</td>
<td>0.1793</td>
</tr>
<tr>
<td>Eucidaris tribuloides</td>
<td>Intercept</td>
<td>8.028</td>
<td>2.079</td>
<td>3.860</td>
<td>$&lt; 0.001$</td>
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<tr>
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<tr>
<td></td>
<td>Urchin test size</td>
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<td>0.084</td>
<td>-4.182</td>
<td>$&lt; 0.001$</td>
</tr>
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<td>0.002</td>
<td>0.001</td>
<td>3.262</td>
<td>0.001</td>
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<tr>
<td>Lithopoma tectum</td>
<td>Intercept</td>
<td>4.185</td>
<td>4.481</td>
<td>0.934</td>
<td>0.35</td>
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<tr>
<td></td>
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<tr>
<td></td>
<td>Snail length</td>
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<td>0.209</td>
<td>-1.621</td>
<td>0.105</td>
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<td>Lobster CL $\times$ Snail length</td>
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<td>0.002</td>
<td>1.486</td>
<td>0.137</td>
</tr>
<tr>
<td>Thais deltoidea</td>
<td>Intercept</td>
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<td>15.25</td>
<td>-0.625</td>
<td>0.532</td>
</tr>
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<td>0.150</td>
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<td>Snail Length</td>
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<td>0.491</td>
<td>0.528</td>
<td>0.597</td>
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<tr>
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<td>0.000</td>
<td>0.005</td>
<td>-0.005</td>
<td>0.996</td>
</tr>
</tbody>
</table>
This could well be a reflection of smaller, faster growing lobsters being more selective in the prey they were consuming, opting for prey with higher organic content. Two of the prey species with the highest organic content - the oyster *C. gasar* and the clam *M. mercenaria* - were most preferred. Whether smaller lobsters prefer prey with higher organic content or whether two of their most preferred prey just happened to have higher organic content is unclear and warrants further investigation.

Gastropods, bivalves, and echinoderms were well represented in the trials using six prey species, but arthropods were notably absent from the experiment. Species such as the spider crab (genus *Mithraculus*), which like *D. antillarum* play an important ecological function as herbivorous grazers on reefs (Butler and Mojica 2012), are known *P. argus* prey (Cox et al. 1997, Butler and Kintzing 2016). It is unclear if offered the choice, whether *P. argus* would have preferentially selected spider crabs as prey. Similarly, given difficulties associated with obtaining sufficient numbers of small Queen conch (*Lobatus gigas*) we were unable to include this much-valued gastropod as prey in the experiment despite strong evidence of lobster predation on juvenile Queen Conch (Davis 1992). In a series of laboratory based experiments using hatchery reared juvenile conch and lobsters up to 100 mm carapace length, Davis (1992, 1999) found that the largest lobsters (80-100 mm CL) consumed every size class of juvenile conch offered them, including the largest juveniles tested (68-72 mm siphonal length). Mature conch can reach approximately 300 mm in length, thus it is unclear whether the largest lobsters can consume large, mature conch. Still, the consumption of juvenile conch by spiny lobsters complicates fishery management. Rebuilding breeding stocks of large spiny lobsters may have negative repercussions for conch recruitment where lobsters are abundant.
One of the more thorough investigations into P. argus feeding habits was conducted by Cox et al. (1997). In that study, the authors analyzed the gut content of P. argus in Dry Tortugas and Biscayne National Parks. Lobsters from these two locations ate a variety of prey: 115 taxa were identified within stomach contents. The majority of prey items were mollusks (75%), primarily gastropods (49%) and bivalves (11%); followed by arthropods (15%) and echinoderms (4%). The size range of lobsters they sampled, however, did not include the largest lobsters that we tested. Lobsters in the Cox et al. (1997) study ranged in size from 49-135 mm CL in Dry Tortugas National Park, compared to 27-104 mm CL in Biscayne National Park. We tested lobsters up to 190mm CL from the Dry Tortugas National Park, thus preferences and rates of consumption could well be different between these two studies. Of note is that species of particular ecological or economic value (namely D. antillarum, L. gigas, or Holothuria sp.) were not identified as significant components of lobster stomach contents. Thus, if the primary concern is of lobster predation on high value species, both the Cox et al. (1997) survey and the current study suggest that there is little threat to these species given P. argus prey preferences.

Although the study sought principally to investigate the direct consumptive effects of size-selective P. argus predation on different prey species and sizes, an increase in the abundance and density of large lobsters may have unintended consequences on prey behavior to the detriment of prey growth and survival. Diadema antillarum for example, consume less algae and move significantly more in the presence of the predatory spotted spiny lobster, Panulirus guttatus (Latreille 1804) (Kintzing and Butler 2014). The behavior and choice of habitat by lobster predators can, in turn, be influenced by the presence of their predators (e.g., octopus), creating a cascade of indirect, non-consumptive effects (Berger and Butler 2001, Butler and Lear 2009). There has been very little research, however, on non-consumptive effects on prey in
marine protected areas where the abundance of large predators often increases, because it is
difficult to untangle non-consumptive and consumptive effects that affect prey in the same
manner (Peckarsky et al. 2008).

Fisheries controls that restore and maintain overharvested species for their long-term
sustainability ought to be encouraged for the sake of the ecosystems that rely on these species
and the fisheries they support. It is necessary, however, to consider the indirect effects of
increasing the abundance and density of what are typically higher trophic level species.
Experiments like those conducted here and field-based assessments of trophic connections
provide invaluable information on the possible unintended negative side-effects of proposed
management. With respect to the potential rebuilding of P. argus spawning stocks in the
Caribbean via protection of large lobsters through fishery management measures such as
maximum size limits, we predict that prey community structure may indeed be altered, but not
necessarily to the detriment of prey that are of special ecological or fisheries value.
CHAPTER 6

CONCLUSIONS

According to the most recent United Nations estimate, the human population currently numbers 7.6 billion (UN 2017). By 2030, this number is set to increase to 8.5 billion (UN 2017). One billion people, many of whom live in the world’s poorest nations, already rely on seafood as their primary source of animal protein (FAO 2000), and the demand for fish is expected to increase by 35 million tons by 2030 (Mora et al. 2009). Thus, as the human population continues to grow, pressure on the marine environment will also, placing the health of these ecosystems and the fisheries they support at risk of collapse. Globally 75 percent of existing marine capture fisheries are fully exploited or overfished (FAO 2018) with many lacking scientific data or any form regulation (Agnew et al. 2009, FAO 2016). For fisheries to stand a chance under projected population scenarios informed fisheries management that explicitly recognizes species biology is essential. Business as usual scenarios will only end in the continued collapse of world fisheries (Costello et al. 2016).

Focusing on one of the Caribbean’s most valuable species, the spiny lobster, *Panulirus argus*, this study looked at the use of two management tools: marine protected areas (MPAs) and harvest slot limits based on the reproductive biology of the species. Like many other harvested species such as the black rockfish *Sebastes melanops* (Berkeley et al. 2004, Stafford et al. 2014), the abalone *Haliotis tuberculata coccinea* (Bilbao et al. 2011), and several brachyurans from the genus *Cancer* (Hines 1991), large *P. argus* (individuals > 110 mm carapace length) have a reproductive output that far exceeds the reproductive output of small *P. argus* (MacDiarmid and Butler 1999, Butler et al. 2015a). The removal of these largest individuals through fishing, as is
occurring throughout the Caribbean, can thus disproportionately affect the total reproductive capacity of *P. argus* populations (Ehrhardt 2005). Marine protected areas, and harvest slot limits offer a way to manage *P. argus* fisheries while conserving the largest individuals. Before one advocates for the use of one or both of these mechanisms however, it is necessary to address a few of the knowledge gaps that exist relative to *P. argus* biology. First the relationship between age, size, and reproductive output. In many species sexual maturity and reproduction are age not size dependent (i.e. examples in Hixon et al. 2014), thus regulations aimed at protecting spawning stock would have to differentiate age classes from size classes, or look to conserve specific areas with older individuals. The lack of a reliable method for directly ageing *P. argus* however, means this distinction is difficult to make. In Chapter 2, I explored the use of a new direct ageing technique developed by Leland et al. 2011, and Kilada et al. 2012, that uses bands deposited in the gastric ossicles of the cardiac foregut. As the technique has proven to have some merit, the logical next step, that goes beyond the scope of this work is to apply this method to breeding individuals in different geographic locations to assess the relationship between size and age and reproduction. In particular it would informative to age individuals from existing protected areas such as the Dry Tortugas National Park, where *P. argus* is found at its largest, where the native mating structure appears to be preserved, and where individuals can be prolific breeders (Bertelsen and Matthews 2001, Maxwell et al. 2009, Butler et al. 2015a.). Additionally, regional sampling of age structure throughout the Caribbean would facilitate more accurate stock assessments for the region as a whole and provide much needed data for management.

Second, if one were to hoping to conserve the largest breeding individuals it is important to ascertain whether the species undergoes reproductive senescence. Reproductive senescence has rarely been documented in invertebrates though this may simply reflect a lack of research in
this area or the relatively short lifespan of many invertebrates (Nussey et al. 2013). Building on previous work by MacDiarmid and Butler 1999, and Butler et al. 2015b, in Chapter 3, I looked for evidence of reproductive senescence in large *P. argus* over multiple spawning events. Instead of observing senescence however, positive parental effects for offspring, relative to parental size were observed, lending further support to the idea of protecting large breeding individuals. One aspect of senescence that was not explored however was behavioral senescence – the idea that despite the physiological capacity to produce thousands of offspring large individuals may not be inclined to do i.e. because they cannot assert dominance in social hierarchies (Nussey et al. 2009, Vanpé et al. 2009). Paternity analysis may be one way to test for the existence of behavioral senescence as well as understand mating structure in shared dens (Sørdalen et al. 2017), particularly in areas such as the Dry Tortugas National Park where the natural mating structure has been preserved and where lobster fishing is prohibited.

Given the absence of reproductive senescence in larger *P. argus* and positive parental effects on offspring, it makes sense logically then, to consider the use of marine protected areas and slot limits to protect large individuals from fishing. Chapter 4, used a two sex matrix model that linked the ten top lobster producing nations in the Caribbean to do so. The modelling clearly demonstrated the utility of these two mechanisms for maintaining spawning stock and reproductive capacity and highlighted how the continual removal of the largest size classes has a detrimental effect on reproductive output. The models predictive power, is limited however by its data inputs. Stock data for *P. argus* is limited, with the exception of the US and Cuba, and accessing data from Cuba has its own challenges. Many of the model values used were US derived values, which is clearly not realistic, given that the US has one of the more regulated lobster fisheries in the region. Being able to access national reports, and data from fisheries
departments may help, but it seems more likely that accurate, comprehensive data, for some countries that harvest *P. argus* just does not exist. Moving forward however, it would be most valuable to empirically test some of the parameters in the model with a small scale selective fishery in an area that can be easily monitored.

The modelling demonstrated the potential for MPAs and slot limits to increase the sizes and densities of *P. argus* on the reef, but this increase could have unintended consequences for the surrounding ecosystem. *Panulirus argus* are ecologically dominant predators that eat a variety of prey (Pederson and Johnson 2006, Briones-Fourzán and Lozano-Álvarez 2013), and an increase in *P. argus* abundance could have a detrimental effect on these prey. In Chapter 5, I examined possible indirect effects on prey through a series of cafeteria trials. Predictably, larger individuals had higher total consumption of prey and were not limited in the size of prey they could consume. They did not however show any appreciable preference for species of high ecological value such as the long spined sea urchin, *Diadema antillarum*, and the Florida sea cucumber *Holothuria floridana*. An increase in *P. argus*, would undoubtedly have an effect on the surrounding benthic community, thus in considering long term management of *P. argus*, managers need to consider these possible trophic linkages and perhaps restrict placement of MPAs to areas that do not contain other species of ecological or economic value. Ultimately it requires thinking more holistically, about the ecosystem as a whole, and recognizing that a management approach that favors one species may not favor another.

This work, provides some of the background information needed to support the use of MPAs and harvest slot limits for the management of *P. argus* in the Caribbean. The implementation of MPAs and harvest slot limits on their own however are unlikely to be the silver bullet that prevents the decline of *P. argus* fisheries. Each mechanism has associated
challenges and pitfalls. If MPAs are too small, are placed in areas that are unsuitable for *P. argus*, or are unenforceable then they are unlikely to be of any benefit (Roberts et al. 2001, Halpern and Warner 2003). Likewise, if the harvest slot limit, is too wide, then too few individuals will make it to protection. Ultimately, as pressure on the marine environment increases in coming decades, an entire suite of changes ranging from a decrease in fishing effort to an increase in scientific research in parts of the Caribbean that are data deficient and political will to enforce existing regulations will be necessary. Given the species’ connectivity, regional cooperation is also imperative. The challenge of managing a transboundary species is not limited to *P. argus* alone. There are a number of species that traverse national boundaries and the challenges associated with managing their harvest have been addressed in a number of ways, with varying degrees of success. Intergovernmental bodies and Regional Fishery Management Organizations exist for a number of shared stocks including whales (International Whaling Commission), and tunas (i.e. the International Commission for the Conservation of Atlantic Tunas, Commission for the Conservation of Southern Bluefin Tuna) and perhaps lessons can be drawn from these. It is heartening to see efforts being made within existing bodies like the Caribbean Regional Fisheries Mechanism, and the Caribbean Community (CARICOM) to ensure that there is collaboration and an open dialogue on the management of such an iconic fishery.
LITERATURE CITED


Investigaciones Pesqueras, La Habana, Cuba.


Davis M. 1992. Predation of hatchery reared juvenile queen conch, Strombus gigas (L.) by juvenile spiny lobsters, Panulirus argus (L.). Master’s Thesis. Florida Institute of Technology, Melbourne, USA.


FAO. 2016. The state of world fisheries and aquaculture. Contributing to food security and nutrition for all. Rome. 200pp


Leland JC, Bucher D. 2017. Direct age determination with validation for commercially important Australian lobster and crab species: Western, Eastern, Southern, and Ornate Rock Lobsters, and Crystal, Giant and Mud Crabs. Southern Cross University (Lismore campus), New South Wales, Australia, CC by 3.0. 136pp


139


Nordeide JT, Folstad I. 2000. Is cod lekking or a promiscuous group spawner. Fish Fish 1(1): 90-93


SEDAR. 2010. Stock assessment of spiny lobster, *Panulirus argus* in the Southeast United States SEDAR 8 Update Assessment Workshop Report, Key West, FL 122pp


Ward EJ, Parsons K, Holmes EE, Balcomb KC III, Ford JKB. 2009. The role of menopause and reproductive senescence in a long lived social mammal. Front Zool 6:4


APPENDIX A

APPLICATION OF A NON-INVASIVE TECHNIQUE FOR ESTIMATING CLUTCH SIZE IN THE CARIBBEAN SPINY LOBSTER *PANULIRUS ARGUS* (LATREILLE 1804)

Introduction

Fecundity is a key parameter in population ecology, conservation biology, and the setting of biological references points in fisheries stock assessment (FAO 1974, Gotelli 2008). Estimates of fecundity, particularly in marine fishes and invertebrates, has traditionally employed invasive and oft-times destructive methods. Gravimetric estimation methods are most common and involve removing and drying egg masses, counting the number of eggs in a weighed subsample, and dividing the total weight of the egg mass by the mean weight of a single egg (Diaz et al. 1983, Chubb 2008). Methods that destroy the entire clutch or that require the death of the female, however, preclude the potential for further study of egg and larval development and runs counter to conservation or management measures that aim to maintain spawning stock or promote reproduction. Non-invasive techniques to estimate fecundity using sonography, endoscopy, and photography with image analysis for externally extruded clutches have been attempted in a number of species (Bryan et al. 2007) but these methods can be cost prohibitive, require specialized equipment, or are time consuming.

We investigated the use of a non-invasive method to estimate clutch size in the Caribbean spiny lobster, *Panulirus argus* (Latreille 1804), using the method devised by Currie et al. (2010)
for the American lobster, *Homarus americanus* (Milne Edwards 1837). The Caribbean spiny lobster is an iconic and economically valuable species; it sustains the primary fishery for 24 Caribbean nations and employs an estimated 50,000 fishers and an additional 200,000 in fishery-related jobs (CRFM 2011). As a consequence of their high value and market demand, however, many populations of *P. argus* are currently fully capitalized or overfished (Ehrhardt et al. 2010). Size-fecundity relationships of *P. argus* have been well studied and there are estimates for populations throughout its range including Brazil (Nascimento and Araújo 1984), Cuba (Cruz et al. 1987), Florida (Cox and Bertelsen 1997), and Mexico (Fonseca-Larios and Briones-Fourzán 1998). Female *P. argus* larger than 100 mm carapace length (CL) are highly fecund, carrying an estimated hundreds of thousands of eggs in a single clutch (Cox and Bertelsen 1997). Methods that rely on counting every egg in a clutch are thus impractical for *P. argus* and other highly fecund species, thus most investigators rely on gravimetric estimations. These methods are still invasive and require not only the removal of the clutch from the female, but also the pleopodal setae to which the eggs are attached. For species like *P. argus* that produce multiple clutches in a single reproductive season, doing so prohibits the attachment of eggs in subsequent clutches until the female molts again into a reproductive condition. Egg removal thus dooms the reproductive success of this individual for months to years, depending on the reproductive and molting dynamics of the species. Moreover, we are unaware of any study using a gravimetric-based fecundity estimate for lobsters that also included counts of all the eggs in a clutch. The accuracy of this commonly used approach is thus unknown for these highly fecund species. In comparison, the non-invasive method used by Currie et al. (2010) offers a means of estimating clutch size quickly and without specialized equipment, and without the need to remove the entire clutch
from an individual, thus allowing the remaining eggs to continue to develop and the females to
continue normal reproductive activities.

Methods

Twenty-two ovigerous females ranging in size from 65.4 to 90.2 mm CL were collected
by hand by divers in the Florida Keys, Florida, USA in July 2016 and 2017. Only females with
eggs that were bright orange in color (i.e., within the first 1.5 weeks of spawning) were selected.
The arrangement of the endopodites (the inner portion of the pleopods) in female P. argus, splits
the egg mass into segments (Fig. 25). Larger females typically have four segments, whereas
smaller females have three. The length of the entire egg mass and the height of each egg segment
was measured using a ruler (Fig. 25). For each egg segment, the ruler was inserted into the center
of the egg mass between each segment until it touched the abdomen surface.
Figure 25. Measurements taken to estimate fecundity: length and height of segments.
The volume of the entire egg mass was then calculated using the formula for the volume of a cylinder:

\[
\text{volume of egg mass} = \left( \frac{\pi H^2 L}{2} \right) \times 0.4225
\]

where \( H \) is the average height of the egg segments and \( L \) is the length of the entire egg mass. The volume \( \times \) length equation is divided by 2 to account for the fact that only half the cylinder contains eggs (i.e., eggs are carried externally). Volume is then multiplied by 0.4225, which is the calculated egg packing density (see below).

To calculate mean egg volume, at least 10 eggs were removed from each female and stored in 20 ml scintillation vials containing a 5% formalin-seawater solution. The longest and shortest axes of 10 eggs were then measured under a compound microscope (40\( \times \) magnification) and averaged to provide an average egg radius. Mean egg volume was thus calculated as:

\[
\text{egg volume} = \frac{4}{3} (\pi r^3)
\]

where \( r \) is the egg radius. Clutch size was then calculated by dividing the volume of the entire egg mass (equation 1) by mean egg volume (equation 2).

**Validation with the traditional method**

To validate estimates of egg counts using the non-invasive method, the entire clutch was removed from the 22 females and preserved in a 5% formalin-seawater solution for 24 h. Eggs were rinsed in freshwater and dried at 60 °C for 48 h. Dried eggs were gently sifted through a
300 µm mesh sieve to remove the funiculae, the connective tissue that attaches eggs to the setae. The eggs were then weighed and five weighed subsamples (of greater than 30 eggs sample⁻¹) were counted under a microscope. Clutch size was calculated as total clutch weight divided by mean average egg weight. A paired t-test was used to compare non-invasive and invasive techniques, with a null hypothesis of no difference in calculated clutch size (R version 3.3.1).

**Comparison of the non-invasive method with previous studies**

The non-invasive method was applied to lobsters caught in the Florida Keys during the summer of 2015 and 2016. The length of egg masses and heights of segment were measured in 102 females (63 to 141 mm CL) with bright orange eggs. The mean egg volume from the 22 sampled lobsters was used in the fecundity estimates, and calculations for clutch size were plotted against female CL. As the relationship between CL and fecundity is typically non-linear, particularly as females get larger, we plotted clutch size relative to female CL (in mm) following log transformation, to obtain the following equation ($R^2 = 0.7805$; standard error = intercept 0.304; slope 0.1520) (Fig 26).

\[
\text{Log clutch size} = 2.8669 \log \text{CL} - 0.3442
\]

Back-transformed estimates of clutch size were then compared with estimates from equations for *P. argus* in the Caribbean: Brazil (Nascimento and Araújo 1984), Cuba (Cruz et al. 1987), Florida (Cox and Bertelsen 1997), and Mexico (Fonseca-Larios and Briones-Fourzán 1998).

**Results**
A reduction in the egg mass volume of 42.25% was incorporated into the egg mass equation to account for egg packing density and to reduce the difference in estimates between the traditional and non-invasive methods. This value was calculated by reducing the density of the egg mass volume by 1% until the smallest percent difference between the traditional method and non-invasive method was found. With this correction, clutch size estimates using the non-invasive method were, on average, only 0.003% lower than gravimetric estimates with a standard error of 13.93% (Table 13).

The average difference was nevertheless 42% with a standard error of 10.50% (Table 13) when calculated with absolute values. The mean differences in clutch size estimates between this method and gravimetric-based estimates, however, did not differ significantly (paired t-test, \( t = 1.3655, df = 21, P = 0.1865 \)). As compared to previously published equations from other locations in the Caribbean, the regression equation produced estimates of clutch size that were markedly lower, a mean percent difference of 62% from the next closest values from Brazil (Fig. 27). Currie et al. (2010) noted that the mean percent difference between traditional gravimetric estimates of fecundity and this non-invasive method was 3.68%, thus concluding that the method was reliable. Using the same approach as Currie et al. (2010) to calculate the mean difference between methods, the difference was even lower at 0.003%, suggesting that the non-invasive method could also be applicable for *P. argus*. If absolute values are used in the calculation the mean difference between the non-invasive and gravimetric methods was 42%, although this difference was still not statistically significant. Unfortunately, we know of no studies that simultaneously provided actual counts of each and every egg in a clutch, a tedious task when each clutch is comprised of tens to hundreds of thousands of eggs and many clutches must be
counted. So at present, there is no way of determining which of the two techniques provides a more accurate estimate of clutch size.

Figure 26. Estimated mean clutch size (non-invasive method) and carapace length (log transformed) for 102 female Panulirus argus (63–141 mm carapace length) sampled from the Florida Keys, FL.
When used to estimate fecundity for *P. argus* from the Florida Keys, the non-invasive method produced estimates that were considerably lower than those previously published and derived from traditional methods. If the non-invasive method of estimating clutch size is indeed more accurate than the gravimetric method, then size-specific fecundity is lower in the Florida Keys than previously estimated there and at other locations.

Table 13. Fecundity estimates using non-invasive and traditional methods.

<table>
<thead>
<tr>
<th>Lobster ID</th>
<th>Lobster Carapace Length (mm)</th>
<th>Traditional Method</th>
<th>Non-Invasive Method</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>84.2</td>
<td>224 712</td>
<td>123 147</td>
<td>45.19</td>
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<tr>
<td>2</td>
<td>82.7</td>
<td>140 329</td>
<td>165 267</td>
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<td>3</td>
<td>79.5</td>
<td>146 033</td>
<td>218 651</td>
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</tr>
<tr>
<td>4</td>
<td>83.8</td>
<td>192 651</td>
<td>170 754</td>
<td>11.37</td>
</tr>
<tr>
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<td>72.9</td>
<td>90 305</td>
<td>213 144</td>
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<td>16 498</td>
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</tr>
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<td>195 021</td>
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<td>22</td>
<td>75.1</td>
<td>158 885</td>
<td>86 504</td>
<td>45.56</td>
</tr>
</tbody>
</table>

| Mean        | 0.003                        |
| Std Error   | 13.93                       |
| Absolute Mean | 41.95                     |
| Std Error   | 10.50                       |
Figure 27. Estimated mean clutch size for different sizes of female *Panulirus argus* using the non-invasive method and previously published studies for Mexico (Fonesca-Larios & Briones-Fourzán, 1998), Florida (Cox & Bertelsen, 1997), Brazil (Nascimento & Araújo, 1984), and Cuba (Cruz *et al.,* 1987).

Discussion
The lower estimates produced by the non-invasive method, however, could be influenced by the non-linear relationship between female size and clutch size and the small range of female sizes we used to generate the regression equation (equation 3). Previous research indicates that non-linearity in the regression equations becomes more apparent as female \emph{P. argus} get larger than 90 mm CL (MacDiarmid and Sainte-Marie 2006). Egg diameters can also vary relative to female size and clutch number where females produce multiple clutches in a year (Gnanalingam and Butler 2018a), and it is possible that packing density differs as female lobsters grow (Koopman et al. 2015). If so, the non-invasive method would have to be validated for larger females before one could reliably estimate clutch size for lobsters greater than 90 mm CL.

As Currie et al. (2010) noted, a slight error in the measurement of egg height can have a disproportionate influence on the total egg volume calculated. A change of \(\pm 1\) mm in average height can alter fecundity estimates by as much as \(\pm 1,000\) eggs lobster\(^{-1}\) (Currie et al. 2010). The estimated clutch size of individual 6 (Table 13) differed by 221\% between methods. Careful measurements are therefore required for precise estimates of clutch size.

Part of the challenge in using the non-invasive method for \emph{P. argus} is the potentially different egg sizes and packing densities relative to egg development stage, clutch number, spawning time (i.e., early or late in the breeding season), and geographic location, all of which could influence egg mass volume and mean egg volume. Although we calculated an egg packing density correction of 0.4225 it may require alterations for females with eggs at different development stages or sizes. These potential confounding factors are rarely accounted for in studies estimating fecundity using traditional methods and, for the sake of consistency, we only used early-stage, first-clutch, bright-orange (i.e., no visible eyespots) eggs.
The variability associated with the non-invasive method is regrettable because the method does offer some clear advantages over other methods: it is quick, inexpensive, and can easily be applied in the field. It only requires measurements of egg mass, length, and height in addition to the removal of a small subsample of eggs with which to calculate individual egg volume. In fisheries stock assessment, a single discrete study is often relied on for the estimates of fecundity that underpin the needed length-fecundity relationship. In these cases, the issue of destructive sampling is perhaps not problematic and gravimetric methods will suffice. In other research settings, however, destructive sampling is undesirable, as in field studies of changes in lobster fecundity through time or among regions as part of annual catch sampling to monitor potential effects of environmental change or sex ratio in the stock. Laboratory studies of lobster fecundity would also benefit from a non-invasive approach, as in cases where multiple clutches of each female must be examined. Non-invasive methods are also more consistent with laboratory animal welfare policies and more in keeping with conservation and management objectives.
APPENDIX B

CO-AUTHORSHIP STATEMENT AND MANUSCRIPT PUBLICATION STATUS

The studies presented in this dissertation were designed by Gayathiri Gnanalingam, with guidance and input from Dr. Mark J. Butler, as well as committee members Drs. Holly Gaff, and Alison MacDiarmid. All data for these experiments were collected and analyzed by G. Gnanalingam. Chapter 2 (Ageing *P. argus*) was carried out in collaboration with Dr. Mark Butler, Tom R Matthews (Florida Wildlife Conservation Commission, Marathon, Florida), Emily Hutchinson (Florida Wildlife Conservation Commission, Marathon, Florida) and Dr. Raouf Kilada (University of New Brunswick, Canada). Chapter 5 (Modeling MPAs and slot limits) was completed as a Modeling and Simulation Fellowship under the guidance of Drs. Holly Gaff and Mark Butler. All manuscripts resulting from these works were written by G. Gnanalingam, with editing and creative assistance from co-authors.

Five manuscripts are expected from this dissertation. The manuscript resulting from Chapter 2 is in review at the *ICES Journal of Marine Science*, co-authored with Dr. Mark Butler, Tom Matthews, Emily Hutchinson and Dr. Raouf Kilada. Manuscripts from Chapters 3 and 4 co-authored with Dr. Mark Butler have already been published in the *Bulletin of Marine Science* and *Journal of Crustacean Biology* respectively. Appendix A has similarly been published in the *Journal of Crustacean Biology*. The manuscript from Chapter 5, co-authored with Drs. Mark Butler and Holly Gaff is in preparation and is aimed for publication in *Conservation Biology*. 
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