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Biology and Diseases of *Panulirus argus* on Saint Kitts and Nevis

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Abstract

The Caribbean spiny lobster, *P. argus*, is a commercially important species found in the Western Atlantic Ocean, the Caribbean Sea, and the Gulf of Mexico. Since *P. argus* fisheries generate approximately US $1 billion annually, regulations are in place to protect the fisheries and ensure sustainability of the resource. Countries within the region have adopted regulations to achieve this objective, including closed seasons and minimum landing size requirements. In a study conducted on Saint Kitts and Nevis from 2017 to 2020, morphological and histological data were collected, it was determined that minimum size at maturity occurs at 87 mm carapace length while peak reproductive activity occurs from March to May.

Although there are few known diseases of *P. argus*, the PaV1 virus has negatively affected several *P. argus* populations in some countries. Disease surveillance functions to determine the health of the fishing stock and to discover emergent pathogens which may threaten the fisheries. In this study, lobsters at the adult, juvenile and postlarval stages were screened grossly and histologically for signs of disease. Molecular techniques were also used to identify the aetiological agents of pathologies observed. Parasites such as *Ameson herrnkindi* (a novel microsporidian parasite), *Carcinonemertes conanobrieni*, an egg-predator previously reported in Florida and Colombia, and previously undescribed *Carcinonemertes* sp. were found. Such scientific data can prove instrumental in the management of *P. argus* stocks including the identification of determination of potential closed seasons, establishing minimum legal size, and assessing aquaculture suitability. Based on our data, March to June could be adopted as the closed season for Saint Kitts and Nevis.

KEYWORDS: aquaculture, Caribbean spiny lobster, fishery regulations, disease

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1. INTRODUCTION

The Caribbean spiny lobster, *Panulirus argus*, is an important resource throughout its geographical range. Fishery of the tropical and sub-tropical species generates approximately US $1 billion throughout the Western Atlantic Ocean, the Caribbean Sea, and the Gulf of Mexico. Based on information provided by the Food and Agriculture Organization (FAO), the three countries with the largest exports are Cuba, the Bahamas, and Nicaragua (Pereira and Josupeit, 2017). This fishery is also important to the subsistence of fishermen in all countries in which *P. argus* is located.

Since this lobster is so important economically and ecologically, a sustainable fishery is of paramount importance. The International Union for Conservation of Nature (IUCN) red list states that the overall population of the Caribbean spiny lobster is decreasing throughout its range (Butler et al., 2011). While overharvesting is traditionally noted as reason for this decline, other factors may exist. These causes may include mortality because of parasites and inadequate fishing regulations. Regulations have been employed throughout the range of this species, including closed seasons, a minimum legal size (MLS) of landed lobsters, and a restriction on landing of berried lobsters. Some territories also restrict the removal of females with spermatophores (or tarspots).

Pathogens in the marine environment can lead to population declines and disruption of the existing ecosystem. Presence of pathogens and parasites can also result in altered appearance and taste of commercially important marine species. A reduction in marketable individuals by death or otherwise can lead to reduced economic yield (Shields, 2012). Pathogens such as Panulirus argus virus-1 (PaV1) have contributed to the decline of populations in Florida since the discovery of the virus in that area (Shields & Behringer, 2004; Behringer et al., 2012). This virus is lethal in juveniles and sub-clinical in adults, causing the hemolymph to become thin and milky white (Behringer et al., 2012). Much research has been done on this virus since 2000, including a study on its distribution. As of 2013, PaV1 is found in the Northern and Western Caribbean and the Gulf of Mexico (Moss et al., 2013). However, there have been no reports of PaV1 in the Eastern Caribbean.

In addition to this, other reported diseases of *P. argus* include microsporidiosis, in Florida (Kiryu et al., 2009). Microsporidiosis, caused by members of the *Ameson* genus, has been reported predominantly in shrimps and crabs both in the wild and in aquaculture (Singh & Singh, 2018). Infection results in 'cotton muscle' which is indicative of microsporidian spores replacing muscle cells (myocytes) within the host (Stentiford & Dunn, 2014; Han et al., 2016). Few reports exist in spiny lobsters, but a microsporidian infection was reported in *P. argus* in Florida in 1976 and again in 2009 (Bach & Beardsley, 1976; Kiryu et al., 2009)

A nemertean worm, *Carcinonemertes conanobrieni* was reported during a study on parental care in *P. argus* in Florida, and was thereafter reported in Colombia (Baeza et al., 2016; Gonzalez-Cueto & Quiroga, 2018). Members of the *Carcinonemertidae* Family, of which *Carcinonemertes* and *Ovicides* are the constituent genera, have been implicated in the collapse of crab fisheries. Particularly, *C. regicides* has been implicated in the collapse of red king crab (*Paralithodes camtschatica*) fisheries in Alaska and an undescribed *Carcinonemertes* sp. caused the collapse of *Cancer magister* in California (Kuris et al., 1991; Shields, 2012). It has now been established that *C. conanobrieni* infestation negatively impacts the reproductive performance of *P. argus* (Berben et al., 2023).

There are deficient data on *P. argus* populations which can adequately inform government and regional organisations and result in regulations that take current status into consideration. From a disease perspective, since particles can spread easily in an aquatic environment, knowledge of the location and dynamics of parasites is important. From a management perspective, since

population connectivity is a well-established phenomenon, a more cohesive strategy includes data and information from as many territories as possible.

The aims of this project were to establish the size at sexual maturity and the peak period of reproductive activity of *P. argus* on Saint Kitts and Nevis, and to conduct a health screen of the population. This paper summarizes the findings of a research project conducted in Saint Kitts and Nevis from 2017 to 2020. We also reference scientific articles in which all methodology and results are more thoroughly explained.

2. MATERIALS AND METHODS

2.1 General sample collection

A permit was obtained from the Department of Marine Resources, Saint Kitts, allowing for the capture of lobster under the legal size of 95mm carapace length (CL). Permission was also granted for the capture of berried females. Three hundred and thirteen lobsters (147 males and 166 females) ranging from 66mm CL to 157mm CL were collected live from fishermen and were anesthetized and euthanized within two hours (Atherley et al., 2020a). For all lobsters collected, morphological data were collected, the carapace length (mm), weight (g). The general health of the lobsters was assessed using gross and microscopic observations, including the colour of the hemolymph. All abnormalities were noted. Samples of the heart, hepatopancreas, gonad, antennal glands and gills were fixed in Davidson's sea water fixative for histological analyses and in lysis buffer or 95% ethanol in preparation for relevant molecular analyses.

2.2 Size at sexual maturity and reproductive activity

As stated in Atherley et al. (2021), the ovaries of all females in this study were assessed grossly to determine the stage of ovarian maturation each individual reached at point of capture. The scale used to determine maturation stage was adapted from Brown-Peterson et al. (2011): Stage 1. Immature; Stage 2. Developing; Stage 3. Spawning capable; Stage 4. Regressing; Stage 5. Regenerating. These data were used to calculate the size at which 50% females were physiologically mature.

The presence of a spermatophore and eggs was also recorded, and these data were used to calculate size at functional maturity. The analyses of size at functional and physiological maturity were completed with the sizeMat package of RStudio statistical software using the following equation (Atherley et al., 2021): $y=1/(1+\exp-(a+b*x))$, where y is the probability of one female being mature at a particular carapace length, denoted by x. The estimated parameters, a and b are the intercept and slope respectively. The sizeMat package in R requires the definition of the 'immature' and 'mature' parameters as follows: immature = Stage 1, mature: Stages 2 to 5). The proportion of functionally mature females was recorded during the duration of the study to determine peak months of reproductive activity. All other information on these methodologies can be found in Atherley et al. (2021).

2.3 Microsporidian study

Three hundred and thirteen lobsters were examined grossly for signs of abnormality. When dissected, muscle tissues were examined under a compound microscope for microsporidian spores using a wet mount. Both healthy and presumptive diseased muscle were fixed and examined histologically. DNA was extracted from diseased tissue and PCR done to amplify microsporidian DNA using primers that target the small subunit (SSU) of ribosomal DNA. A phylogenetic tree based on the SSU subunit of several *Ameson* and other microsporidian species were generated.

2.4 Nemertean study

The egg masses of 31 berried females were examined using micro forceps under a dissecting microscope (Olympus SZX7 dissecting microscope. Olympus, Tokyo, Japan) for evidence of nemertean infestation. If found, adult specimens were relaxed in a 1:1 solution of 1M $MgCl_2$ and sea water, photographs were taken, and samples were collected for molecular analysis. The Folmer primers, (5 ′ GGTCAACAAATCATAAAGATATTGG 3 ′) and HCO2198 (5 ′ TAAACTTCAGGGTGACCAAAAAATCA 3 ′) (Folmer *et al*., 1994) were used to amplify the cytochrome oxidase subunit gene of mitochondrial DNA for identification of worms found. The PCR reaction and conditions are documented in depth in Atherley et al., 2020b, the article in which the study on *Carcinonemertes* sp. on Saint Kitts has been reported. All PCR product approximately 700bp in length were sent to an external service for Sanger sequencing. All sequences were compared to those on the NCBI database. A phylogenetic tree was constructed by including sequence sourced from the database, such as other members of Carcinonemertidae and monostiliferan and polystiliferan nemerteans (Atherley et al., 2020b).

2.5 PaV-1 study

In addition to the 313 fishery-caught lobsters in this study, 97 juvenile *P. argus* were hand-collected from sea grass nurseries located off Cockleshell beach in Saint Kitts between October 2019 and February 2020. Post-larval collectors were deployed in the Narrows and the Western side of the island of Saint Kitts for collection of post-larvae. Two collector designs, one of which was designed after Jaimes et al. (2016) and the other with the assistance of Donald Behringer and D. Pharo, were deployed at 17-20 meters and 3-5 meters respectively. They were deployed from a vessel owned by the Department of Marine Resources.

For adults and juveniles, after specimens were euthanized, approximately 0.2 ml hemolymph samples were collected from the arthrodial junction between the fourth and fifth walking leg of adults using a 25G needle. The hemolymph was stored in 1ml of 95% ethanol for molecular analysis. Gill, hepatopancreas and muscle were removed and immediately placed in Davidson's sea water fixative for later histological analysis. Lobsters taken from postlarval collectors were divided in half along the midsagittal plane and fixed to facilitate molecular and histological analyses respectively.

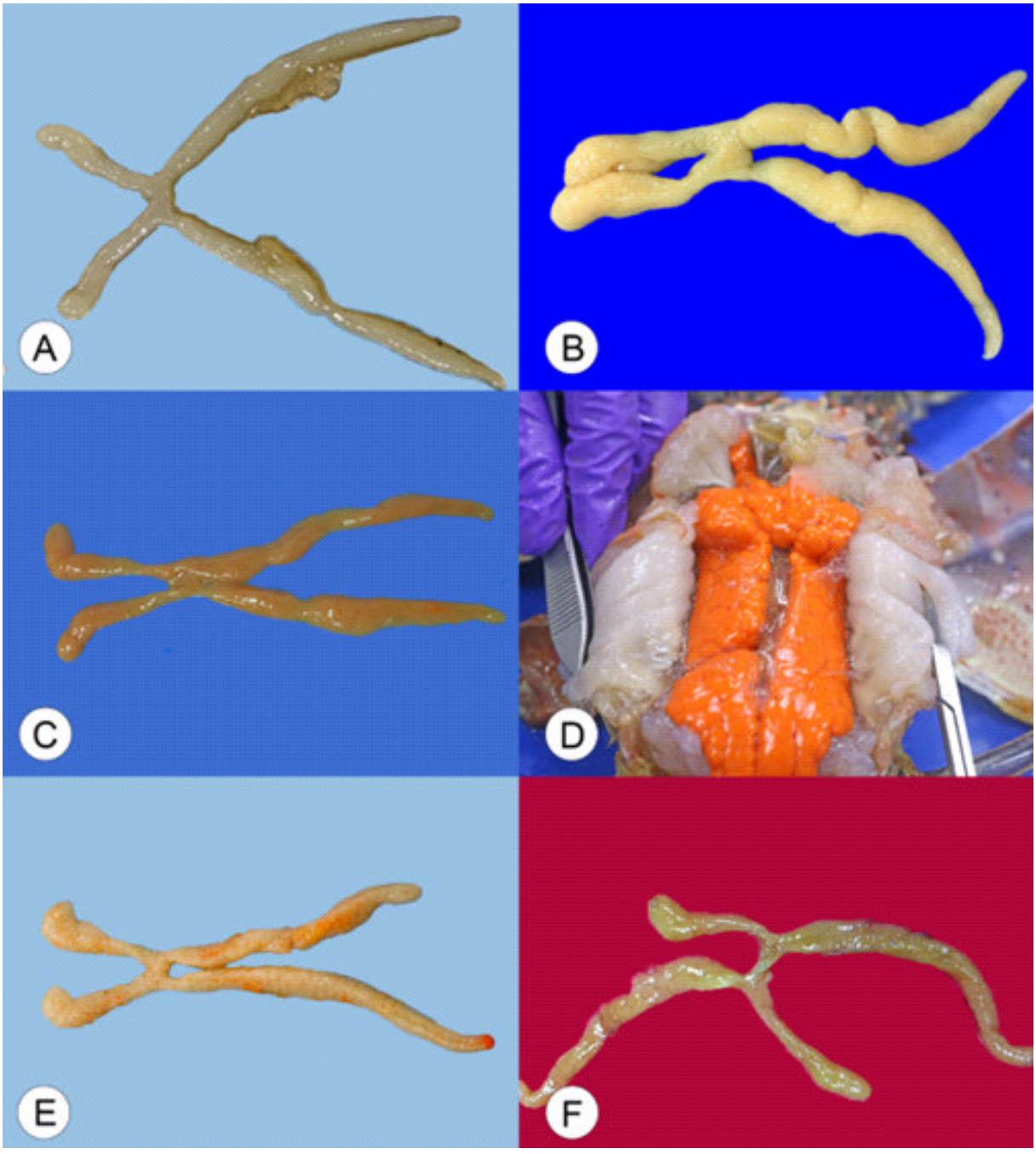
DNA was extracted from either hemolymph or muscle samples collected during postmortem examination. Total DNA was extracted using the GeneMATRIX tissue DNA purification kit. PaV1 DNA was amplified using the primers 45aF (5′ TTCCAGCCCAGGTACGTATC 3′) and 543aR (5′ AACAGATTTTCCAGCAGCGT 3′). The total volume of each reaction (20 µl) contained, 1× PCR buffer (20 mM Tris- HCl, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM each of forward and reverse primers and 1 unit of Taq polymerase after Moss et. al. (2013). PaV1 was extracted from the muscle of a heavily infected lobster supplied by Dr. Donald Behringer and used as the positive control. Millipore Q water was used in the non-template negative control reaction. PCR reactions were performed under the following conditions: initial denaturation at 94 ˚C for 5 min, then 40 cycles of denaturation at 94 ˚C for 45 s, annealing 63 ˚C for 45 s, and extension at 72 ˚C for 1 min, followed by a final extension at 72 ˚C for 10 minutes. The PCR product was visualized by gel electrophoresis on a 1% agarose gel.

3. RESULTS

3.1 Sexual maturity study

One hundred and sixty-six females were examined in this study, 47 of which were smaller than 95mm CL. Twenty females were ovigerous (carrying eggs). Gross assessment of the ovarian maturation stages based on ovarian colour were as follows and is further reported in Atherley

et al (2021): immature = white (Figure 1A), developing = yellow to orange (Figure 1B and 1C), spawning capable = bright orange (Figure 1D), regressing = cream with regions of orange (Figure 1E) and regenerating = cream (Minagawa & Sano 1997; Figure 1F).



Of the 166 females examined for cues of functional maturity, 85 (51%) were mature, while 64.8% (105 of 162 females) were physiologically mature based on gross assessment. Statistical analyses in R revealed that functional maturity is reached at 87.8 mm carapace length (r^2= 0.36, CI 85.8 to 91.3, N = 160) while physiological maturity based on gross assessment of ovarian maturity was 82.8mm CL (r^2 = 0.53, CI 80.7 to 84.5, N = 160).

The proportion of functionally mature females was highest from March to May in 2018 and during the same period in 2019 (Figure 2).

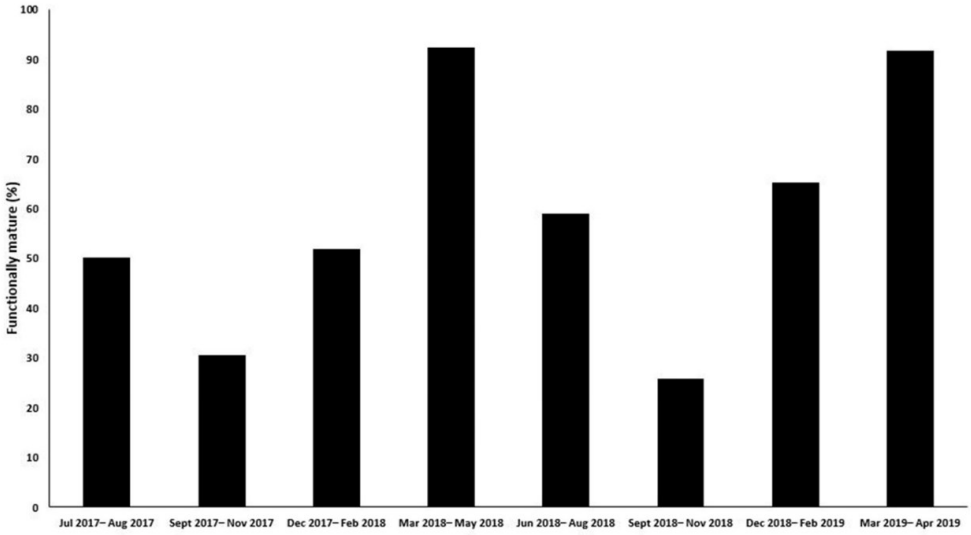


Figure 2

3.2 Microsporidian study

Microsporidian infection was detected in 2 (0.6%) lobsters included in this study. Infection was characterized by white skeletal muscle (Figure 3). Standard H & E stains revealed spores replacing the sarcoplasm in the skeletal muscle (Figure 4). The microsporidian isolated from this study groups with members of the *Ameson* genus and has been characterized as a novel species: *Ameson herrnkindi* sp. nov. (Small, Stentiford, Behringer, Freeman, Atherley, Reece, Bateman & Shields).

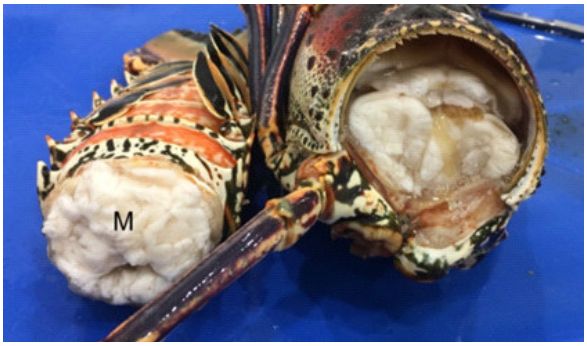


Figure 3

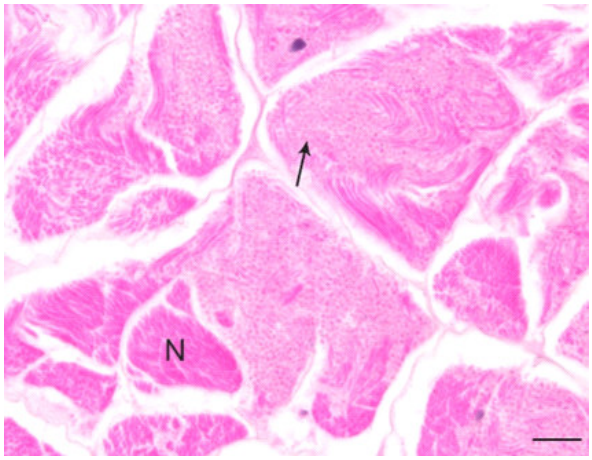


Figure 4

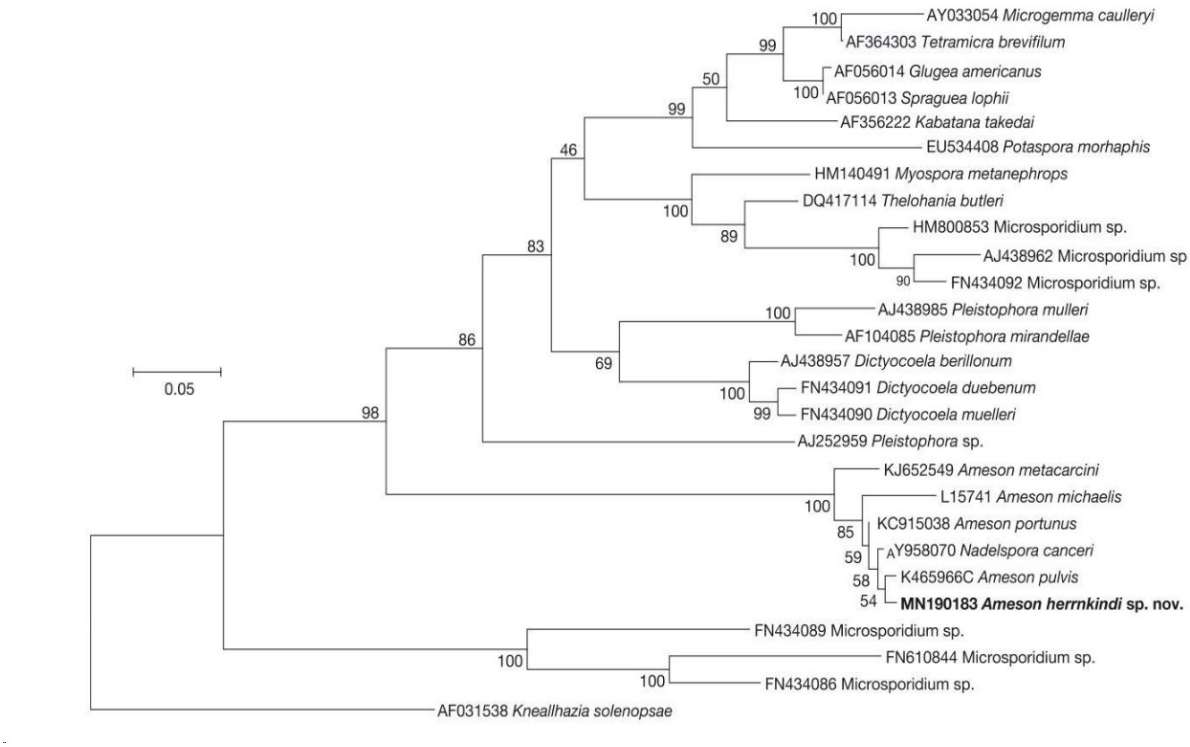


Figure 5

3.3 Nemertean Study

The presence of nemertean infestation (e.g., Figure 6) was observed in 87% (27 of 31) of all berried females screened. Phylogenetic analyses reveal the presence of two species of nemerteans on *P. argus* in this territory, one of which is confirmed to be *C. conanobrieni* (Figure 7). Therefore, one species in this study is identified as *C. conanobrieni*.

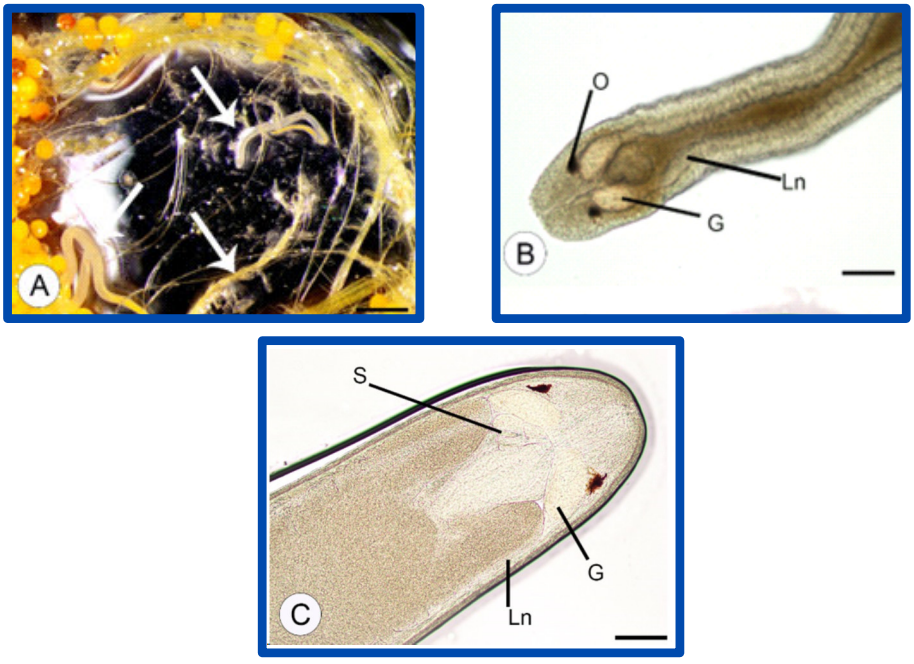


Figure 6

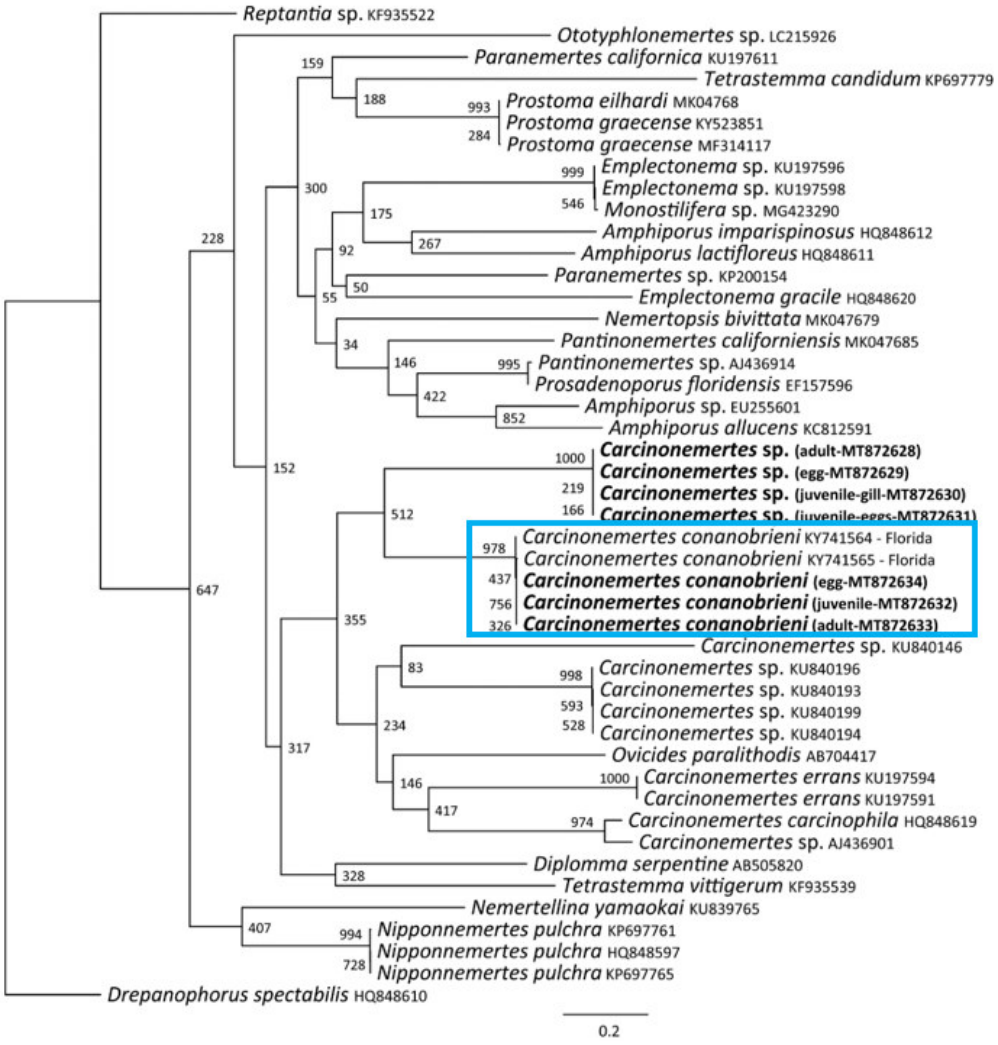

Reptantia sp. KF935522
Ototyphlonemertes sp. LC215926
Paranemertes californica KU197611
Tetrastemma candidum KP697779
Prostoma eilhardi MK04768
Prostoma graecense KY523851
Prostoma graecense MF314117
Emplectonema sp. KU197596
Emplectonema sp. KU197598
Monostilifera sp. MG423290
Amphiporus imparispinosus HQ848612
Amphiporus lactifloreus HQ848611
Paranemertes sp. KP200154
Emplectonema gracile HQ848620
Nemertopsis bivittata MK047679
Pantinonemertes californiensis MK047685
Pantinonemertes sp. AJ436914
Prosadenoporus floridensis EF157596
Amphiporus sp. EU255601
Amphiporus allucens KC812591
Carcinonemertes sp. (adult-MT872628)
Carcinonemertes sp. (egg-MT872629)
Carcinonemertes sp. (juvenile-gill-MT872630)
Carcinonemertes sp. (juvenile-eggs-MT872631)
Carcinonemertes conanobrieni KY741564 - Florida
Carcinonemertes conanobrieni KY741565 - Florida
Carcinonemertes conanobrieni (egg-MT872634)
Carcinonemertes conanobrieni (juvenile-MT872632)
Carcinonemertes conanobrieni (adult-MT872633)
Carcinonemertes sp. KU840146
Carcinonemertes sp. KU840196
Carcinonemertes sp. KU840193
Carcinonemertes sp. KU840199
Carcinonemertes sp. KU840194
Ovicides paralithodis AB704417
Carcinonemertes errans KU197594
Carcinonemertes errans KU197591
Carcinonemertes carcinophila HQ848619
Carcinonemertes sp. AJ436901
Diplomma serpentine AB505820
Tetrastemma vittigerum KF935539
Nemertellina yamaokai KU839765
Nipponnemertes pulchra KP697761
Nipponnemertes pulchra HQ848597
Nipponnemertes pulchra KP697765
Drepanophorus spectabilis HQ848610
0.2

Figure 7

3.4 PaV1

No lobsters screened for PaV1 displayed gross clinical signs typical of infection, i.e., no lobsters were lethargic and did not possess milky white hemolymph. Histological sections of 134 lobsters ≤80mm CL, 276 lobsters ≥ 80mm CL and 17 post larvae were screened for indications of PaV1 infection. The epithelial cells lining the cuticle of this lobster were also examined histologically and analyzed sections did not provide clear evidence of viral infection. No PaV1 DNA was amplified in PCR reactions despite screening 134 lobsters <80mm CL, 252 lobsters ≥ 80mm CL, and 18 post-larvae (Figure 8).

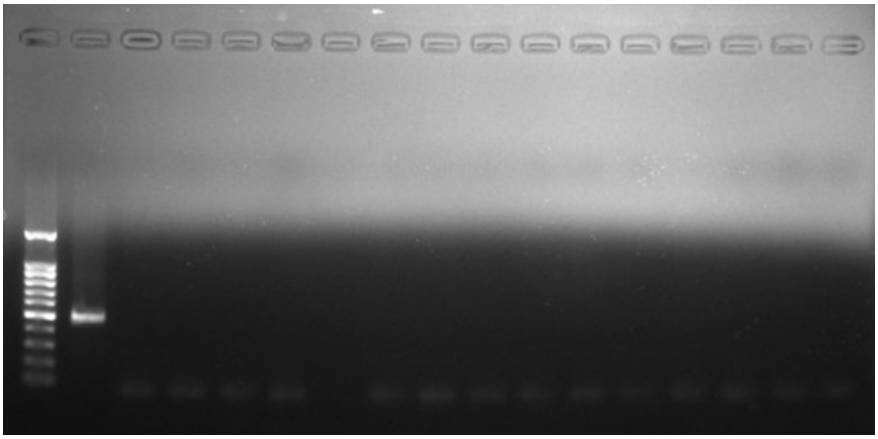


Figure 8

4. DISCUSSION

This study on *Panulirus argus* captures factors that affect sustainable fisheries. The size at sexual (functional) maturity on Saint Kitts and Nevis was lower than the current minimum landing size (MLS) of 95mm. While Belize recently discovered that size at sexual maturity (86mm CL) was higher than the MLS of 76 mm CL (Tewfik et al., 2020). Some lobsters were removed before breeding at least once, and this could have detrimental effects on fisheries. The data generated in this study indicate a peak reproductive activity from March to May. Reproductive studies should be conducted, and management protocols updated to account for changes in temperature and fishing pressure throughout the range of *P. argus*, which are two drivers of size at maturity in marine organisms. This knowledge is expected to improve fisheries management strategies.

New emergent pathogens are reported in several commercially important aquatic species (Bojko *et al.*, 2017; Hooper *et al.*, 2020). Furthermore, it is projected the incidence of emergent aquatic diseases will increase over the next century (Shields, 2019). Studies have shown that host-pathogen-environment dynamics play a large role in the transmission and proliferation of disease in a population, and the progression of disease in an individual. For example, WSSV does not cause mortality at low temperatures, but does so at 22°C in *Pacifastacus leniusculus*, a freshwater crayfish (Korkut et al., 2018). Elevated ocean temperatures alter immunity in crustaceans, rendering them unable to mount an immune response against a pathogen. Conversely, elevated temperatures provide optimum conditions for proliferation within a host (Shields, 2019). Long-term screening of *P. argus* populations should therefore be conducted not only to quickly detect emergent pathogens but also to detect the increase of pathogenicity due to changes in biotic and abiotic factors.

This research continues to show the importance of disease surveillance and the necessity of subsequent management strategies. Surveillance of economically importance marine species increases awareness of pathogens, parasites and non-infectious conditions that may threaten survival of the species. Susceptible but naïve populations should be screened for disease to ensure known pathogens are not present in the local fishing stock. Management of emergent diseases and possible disease outbreaks should therefore be included in the management strategy for any commercially and ecologically important organisms (Groner *et al.*, 2016). For example, any newly discovered pathogens should be closely studied to determine whether there is potential threat to the P. *argus* population in Saint Kitts and indeed beyond this territory. Once a threat has been established, a management plan should be developed, if necessary. Human health impact, economic and ecological impact should also be assessed to aid management protocols.

A recently discovered egg parasite, *Carcinonemertes conanobrieni*, causes reduced fecundity in gravid *P. argus* (Baeza *et al.*, 2016). This nemertean worm was reported in Florida and Colombia in 2017 and 2018 respectively, and in Saint Kitts and Nevis. These results indicated a widespread distribution of *C. conanobrieni* throughout the - geographical range of the host. Since this may be the case, screening should be conducted in other countries for the presence and prevalence of infestation in *P. argus*. Molecular techniques were instrumental in the discovery of two *Carcinonemertes* spp. on *P. argus* from Saint Kitts. While this study is the first to report infestation of *P. argus* by more than one *Carcinonemertes* species, one is currently not identified or characterized. The presence *Carcinonemertes* spp. is however concerning since some species in the Carcinonemertidae have been responsible for the collapse of some fisheries (Wickham, 1980; Shields *et al.*, 1990; Kuris *et al.*, 1991. Previous studies indicate significant reductions in *P. argus* fecundity when infested by *C. conanobrieni* (Baeza *et al.*, 2016; Berben et al., 2023). Determining the feeding and reproductive patterns of both nemertean species may aid in the development of possible control methods for this parasite in the wild. Control methods may include an adjustment of fishing pressure by changing regulations.

Yet another parasite discovered in this study, *Ameson herrnkini* sp. nov., may have a negative impact on fisheries. Although the prevalence of infection is low (0.6%) and confirms the rare incidence of microsporidiosis in *P. argus*, there were some limitations to this study. The samples collected were fishery-caught, excluding juveniles and adult lobsters which may have been too morbid to enter the lobster trap. Light infections may not be detected by gross or histological methods and thus future research should focus on the use of molecular methods to elucidate the prevalence of A. herrnkindi more clearly in a population. More studies may increase the depth of knowledge on *A. herrnkindi* in *P. argus*.

We did not detect PaV1 during this study using standard PCR techniques and gross observation of postlarvae, and of juvenile and adult lobsters. The presence of this virus has the potential to decrease the fishing population of *P. argus* by causing mortality in juveniles. PaV1 may reduce landing of *P. argus* not only due to population decline through mortality (Behringer *et al*., 2012), but also possibly due to a change in the behaviour of healthy individuals. Healthy individuals avoid diseased individuals (Behringer, Butler and Shields, 2006), therefore they will avoid traps if infected conspecifics are present therein (Behringer *et al*., 2012). Although ocean currents and fishing practices may limit the spread of virus in this territory (Kough *et al.*, 2015), the *P. argus* population should be screened regularly.

Parasites and pathogens reported in this study may have lasting effects on fisheries. Presence of disease results in loss of revenue due to mortality and change in appearance (e.g., epizootic shell disease, *Homarus gammarus*) and taste (e.g., bitter crab disease, *Chionoecetes opilio*) in commercially important aquatic species (Shields, 2012). Mortality in juvenile crustaceans decreases recruitment to breeding and fishing stock, while egg-mortality in gravid females ultimately through reduced fecundity, lowers recruitment of post larvae to coastal regions (Behringer, 2012; Shields, 2012; Baeza *et al.*, 2016).

Anthropogenic activity has led to global climate change, and more specifically, ocean temperatures in the Caribbean region and surrounding waters have increased over the past 15 years (Glenn *et al.*, 2015). The environmental factors driven by climate change, high temperature, salinity, and pH affect the immune system, survival, and behaviors of organisms in the aquatic environment (Nasrolhai *et al.*, 2013; Ross and Behringer, 2019). In *P. argus*, elevated temperature (32°C), salinity (45ppt) and pH (7.65) reduce chemosensory ability, impairing the ability to avoid diseased lobsters and select shelter (Ross and Behringer, 2019). This phenomenon has implications on predation of the species and the spread of pathogens such as PaV1 throughout a population. Disease surveillance should thus be conducted continuously considering a changing environment.

We report parasites in the *P. argus* stock on Saint Kitts and Nevis, some of which have the potential to negatively impact fisheries. Disease surveillance and pathogen identification and characterization are necessary steps which will lead to proper management of the species. In addition, this research shows the need to collect data on sexual maturity, environmental parameters and anthropogenic activity. Successful management of the species must also incorporate information on disease and sexual reproduction for sustainable *P. argus* fisheries and conservation. The novel findings generated from this study also indicate there is potential to conduct additional research on this economically important organism in the Caribbean in the coming years.

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Figure 1. Gross observation of ovarian development in *Panulirus argus*. A. Immature; B: Developing, early. C: Developing, late; D. Spawning capable; E. Regressing; F. Regenerating

Figure 2. Percentage of functionally mature female (i.e. females either ovigerous or with a spermatophore) *Panulirus argus* observed from St. Kitts, July 2017 to April 2019.

Figure 3. Skeletal muscle of *Panulirus argus* infected with microsporidian

Figure 4. Microscopic observation of microsporidian-infected muscle of *Panulirus argus*. A. Histological section stained with hematoxylin & eosin, showing myofibers with microsporidian spores (arrow; scale bar = 30 µm). Normal muscle histology is also apparent (N).

Figure 5. Phylogenetic tree (Maximum Likelihood) comparing sequences of the SSUrRNA gene of several aquatic microsporidian species. The isolate from this study, *Ameson herrnkindi* sp. nov., is noted in bold (Small *et al.*, 2019).

Figure 6 . Adult nemertean worms in host brood mass (A). Adult worm viewed using compound microscope (B); adult worm viewed under microscope slide using compound microscope (C). Ln, lateral nerve; O, ocelli; S, stylet; G, cephalic ganglion. Scale bars: A = 1.3 mm, B = 100 µm, C = 100 µm. Image taken from Atherley et al., 2020b.

Figure 7. Maximum likelihood phylogenetic tree, depicting phylogenetic relationships between COI sequences of the life stages of two *Carcinonemertes* spp. from *Panulirus argus* on Saint Kitts and other nemertean worm species. One of the species found in this study is very similar *Carcinonemertes conanobrieni* from Florida, thus grouping with the species reported from that territory (highlighted by box). Accession numbers of sourced sequences are noted in brackets. Sequences generated from this study in bold. Nodal values denote support from 1,000 bootstrap samplings. Image taken from Atherley et al., 2020b.

Figure 8. Gel electrophoresis results from molecular screening of *Panulirus argus* for PaV1. The positive control (~499bp) is visualized. All lobster samples are negative.