A study of self recruitment amongst populations of *Stegastes flavilatus* in the Las Perlas Archipelago

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TABLE OF CONTENTS

Acknowledgements 4
Abstract 5

1 Introduction 6
1.1 Larval Connectivity 6
1.1.1 Background to Larval Connectivity 6
1.1.2 Retention and Self Recruitment 7
1.1.3 Tools and methods for analysing dispersal 9
1.2 Las Perlas 11
1.2.1 Introduction to Las Perlas 11
1.2.2 The Islands 13
1.3 Aims of Dissertation 15

2 Methods 17
2.1 Capture and sampling methods 17
2.2 DNA extraction and amplification 17
2.2.1 Preparation of samples 18
2.2.2 Monitoring of DNA quality using gel electrophoresis . . . 19
2.2.3 Amplification of microsatellites using polymerase chain reaction . . 19
2.3 Analysis and interpretation 21

3 Results 23
4 Discussion 28
5 Conclusion 31
6 Recommendations 32
7 Bibliography 34

Appendix I – Productivity in the Gulf of Panama 38
Appendix II – Declaration of Las Perlas Special Management Zone . 42
Appendix III - Primer details and microsatellite loci . . . 50
Appendix IV – United States Geological Survey map of Gulf of Panama . . 56

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ABSTRACT

The question of larval connectivity between populations has received much attention in recent years. As scientific attitudes shift away from the assumption of passive dispersal towards more dynamic and situation-specific models of settlement, marine biologists have had to adapt existing methodologies and develop new tools to test hypotheses of larval settlement. This dissertation aims to address the possible larval connectivity of the tropical damselfish *Stegastes flavilatus* in the Las Perlas archipelago, Panama, using microsatellite analysis of DNA from distinct populations as a primary tool. The purpose of this work is to consider the physical and biological factors in this specific case, in order to provide a framework which may be adapted to examine the larval connectivity of other biologically and commercially important species found within the area. This could potentially be used in future protection and sustainable resource use of the recently created Las Perlas special management zone.

1 INTRODUCTION

1.1 LARVAL CONNECTIVITY

1.1.1 Background to Larval Connectivity

Scientific attitudes towards marine larval distribution have changed dramatically over the last 20 years (Levin, 2006). Historically, scientists have taken the view of "open populations," i.e., that marine dispersal is both passive and random, and therefore widespread, and that subsequent recruitment is entirely dependent on the movement of currents and the limitations of predation and habitat suitability (Shanks, 1995). Recently, however, a significant number of studies have begun to show that there may be many other factors in the determination of larval settlement (Levin *et al,* 2005), which may suggest reasons for some of the many recent examples of evidence for self-recruitment (e.g., Jones *et al,* 1999). Neubert and Caswell (2000) write extensively about the statistical probabilities of the settlement of the final location of larvae as a function of their original starting point, which is referred to in successive literature as the dispersal kernel of a species.

Some of the most important considerations within this emerging field current include: the role of biogeography and oceanography as either promoting or limiting dispersal; the biology of species, and the consideration of both general and specialised behavioural practices, where they are known (see Levin 2006, Simpson *et al,* 2004, Wellington and Victor, 1992).

The purpose of breaking down the question of larval dispersal into each potential factor is vital for the possible future development of a framework in which interested

parties can calculate the potential for self recruitment in any given population. Examination of the myriad potential variables involved in larval dispersal is important for a number of reasons: for the examination of ecology and community structure in the designation of marine protected areas and compliance with legislation (Lubchenco *et al,* 2003), and for the continuation and development of sustainable harvest within commercially important stocks and fisheries (Mazzarelli, 1992).

1.1.2 Retention and Self Recruitment

The role of biogeography in patterns of larval dispersal and recruitment has only recently begun to be analysed. Wellington and Victor (1992) conducted studies on a number of damselfish (*Thalassoma lucasanum, Stegastes flavilatus,* and *Microspathodon dorsalis*) in the eastern Pacific, to measure variance of populations in biogeographically separated areas over a spatial scale of 3500km. The results showed statistically significant difference in age, size and larval lifespan at each different site, with shorter larval durations occurring at more northerly sites.

The difference in heterozygosity at varying spatial scales within populations of the same species has been discussed in depth in a number of studies and reviews over the last 10-15 years (Levin, 2006). Three of the most important reviews include those by Palumbi (2001), Hellberg *et al,* (2002), and Palumbi (2003), in which the importance of spatial scale in genetic similarity was critically examined. The reviews conclude that the distance at which dispersal is most active is at scales of around 100km from the initial site. At scales of less than a km there is no visible genetic variability, and at scales of over 1000km factors of habitat suitability become the dominant factors. This corresponds

to recent studies undertaken in the Caribbean, which show that genetic retention occurs in some reef fish at spatial scales of approximately 10-100km (Taylor and Hellberg, 2003).

Levin (2006) states clearly that retention is not solely a product of life history. However, there are clearly a number of specific biological conditions which make self-recruitment a more viable and likely option. This is especially important as the traditional view of larval stages being biologically advantageous is being challenged (Palmer and Strathmann, 1981), and replaced with evidence to suggest that retention may be of more evolutionary value (Strathmann *et al*, 2002).

There is much evidence to suggest that the pelagic larval duration (PLD) can vary quite dramatically even in separate populations of the same species. For example, Wellington and Victor (1989) classified the pelagic larval stages of 100 species of Pomacentridae (damselfish), in a variety of different locations, and found that larval stages vary greatly depending on other factors, such as oceanographic factors, habitat suitability, and behaviour. The pelagic larval duration of *Stegastes flavilatus,* for example, ranged from 25-39 days depending on location, with populations in the eastern Pacific generally having the longest PLD. Studies by Siegel *et al,* (2003) and Shanks *et al,* (2003) and examined the genetic differences of populations specifically in respect to PLD. The findings showed a correlation with genetic relationships over distance compared to the length of PLD (see fig 1.1.2). This would suggest that populations with longer dispersal periods are more homogeneous over medium distances than populations with shorter dispersal periods.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

Fig 1.1.2 – PLD and dispersal distance. Reproduced from Levin, 2006, from work by Shanks *et al* (2003) and Siegel *et al,* (2003).

Levin (2006) states that egg size is of importance, as larger egg sizes are more likely to be retained in the natal habitat. As larger egg sizes are more metabolically expensive to produce, and less are released, there is less biological advantage for distribution over large distances (Sewell and Young, 1997). This shows that self-recruitment is related to ordinary r-selection and K-selection reproductive strategies (Levington, 2005).

Simpson *et al,* 2004, studied the effect of sound on settlement-stage reef fish at Lizard Island in the Great Barrier Reef, and concluded that the familiarity of reef noises was a factor in the process of dispersal versus retention. Work continues to prove whether or not specific reefs at separated islands have separate sound signatures (Kennedy, 2007).

1.1.3 Tools and Methods for Analysing Dispersal

The tools available for the modelling of larval dispersal have changed considerably over the last 50 years (Levin, 2006). Initial forms of mathematical modelling within the field of larval dispersal were implemented and developed from simple reaction and diffusion models such as those used by Fisher (1937) and Skillam (1951). These models were imperfect due to their inability to handle complex variables. Recent developments such as those implemented by Neubert and Caswell (2000) have improved the situation. These more recent models are based upon a probability density function, or dispersal kernel. Instead of following the calculation of diffusion time alone, the modelling of dispersal kernels allows for discrete time analyses based upon integrodifference equations. Work continues to create more sensitive models.

The use of tags and markers is widespread in larval dispersal monitoring. The most common involve the use of otoliths (ear stones), which are marked with fluorescent dye prior to dispersal (Levin, 2006). These studies can also combine with the examination of trace elements in order to calculate the natal habitat of dispersed organisms. This is more useful for the monitoring of dispersal, as opposed to retention (Levin, 2006).

There have been a considerable number of studies on all sorts of larvae which estimate the biogeographic distance required for populations to develop noticeable genetic differences from each other (see reviews in Levin, 2006). An important study on populations of the bicolour damselfish *Stegastes partitus,* in the Caribbean Sea was conducted by Taylor and Hellberg in 2003. The researchers concluded that the optimum distance for retention was between 10km and 100km. This corresponds to more general work conducted by Kinlan and Gaines, 2003, Palumbi in 2003, and Shanks *et al,* 2003,

who all come to similar conclusions regarding optimal distances for dispersal. Retention is not likely to be seen over very small scales, such as less than 10m (Levin, 2006). Additionally, over very wide distances, such as the whole Eastern coast of the Americas, there are likely to be other factors regarding settlement, speciation and retention – such as large-scale climate events and biogeography.

1.2 LAS PERLAS

1.2.1 Introduction to Las Perlas

The Las Perlas archipelago is located within the Gulf of Panama, on the Western side of the Isthmus of Panama, and is a collection of around 253 largely uninhabited basaltic islands, shoals and islets, located approximately 31km at the nearest point from the Pacific coast of Panama, and 37km from Panama City (Anderson, 2005; Usan, 2006; Berman, 2004). Translated as the Pearl Islands, this archipelago is home to a high diversity of marine fish species, consisting of 737 of the total 780 found along the entire Panamanian stretch of the Pacific coast (Anderson, 2005; Robertson and Allen, 2002).

The Isthmus of Panama began to form around 15 million years ago, towards the end of the Tertiary period during the middle to late Miocene era. The land bridge developed when the tectonic plates of the Pacific and Caribbean collided, forcing the Pacific plate downwards. This caused the development of a chain of underwater volcanoes; some of which eventually broke the surface. The large quantities of water (the circulation of what is now separated as the Atlantic and Pacific) being forced through such a small area resulted in extreme levels of silt and sedimentation forming amongst the island chain.

This process increased as the space reduced. By around 3.5 million years ago, in the middle of the Pliocene era, the land bridge of the Isthmus had broken through the surface, separating the Atlantic from the Pacific and creating the continental shelf as it is seen today (NASA, 2006).

Fig.1.3. – The Isthmus of Panama in the context of the Caribbean and East Pacific.

Figure 1.3.1 shows the width of the continental shelf around the coast of Panama and also some of the general topography of the wider area. This is counteracted by the USGS image below (fig. 1.3.2) which shows some of the geological features which affect the Las Perlas area, including small fault-lines approximately 10,000 years old. This tectonically-formed continental shelf, upon which the shallow archipelago is a part,

occurs just above the deep Columbian Trench and South Panama Deformed Belt (see Appendix IV).

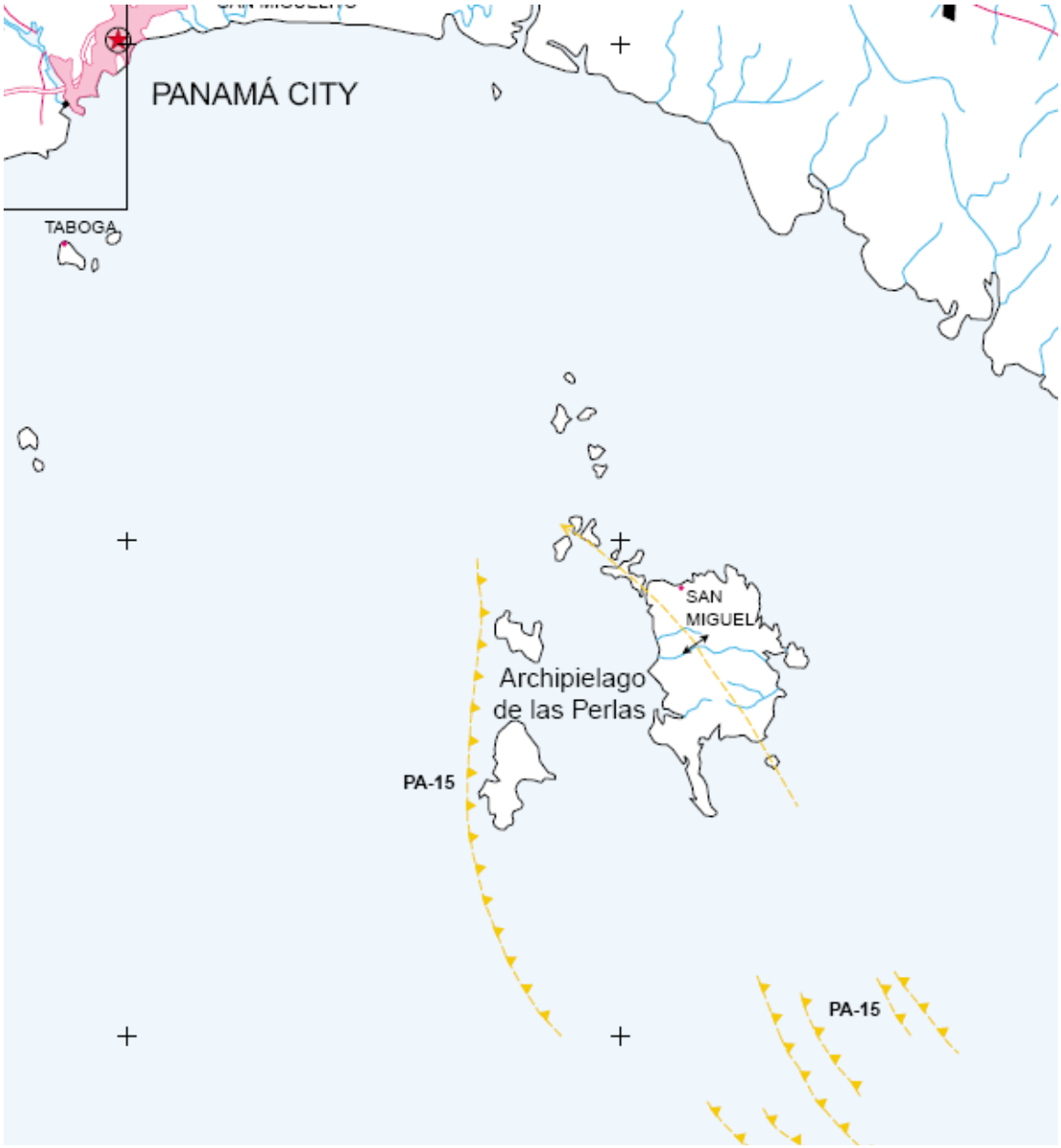

Fig. 1.3.2. Minor geological faults along the Las Perlas Archipelago (USGS, 2005).

1.2.2 The Islands

There are a wide variety of key habitats supported throughout the archipelago. The main marine habitats include coral reefs, mangroves, and stretches of sand and gravel which support a wide range of benthic flora and fauna (Benfield, 2005).

Las Perlas is characterised by considerable diversity of species, comprising 737 of the 780 fish species known to inhabit the Pacific coast of the Gulf of Panama (Anderson, 2005; Robertson and Allen, 2002). The 2001 SeaWiFS satellite data mapping the distribution of chlorophyll intensity throughout the gulf of Panama shows the archipelago as one of the highest regions along this section of the Pacific coast, especially during the highly productive winter months, when Las Perlas exceeds the chlorophyll concentrations for the entire gulf of Panama (see Appendix 1). This shows the regional and national importance of the Pearl Islands productivity. It is this very diversity, stemming from key habitats such as mangroves and coral reefs, which support the range of artisanal and commercial fisheries in the region. The fluctuating patterns of nutrient supply throughout the year controls the marine life of the region, and should be of major importance to the recruitment process of both biologically and commercially important species (Levin, 2006).

The International Tropical Convergence Zone, or ITCZ, is an annual occurrence of high pressure which moves cyclically around the equator (Anderson, 2005).The location of the ITCZ changes regularly, with respect to the zenith point of the sun over the equator (Garrison, 2005). Throughout the Panamanian dry season, which runs from January to

April in correspondence with the absence of the ITCZ (see fig. 1.1), the

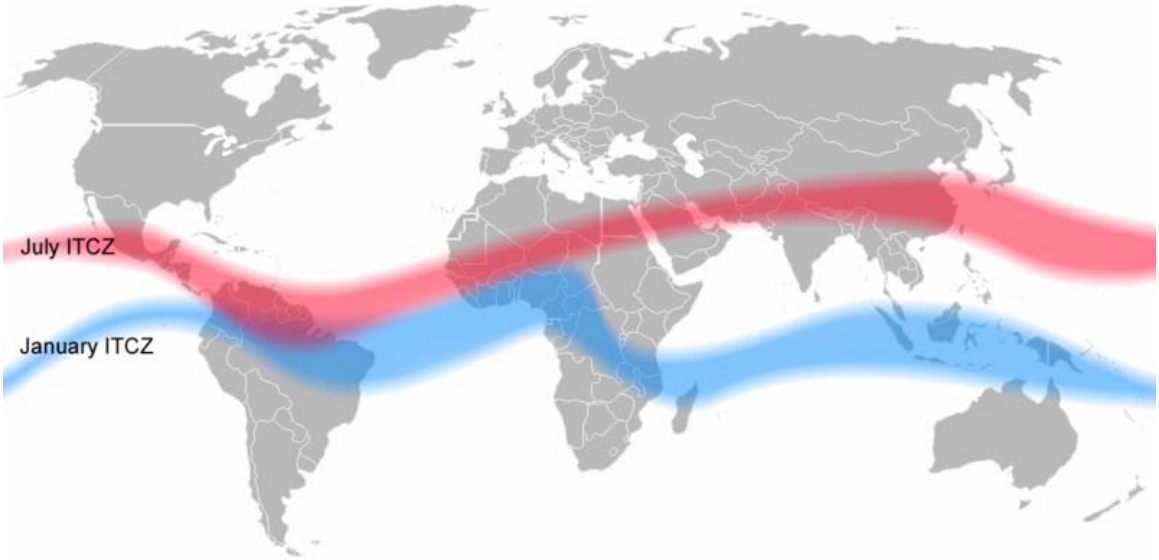

Fig 1.1. Annual movement of ITCZ showing Panamanian presence (red) and absence (blue).

seas around Las Perlas are dominated by the effects of the trade winds, which move in a Westerly direction over the gulf of Panama, resulting in seasonal upwelling events which form the basis of marine productivity. For the rest of the year, from May to December, when the ITCZ is positioned over Panama, the seas around Las Perlas are characterised by warm, nutrient-poor waters with reduced diversity and productivity (Anderson, 2005; Kwiecinski and Chial, 1987).

The irregular occurrence of the El Nino Southern Oscillation (ENSO) event affects all areas of the Pacific Ocean (Garrison, 2005). ENSO occurs when, due to a range of poorly-understood environmental factors, the intensity of the trade winds become disrupted and the Pacific ocean experiences elevated levels of warming. In Las Perlas, this can cause the average water temperature to rise from around 15º-20ºC, as expected

during the cold dry seasons of upwelling, to around 30º-31ºC (D'Croz *et al,* 2001; Baxter, 2004; Anderson, 2005). This can have far reaching and sometimes unexpected implications on community composition and productivity (Lavaniegos *et al,* 2003). During the 1982-83 ENSO event the Gulf of Panama witnessed 85% coral mortality (Baxter, 2005).

1.3 Aims of Dissertation

i) Examination of population connectivity of *S. flavilatus*

ii) Relation of existing literature to observed results

iii) Suggestion of future studies for commercial fish stocks as part of Las Perlas Special Management Zone

2 METHODS

2.1 Capture and sampling methods

Stegastes flavilatus tissue was collected from a number of sub-populations for genetic analysis (Clifton). After trialing a number of capture methods (Baldwin), clove oil (90% eugenol) was diluted with five parts 95% ethanol to anesthetise fish, used in squeezable bottles with two divers with hand held nets used to collect specimens. Later clove oil was substituted for a quinaldine (2-methyl quinoline) solution in 95% ethanol (Cuna and Rosa, 2006). This chemical was found to act faster and more effectively, since clove oil is positively buoyant, and resulted in less escapes and shorter dive times. Fish were put into plastic bags and brought to the boat and put in a bucket of seawater so that a small (1-2mm) clip could be taken from the ventral fin, using ordinary nail clippers, before being and transferred into ethanol and put on ice.

2.2 DNA extraction and amplification

The purpose of this stage was to produce a raw product of DNA from the preserved fin clips for the proposed amplification of microsatellites. The technique was based upon a methodology produced by the Centre for Marine Biodiversity and Biotechnology, Heriot Watt University, for the extraction of DNA from mussels (*Mytilus edulis*). With the assistance of Dr. Peter Morris, and relevant scientific literature, this method was modified

where appropriate. The preparation of samples was undertaken using the commercially available DNeasy kit from Qiagen.

2.2.1 Preparation of Samples

Refrigerated fin clips were removed from ethanol using a pair of forceps and weighed. Each sample was placed in a labelled 1.5ml centrifuge tube, and 180 µl of lysis buffer ATL was added to disrupt cells. The fin clips were then homogenised using plastic pestles until each sample had achieved the consistency of a milky liquid, with no visible tissue remaining.

20 µl of Proteinase K solution was added, and the samples were mixed via a vortex before being placed in a plastic float and incubated in a 55ºC water bath for 90 minutes to allow for digestion of cellular tissue and release of genomic DNA.

The samples were vortexed again on removal from the water bath, and 200 µl of chaotropic buffer AL was added to the solution. The samples were then vortexed once again and placed in a 70ºC heating block for 10 minutes. On removal, the samples were vortexed again before 200 µl of 96% ethanol was added and vortexed again.

The sample, including any solid tissue and precipitate was added to a mini-column in a 2 ml collection tube. Each tube was microfuged for 1 min at 8000 rpm. The column was then placed in a new tube before being added with 500 µl of wash buffer AW1,

microfuged as before, removed to a new tube and washed with 500 µl of wash buffer AW2. The samples were microfuged for 3 minutes at 13000 rpm to dry.

The columns were added to labelled 1.5 ml centrifuge tubes with the lids removed and retained, and 200 µl of elution buffer AE was added. The samples were centrifuged for 1 min at 8000 rpm. The columns were removed, leaving a raw product of genomic DNA.

2.2.2 Monitoring of DNA quality using Gel Electrophoresis

An agarose gel was prepared using 0.5g agarose weighed and added to a conical flask. 50 ml 0.5x TBE buffer was added. The flask was heated in a microwave for a maximum of 2 minutes until all agarose was dissolved. 1 µl ethidium bromide was added to the solution with a pipette, before being cooled to 60ºC and poured into a gel tray and mould. A 12-well plastic comb was added and the gel was left for 15-20 minutes.

10 µl of each DNA sample was added into separate microcentrifuge tubes, and combining with 2 µl of sample buffer. Each sample was vortexed and loaded into an agarose gel. A DNA ladder was always added to the first well. In initial tests, the DNA ladder was λ *Hin*dIII. The gel electrophoresis unit was activated and left to run for approximately 20 minutes before being analysed under an ultraviolet transilluminator and photographed. Once DNA had been detected, the product was prepared for PCR amplification.

2.2.3 Amplification using Polymerase Chain Reaction

The samples were amplified in batches using PCR, using three pairs of primers adapted for Caribbean species of *Stegastes*. These primers were AAC33-F and AAC33-R; GATA16-F and GATA16-R, and GATA40-F and GATA40-R.

The volumes of water added to each primer, along with the optimum annealing temperature, are shown in table 2.1.

Primer	Vol (µl)	Temp (ºC)
AAC33-F	711	56.7
AAC33-R	612	53.7
GATA16-F	479	53.1
GATA16-R	375	57.9
GATA40-F	440	53.2
GATA40-R	383	53.5

Table 2.1: Primers with added volumes of pure water and annealing temperature.

Each sample consisted of10 µl of DNA, 5 µl of PCR buffer, 8 µl dNTP, 1 µl each of both forward and reverse primers, 1 µl Taq polymerase, and the remaining 34 µl comprised of pure water. These samples were run on a gel with an additional sample as a negative control. This consisted of primer AAC33-F and R and 10 µl water. The samples were analysed using a UV transilluminator after 15 minutes and photographed (see appendix

III). The PCR products were run on a cycle as laid out by Williams et al (2003) with an annealing temperature of 50ºC.

The PCR products were then run on a gel to determine initial results. The gel was run a second time using 0.9 µl ethidium bromide and 30 µl DNA product. This was analysed. The PCR process then was re-run for the pairs of primers GATA-16 and GATA-40. In this instance, the negative control was run using primer pair GATA-40.

2.3 Analysis and Interpretation

Materials used for this project included:

Acrylamide gel electrophoresis unit, using 20x20cm glass plates

40% acrylamide

10x TBE buffer

25 µl TEMED diluted in 250 µl ammonium persulphate

24ml water

100bp DNA ladder

DNA samples

The acrylamide gel electrophoresis unit was set up in order to analyse the diversity of alleles isolated and amplified by the primer pair AAC33 (see Appendix III for sequence). This involved the creation of a polyacrylamide gel and set up following the method laid down by Ausubel *et al* (1998). A 12% acrylamide gel was prepared as the DNA fragments were considered to be between 50-200 bp (see initial electrophoresis results).

A spacing comb was used to create 25 wells, which were loaded with a 100bp DNA ladder, 20 DNA samples (10 from Site 1 and 10 from Site 2), and a negative control made up of water instead of DNA. The polyacrylamide gel was run at 5ev/cm for approximately 3 hours until the loading buffer had reached the end of the plates. The samples were then stained with ethidium bromide and photographed under an ultraviolet transilluminator. The samples were then analysed in order to determine whether or not there were any allele differences within the populations (see Results).

3 RESULTS

Preliminary results

Test runs of extraction and PCR amplification using rRNA proved to be successful. This showed that DNA had been extracted from the fin clips.

Initial amplification of two test samples (one from Contadora and one from Taboga) showed that primer pair AAC33 amplified data from both sites. Potential anomaly results occurred in GATA16 pair at one site. No results were seen using GATA40 pair. The experiments were repeated for GATA16 and GATA40 primer pairs, and this removed the anomaly and showed that the genes amplified by these two primers are not present in *Stegastes flavilatus*, whilst the gene amplified by primer pair AAC33 is still present in both populations.

The initial acrylamide gel results appeared inconclusive, despite the appearance of features (see appendix x) on several samples which required further analysis. Strong banding was seen across all samples at approximately 100 bp, with non-specific binding at higher sites. There were also faint bands seen at approximately 50bp (see appendix x). Non-discriminate amplification was noticed and any bands above approximately 120bp were considered to be artefacts and ignored (see method).

Initial comparison of sites from Taboga and Contadora suggest no discernable differences in genetics between the two populations. However, the initial results require significantly more detailed forms of analysis in order to successfully determine the true extent of connectivity and retention amongst the Las Perlas reefs.

Figures 1 and 2 below show the final PCR reactions as used with primer pair AAC33-F and AAC33-R, as run on agarose gel using electrophoresis. Whilst this appears to show some difference in a second band of DNA at approximately 50-70bp, this could be due to slight differences in timing of the separate runs. The differences appear to disappear when run on an 8% polyacrylamide gel (Figure 3), however there appears to be excess staining using ethidium bromide which makes full visual analysis difficult to determine (see discussion).

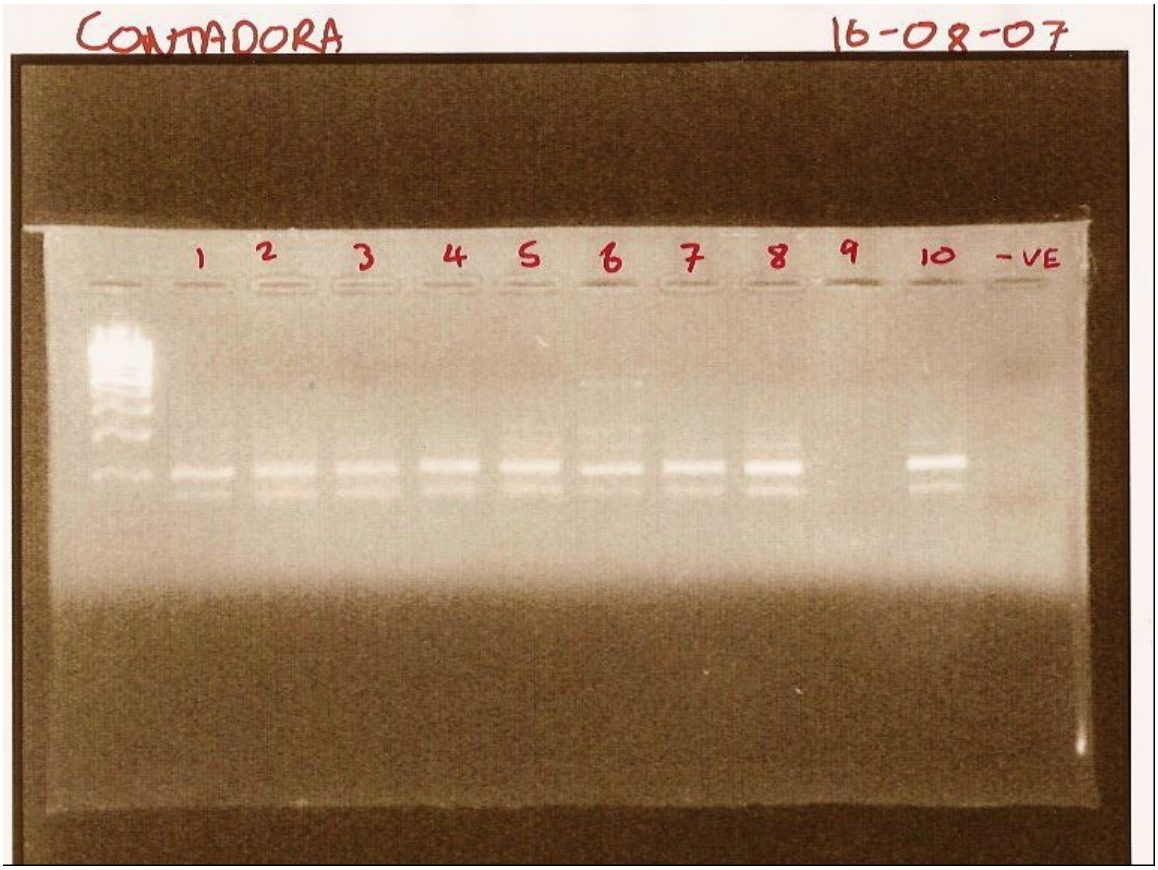

Figure 1. Photograph of PCR product of 10 DNA samples (plus 100bp ladder and negative control) from the island of Contadora. Sample 9 appears to have yielded no DNA.

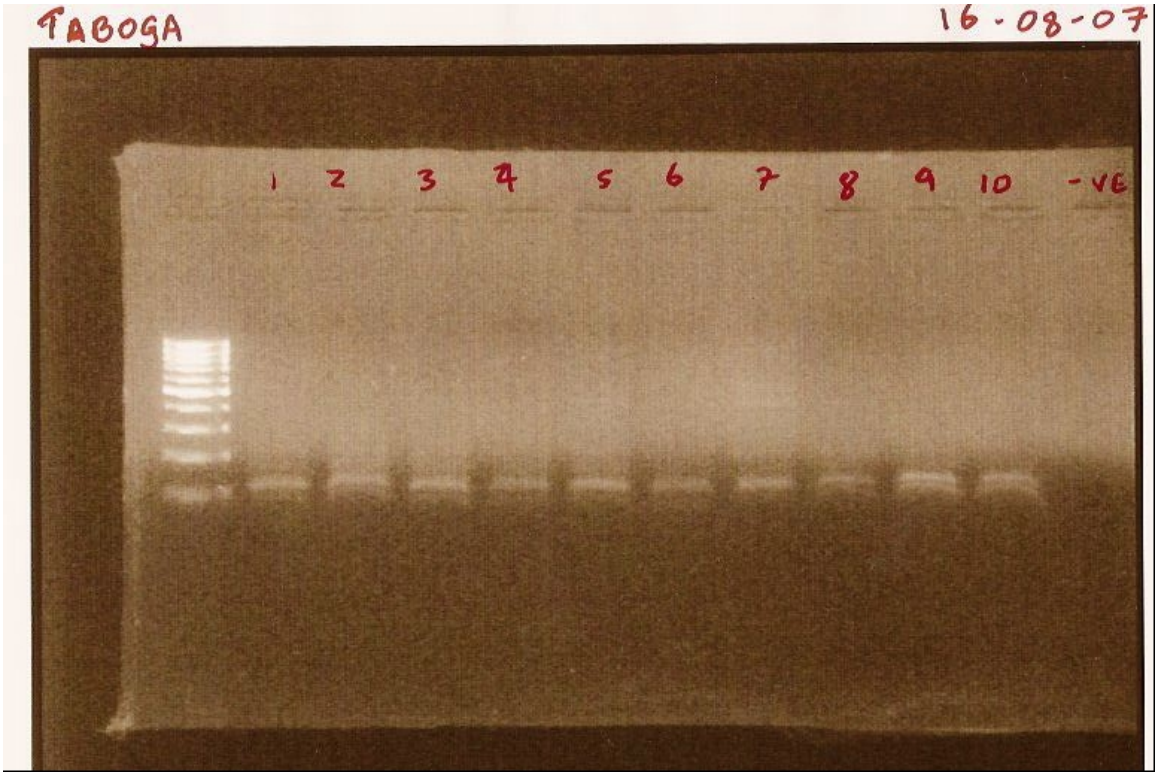

Figure 2. Photograph of PCR product of 10 DNA samples (plus 100bp ladder and negative control) from the island of Taboga. The gel was run separately to samples collected from Contadora (see fig. 1), however the PCR reactions were undertaken at the same time to reduce the chances of error.

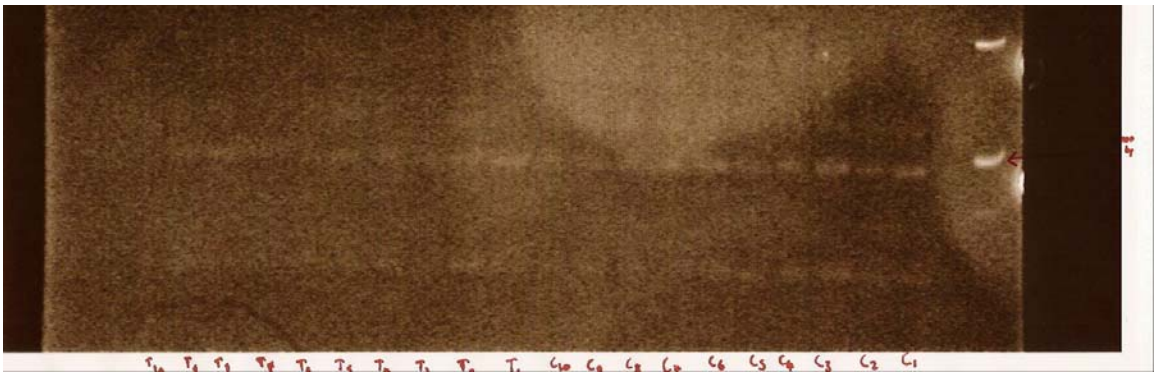

Figure 3. Photograph of banding seen below 100bp marker (noted on ladder to the right of image) of samples from Taboga and Contadora as run on an 8% polyacrylamide gel. No discernable difference appears to occur between either site.

4 DISCUSSION

The preliminary laboratory results showed no significant difference between populations. There are many different reasons for this, and this should not be considered to be conclusive or definitive proof that self-recruitment does not occur throughout reefs in the Las Perlas archipelago. This set of experiments should be seen as preliminary only, and much more work should be carried out to determine the true extent of connectivity throughout the archipelago. There are a number of reasons why the initial experiments may appear to show inconclusive results, and many questions which remain unanswered until further study yields more results.

Since only a single pair of primers out of the initial three appeared to be effective, it is possible that this microsatellite loci may not polymorphic. The primers identified were for *Stegastes partitus,* a Caribbean damselfish biogeographically separated from *Stegastes flavilatus* by the development of the Isthmus of Panama approximately 3.5 million years ago (NASA, 2006). Since microsatellites evolve rapidly, this amount of time is likely to be significant. Additional primers developed for microsatellites found in *S. partitus* by Williams *et al* (2003), and Thiessen and Heath (2007) could be used in future PCR reactions to determine possible effectiveness. These two previous studies yield, between them, a much higher number of microsatellites which range in size from 16-200bp, and may reveal polymorphic loci as retained in *S. flavilatus.*

Of course, the possibility should not be ruled out that there may be no real self-recruitment occurring in specific populations of *Stegastes flavilatus* in the Las Perlas archipelago. As discussed by Purcell and Hertzog (2006), Wellington and Victor (1992) and reviewed in detail by Levin (2006), the factors affecting self recruitment amongst populations are extremely complex. The Las Perlas archipelago is a dynamic environment with a relatively poorly understood internal current circulation (D'Croz and Robertson, 1997), known to flow in a south-westerly direction through the Gulf of Panama (Barrios, *pers. comm*). Although not properly studied, it is possible that the combination of strong Pacific currents moving in a Northerly direction through the Columbian trench (see Appendix IV), the ITCZ causing seasonal upwelling affecting productivity and distribution (see Appendix 1) and a 6m tidal range in parts of the Gulf of Panama (D'Croz and Robertson, 1997, Anderson 2005), strongly suggest that opportunities for passive dispersal throughout the archipelago may be high. It is not clear, either from a review of existing literature nor from the preliminary results of this investigation, whether or not the island of Taboga to the north-east of the Archipelago receives passive larval dispersal from the islands, or whether there is active retention or dispersal occurring over this spatial scale.

Additionally, not enough samples were able to be analysed due to experimental constraints. Only ten samples were analysed per site, whilst at least 30 should be required for meaningful statistical examination using Wrights Coefficient of Inbreeding (Kennedy, *pers. comm.*). It is possible that a full set of extractions and more detailed investigation of different polymorphic loci may yield more conclusive results. Visual examination of

some photographs taken throughout this research do appear to show allele differences between individuals – a greater examination using more sensitive polyacrylamide gels, and any of the other techniques available may well reveal statistical population differences between the islands of Taboga, Contadora and perhaps any of the other islands in the archipelago from where samples were collected. Even if there is no difference between species of *Stegastes flavilatus,* within Las Perlas, does not mean that this research is unimportant. Data should be collected populations of *S. flavilatus* from Coiba Island and compared with the Las Perlas sites. Due to the geographic location of Coiba Island slightly beyond the Gulf of Panama, and subjected to biogeographical and geological pressures different to those affecting Las Perlas (see appendix IV), a comparison of this scale would yield more conclusive results and perhaps serve to highlight areas of key importance for the long-term management plan of the Pearl Islands Marine Protected Area (see appendix II).

5 CONCLUSION

Based solely upon the preliminary results ascertained throughout the duration of this study, it appears that there is no discernable difference between populations of *Stegastes flavilatus* as found on the island of Contadora in the Las Perlas Archipelago, and those found on the slightly removed island of Taboga, close to the Panamanian mainland. Due to the previously discussed limitations affecting the course of this study, these results should not, on any account, be interpreted as either conclusive or authoritive. Significant amounts of further analysis will be required, as will additional work into the determination of local and near-shore currents affecting pelagic larval dispersal. This study should also be seen as just a single part of a much wider study examining population connectivity of additional species throughout the Las Perlas Archipelago, especially those which are commercially valuable or threatened due to overexploitation, pollution or other anthropogenic factors.

6 RECOMMENDATIONS

1 Detailed analysis of reef fish DNA from the existing sites, following an initial process of extraction, PCR, agarose and acrylamide gel analysis.

2 Examination of existing samples with additional primers developed for *Stegastes partitus* in order to determine suitability.

3 In-depth microsatellite analysis of *Stegastes flavilatus* to attempt to search for more suitable loci and to determine any possible allele differences within the specified populations.

4 Statistical analysis of results, rather than mere visual determination of similarity.

5 Sampling of additional populations from slightly more biogeographically distinct areas, for example from Coiba Island, located to the North-West of the Archipelago.

6 Repetition of the project for more commercially important species, in order to determine management plans from specific areas.

7 Development and maintenance of functional and detailed GIS database regarding the biological potential of the Las Perlas archipelago – including oceanographic

data, biological records, distribution and abundance, habitat classification and associated biota.

8 Implementation of biological monitoring and record keeping procedures in keeping with the management of an MPA.

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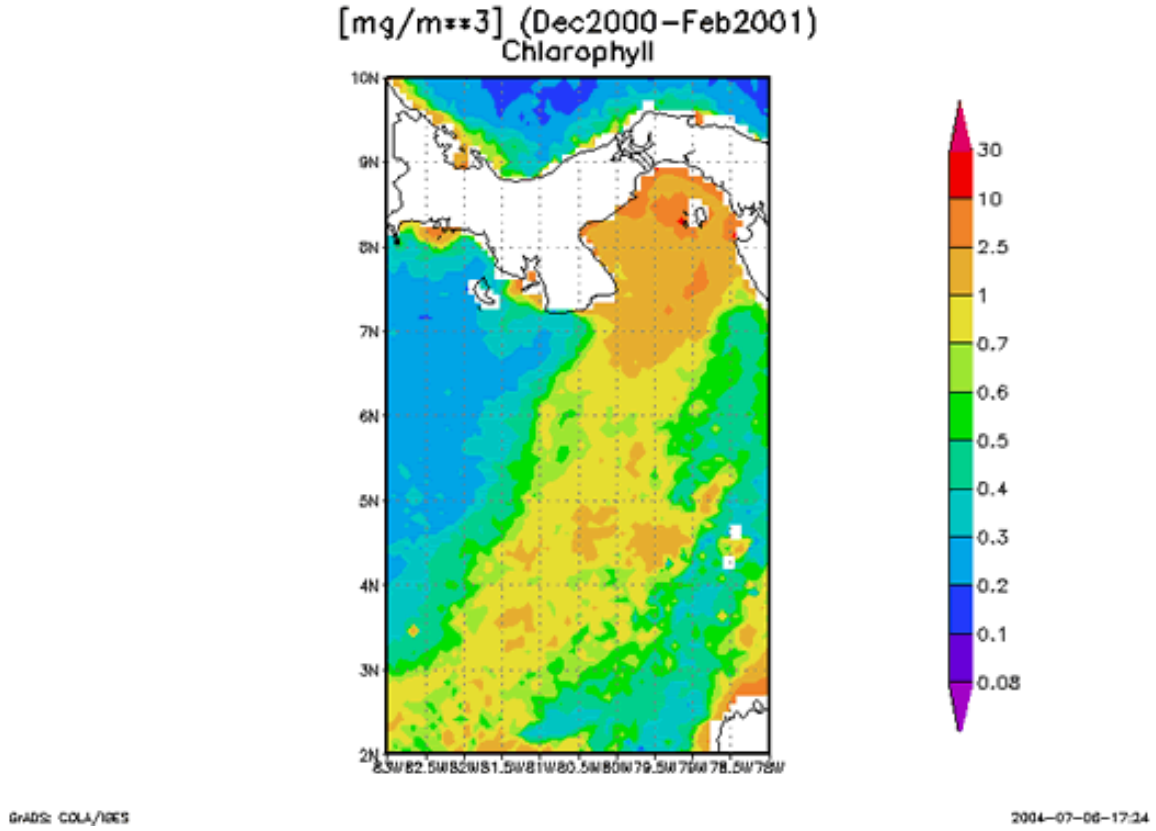
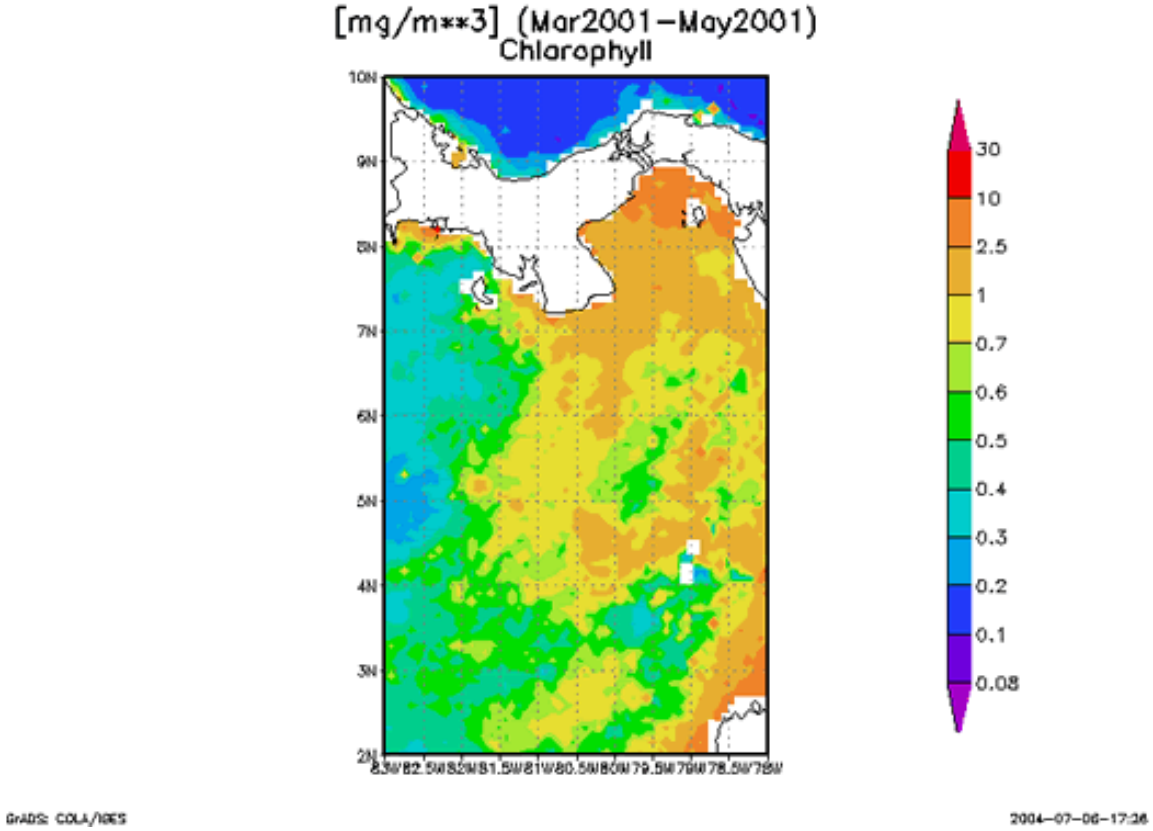
8 APPENDICES

Appendix 1

SeaWiFS satellite data showing marine chlorophyll concentrations from December 2000 to November 2001 (Goddard Earth Sciences Data and Information Centre, 2007). Each image covers a three-month period, and shows the productivity of the Gulf of Panama. The Las Perlas Archipelago is as shown. White pixels outwith land areas represent cloud cover at the time of image capture.

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9N
8N
7N
6N
5N
4N
3N
2N
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30
10
2.5
1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
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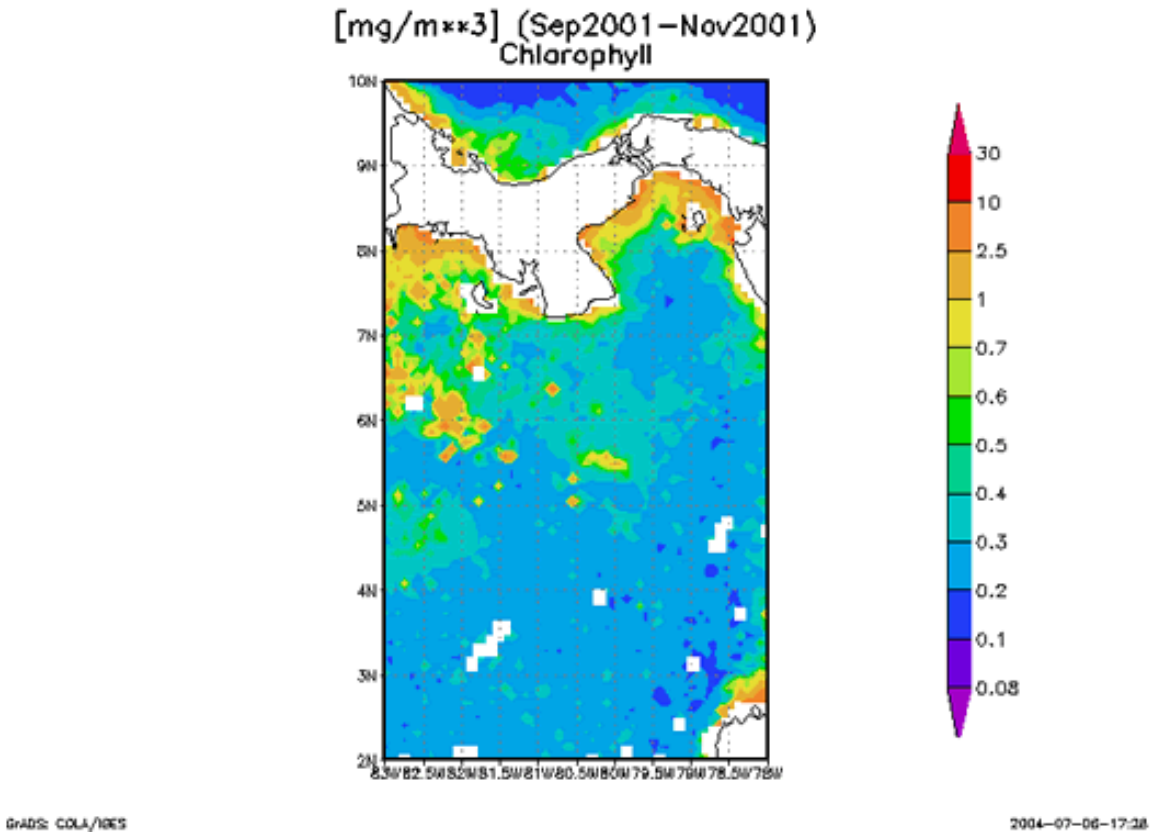
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9N
8N
7N
6N
5N
4N
3N
2N
83W 82.5W 82W 81.5W 81W 80.5W 80W 79.5W 79W 78.5W 78W
30
10
2.5
1
0.7
0.6
0.5
0.4
0.3
0.2
0.1
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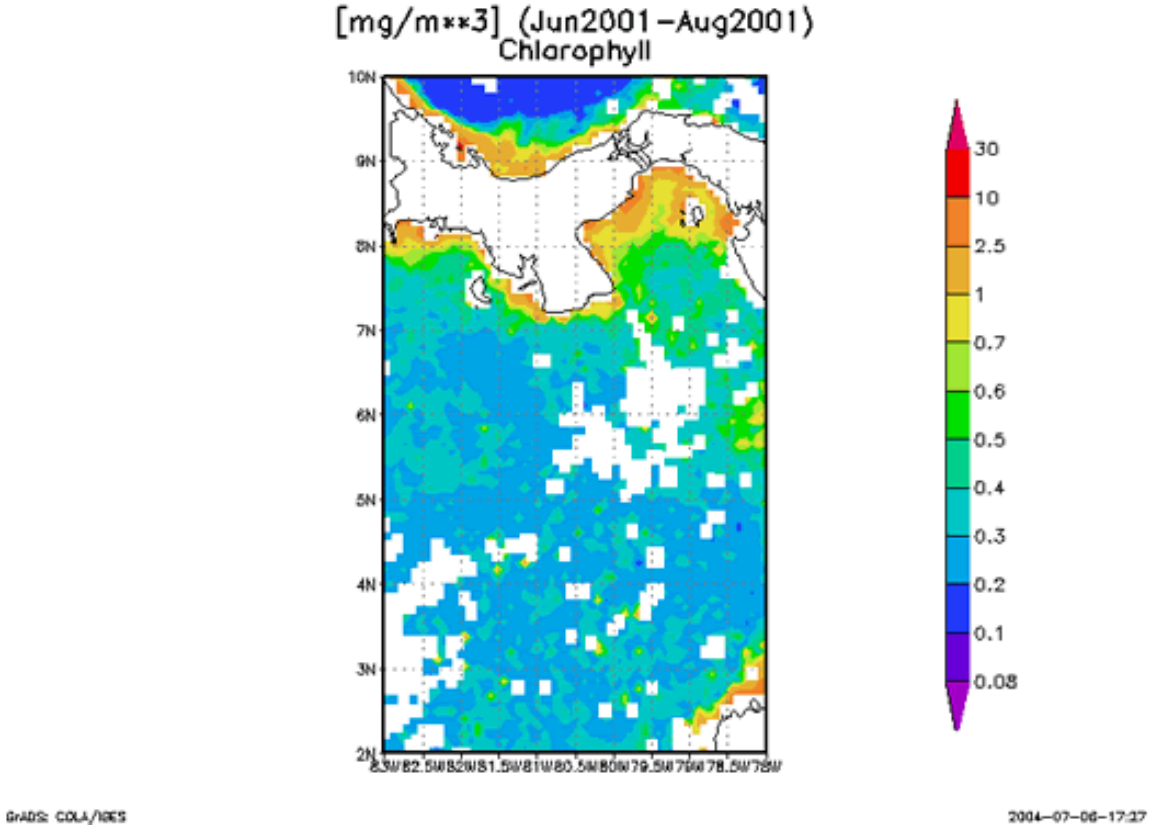
z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2N
83W 82.5W 82W 81.5W 81W 80.5W 80W 79.5W 79W 78.5W 78W
30
10
2.5
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.08
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Appendix II – Declaration of the Las Perlas Special Marine Coastal Management Zone.

Digitalizado por la Asamblea Nacional

GACETA OFICIAL

AÑO CIII PANAMA, R. DE PANAMA LUNES 4 DE JUNIO DE 2007 N°25,805

CONTENIDO

ASAMBLEA NACIONAL
LEY No. 18
(de 31 de mayo de 2007)
"QUE DECLARA ZONA ESPECIAL DE MANEJO MARINO – COSTERA AL ARCHIPIELAGO DE LAS PERLAS Y DICTA OTRAS DISPOSICIONES." ..... PAG. 3

CONSEJO DE GABINETE
RESOLUCION DE GABIENTE No. 66
(de 30 de mayo de 2007)
"QUE EMITE CONCEPTO FAVORABLE AL CONTRATO No. 29-2007, A SUSCRIBIRSE ENTRE EL INSTITUTO DE ACUEDUCTOS Y ALCANTARILLADOS NACIONALES Y LA EMPRESA MNV-COGEFAR – PANAMA, S.A., PARA LLEVAR A CABO EL DISEÑO Y CONSTRUCCION DE TUBERIA DE CONDUCCION DE CHILIBRE – SABANITAS, PROVINCIA DE PANAMA Y COLON. POR UN MONTO TOTAL DE CUATRO MILLONES SEISCIENTOS SETENTA Y DOS MIL SETECIENTOS TREINTA Y TRES BALBOAS CON 00/100 (B/. 4,672,733.00)." ..... PAG. 9

MINISTERIO DE LA PRESIDENCIA
DECRETO No. 54
(de 7 de mayo de 2007)
"POR EL CUAL SE DESIGNA A LA MINISTRA Y VICEMINISTRO DE SALUD, ENCARGADOS." ..... PAG. 11

DECRETO No. 55
(de 10 de mayo de 2007)
"POR EL CUAL SE DESIGNA AL MINISTRO Y VICEMINISTRO DE DESARROLLO AGROPECUARIO, ENCARGADOS." ..... PAG. 12

DECRETO No. 56
(de 10 de mayo de 2007)
"POR EL CUAL SE DESIGNA AL MINISTRO DE RELACIONES EXTERIORES, ENCARGADO." ..... PAG. 13

RESOLUCION No. 28
(de 14 de mayo de 2007)
"ADJUDICAR A LA EMPRESA SISTEMAS Y SOLUCIONES TECNOLOGICAS INTERNACIONALES, S.A., (SSTI) LA LICITACION PUBLICA 2007-0-03-0-08-LP-000755." ..... PAG. 13

MINISTERIO DE COMERCIO E INDUSTRIAS
DIRECCION GENERAL DE RECURSOS MINERALES
RESOLUCION No. 02
(de 13 de enero de 2006)
"CANCELAR LA CONCESION OTORGADA MEDIANTE CONTRATO No. 69 DE 10 DE SEPTIEMBRE DE 1996, DE EXPLORACION DE MINERALES METALICOS (ORO Y OTROS) DE LA EMPRESA FOMENTO MINERO, S.A." ..... PAG. 15

MINISTERIO DE EDUCACION
DECRETO EJECUTIVO No. 160
(de 1 junio de 2007)
"QUE ORDENA EL CIERRE DE LOS CENTROS EDUCATIVOS OFICIALES Y PARTICULARES, UBICADOS EN LOS DISTRITOS DE PANAMA Y SAN MIGUELITO, EL DIA 4 DE JUNIO DE 2007." ..... PAG. 18

CONTINUA EN LA PAGINA 2

Digitalizado por la Asamblea Nacional

ASAMBLEA NACIONAL
LEY No. 18
(de 31 de mayo de 2007)

Que declara Zona Especial de Manejo Marino-Costera al Archipiélago de Las Perlas y dicta otras disposiciones

LA ASAMBLEA NACIONAL

DECRETA:

Capítulo I

Objetivo y Definiciones

Artículo 1. Se declara Zona Especial de Manejo Marino-Costera al Archipiélago de Las Perlas, ubicado en el distrito de Balboa, provincia de Panamá, que se incorporará al Programa de Manejo Costero Integral según lo establecido en la Ley 44 de 2006, con el propósito de proteger los recursos marino-costeros, aumentar su productividad y mantener la biodiversidad de sus ecosistemas, a fin de mejorar la calidad de vida de las comunidades que dependen de dichos recursos.

Artículo 2. Para el cumplimiento de los objetivos de la presente Ley, las autoridades competentes establecerán las medidas de coordinación necesarias que aseguren un desarrollo social y económico de la Zona, mediante un Plan de Manejo Costero Integral que garantice la sostenibilidad de las actividades extractivas y pesqueras.

Artículo 3. Para los efectos de esta Ley, los siguientes términos se entenderán así:

1. *Plan de Manejo Costero Integral.* Documento planificador del ordenamiento territorial marino de la zona marino-costera, que incorpora tareas técnicas, administrativas y científicas para la conservación de los ecosistemas y el aprovechamiento sostenible de los recursos naturales, cuyos resultados deben producir el ordenamiento especial y de uso de la Zona Especial de Manejo Marino-Costera.
2. *Unidad de Conservación y Vigilancia.* Órgano de apoyo interinstitucional local, creado para coordinar y agrupar a todas las autoridades relacionadas con las zonas especiales de manejo marino-costeras en sus funciones y operaciones de control en el uso de los recursos marino-costeros y la solución de conflictos, establecidas en el Plan de Manejo Costero Integral.
3. *Zona satélite.* La que constituye una extensión de una zona principal de protección, conservación y manejo, la cual adopta las mismas disposiciones legales que la zona principal.

Capítulo II

Estructura del Manejo Costero Integral

Artículo 4. Se establece el Programa de Manejo Costero Integral para el Archipiélago de Las Perlas, cuya función será diseñar el Plan de Manejo Costero Integral, a través de la Dirección General de Ordenación y Manejo Integral de la Autoridad de los Recursos Acuáticos de Panamá, con el apoyo de los actores y usuarios involucrados en el tema. Este Programa tendrá la función de ejecutar, fiscalizar, evaluar, monitorear y elaborar el plan de desarrollo y uso sostenible de los recursos marino-costeros, guardando concordancia con los lineamientos y planes de desarrollo turístico sostenible.

Artículo 5. Se establece la Unidad de Conservación y Vigilancia de la Zona Especial de Manejo Marino-Costera del Archipiélago de Las Perlas, cuyas funciones son las de coordinar y ejecutar las acciones de conservación y vigilancia acordadas en el Plan de Manejo Costero Integral, con todos los actores con injerencia en la zona.

Artículo 6. La Unidad de Conservación y Vigilancia estará integrada por los siguientes miembros con derecho a voz y voto:

1. Un representante de la Autoridad de los Recursos Acuáticos de Panamá, quien la presidirá.
2. Un representante de la Comisión de Población, Ambiente y Desarrollo de la Asamblea Nacional.
3. Un representante de la Autoridad Nacional del Ambiente.
4. Un representante de la sociedad civil ambiental.
5. Un representante de las instituciones gubernamentales y/o no gubernamentales de investigación científica.
6. Un representante de una Asociación de Pescadores del Archipiélago de Las Perlas.
7. Un representante del sector pesquero de la Comisión Nacional de Pesca Responsable, que no pertenezca a las entidades mencionadas dentro de la Unidad.
8. Un representante del Consejo Municipal del distrito de Balboa.

Esta Unidad considerará en sus reuniones la participación de otras instituciones gubernamentales y no gubernamentales, las cuales solo tendrán derecho a voz.

En el caso de los numerales 4, 5 y 6, las organizaciones interesadas, además de estar legalmente constituidas, deberán registrarse en la Autoridad de los Recursos Acuáticos de Panamá mediante simple comunicación expresa en la que indiquen su interés en formar parte de

esta Unidad, de manera tal que sus representantes sean escogidos cada cinco años entre quienes se hayan registrado y cumplan los requisitos establecidos en la presente Ley y su reglamento.

Artículo 7. El Administrador de la Autoridad de los Recursos Acuáticos de Panamá escogerá, de una terna de candidatos presentada por la asociación o las asociaciones de gremios pesqueros y por las organizaciones no gubernamentales, a los miembros principales y suplentes de la Unidad, los cuales al momento de su designación no deberán tener parentesco, dentro del cuarto grado de consanguinidad o segundo de afinidad, con los otros miembros de la Unidad o con el Administrador de la Autoridad de los Recursos Acuáticos de Panamá. Además, los representantes de la sociedad civil que integren la Unidad no podrán ser funcionarios de las instituciones del Estado que la conforman.

Capítulo III

Zona Especial de Manejo Marino-Costera

Artículo 8. El polígono que define la Zona Especial de Manejo Marino-Costera del Archipiélago de Las Perlas está situado dentro del Golfo de Panamá en el Océano Pacífico, y consta de un área total aproximada de 168,771 hectáreas que abarca sus dos zonas satélites, divididas en 33,153 hectáreas de área insular, que incluye la zona marino-costera de todas las islas e islotes, y 135,618 hectáreas de área marina parte de la Plataforma Continental, la cual conforma un perímetro de 163 kilómetros.

Todo el límite de la Zona Especial de Manejo Marino-Costera está en el mar. El polígono imaginario se inicia en el Punto 1, con latitud 8°40'48.29" Norte y longitud 79°06'05.59" Oeste. Se sigue una línea imaginaria con rumbo Este 89° una distancia de 3.45 millas náuticas hasta el Punto 2. Desde este punto, con latitud 8°40'49.82" Norte y longitud 79°02'37.46" Oeste, se continúa con rumbo Sur 138° Este una distancia de 22.82 millas náuticas hasta el Punto 3. Desde este punto, con latitud 8°23'44.54" Norte y longitud 78°47'14.04" Oeste, se prosigue con rumbo Sur 180° una distancia de 6.83 millas náuticas hasta el Punto 4. Desde este punto, con latitud 8°16'53.01" Norte y longitud 78°47'16.35" Oeste, se continúa con rumbo Sur 226° Oeste una distancia de 7.97 millas náuticas hasta el Punto 5. Desde este punto, con latitud 8°11'17.79" Norte y longitud 78°53'00.85" Oeste, se continúa con rumbo Oeste 271° una distancia de 17.31 millas náuticas hasta llegar al Punto 6. Desde este punto, con latitud 8°11'23.33" Norte y longitud 79°10'28.32" Oeste, se continúa con rumbo Norte 9° Este una distancia de 29.66 millas náuticas hasta encontrar nuevamente el Punto 1.

Artículo 9. Se establecen dos zonas satélites de protección, que estarán localizadas fuera del polígono principal de la Zona Especial de Manejo Marino-Costera, pero vinculadas a esta, con las siguientes coordenadas:

1. Zona Satélite Roca Trollope, localizada en coordenada con latitud 08°06'53.95" Norte y longitud 078°38'51.23" Oeste, con un área de 4,316 hectáreas, formando un círculo imaginario con distancia de dos millas náuticas alrededor desde la roca.
2. Zona Satélite Isla Galera, localizada en coordenada con latitud 08°11'41.10" Norte y longitud 078°46'32.71" Oeste, con un área de 4,288 hectáreas, formando un círculo imaginario con distancia de dos millas náuticas alrededor desde la costa de la isla.

Capítulo IV

Prohibiciones y Veda Temporal

Artículo 10. Dentro de la Zona Especial de Manejo Marino-Costera del Archipiélago de Las Perlas, está prohibido lo siguiente:

1. La tala, el uso y la comercialización de los bosques de manglar, sus productos, partes y derivados. Se exceptúa la tala en proyectos de desarrollo turístico, previa aprobación del estudio de impacto ambiental y el cumplimiento de la legislación vigente.
2. La extracción de corales y peces de arrecifes coralinos.
3. El uso de trasmallos de cualquier tipo o denominación, de chuzos, así como de otras artes y prácticas de pesca prohibidas por la legislación vigente.
4. El uso de palangres horizontales superficiales y a fondo. Se permite el uso de palangre vertical hasta un máximo de quince tanques por embarcación y máximo de cinco anzuelos por tanque.
5. El uso de redes de arrastre y de cerco mecánico industrial en toda la Zona Especial de Manejo Marino-Costera del Archipiélago de Las Perlas.
6. La pesca con tanques para buceo o cualquier otro método que provea al buzo de aire.
7. El uso de arpones, con excepción de los arpones de liga en la pesca a pulmón para fines deportivos no comerciales.
8. La pesca de tiburones y de rayas (Elasmobranquios).
9. La captura y comercialización de carne y huevos de todas las especies de tortugas marinas.
10. La pesca de langosta desde el 1 de diciembre hasta el 15 de abril de cada año.
11. El asedio de las poblaciones de cetáceos que utilizan las aguas de la Zona, en contravención al Reglamento para el Avistamiento de Cetáceos en Aguas Territoriales Panameñas.

12. La extracción de cualquier especie que la Autoridad de los Recursos Acuáticos de Panamá tenga a bien regular con el objetivo de mantener niveles racionales y sostenibles en la pesca comercial, con la cual podrá establecer sistemas de licencia, vedas y cuotas de extracción basadas en la mejor evidencia científica disponible.

13. Cualquier otra actividad que atente contra los objetivos de la presente Ley.

En la reglamentación de la presente Ley, se establecerán áreas exclusivas de pesca de cojinda para uso de redes bolicheras artesanales o de cerco manual.

La Dirección General de Ordenación y Manejo Integral podrá ampliar, modificar o reducir periódicamente las prohibiciones y vedas, de acuerdo con estudios e informes técnicos realizados en apoyo al Plan de Manejo Costero Integral, consulta ciudadana y previa consulta con la Unidad de Conservación y Vigilancia.

Capítulo V
Competencia

Artículo 11. La Dirección General de Ordenación y Manejo Integral de la Autoridad de los Recursos Acuáticos de Panamá será la encargada de establecer e implementar las estructuras organizativas necesarias y requeridas en la ejecución del Plan de Manejo Costero Integral, a fin de que se hagan las consultas necesarias entre los administradores de los recursos acuáticos, las autoridades del distrito de Balboa y los usuarios, y se establezcan las medidas de ordenamiento y conservación de los recursos marino-costeros.

Artículo 12. La Autoridad Nacional del Ambiente establecerá en esta Zona áreas protegidas bajo las categorías de manejo que correspondan y que resulten armónicas con el Plan de Manejo Costero Integral para esta Zona, con los objetivos de esta Ley y de acuerdo con las disposiciones legales y reglamentarias vigentes en esta materia.

Artículo 13. Para la protección de yacimientos arqueológicos u otras áreas de recursos culturales subacuáticos, el Instituto Nacional de Cultura establecerá sitios bajo las categorías de manejo de monumentos nacionales o zonas de interés cultural, los cuales deberán cumplir con las directrices técnicas establecidas en la Ley 32 de 2003 y la Ley 58 de 2003.

Capítulo VI
Disposiciones Finales

Artículo 14 (transitorio). Se establece un periodo de veda temporal por doce meses, a partir de

la entrada en vigencia de la presente Ley, para la extracción de la concha negra (*Anadara tuberculosa*) y casco de burro (*Anadara grandis*) de todas las áreas de manglar del Archipiélago de Las Perlas, para permitir la recuperación de las poblaciones naturales que serán reguladas detalladamente dentro del Plan de Manejo Costero Integral.

Artículo 15. El Órgano Ejecutivo reglamentará la presente Ley dentro de los seis meses siguientes a su promulgación.

Artículo 16. Esta Ley comenzará a regir desde su promulgación.

COMUNÍQUESE Y CÚMPLASE.

Aprobada en tercer debate en el Palacio Justo Arosemena, ciudad de Panamá, a los 18 días del mes de abril del año dos mil siete, en virtud del Proyecto 151 de 2005.

El Presidente,

Elías A. Castillo G.

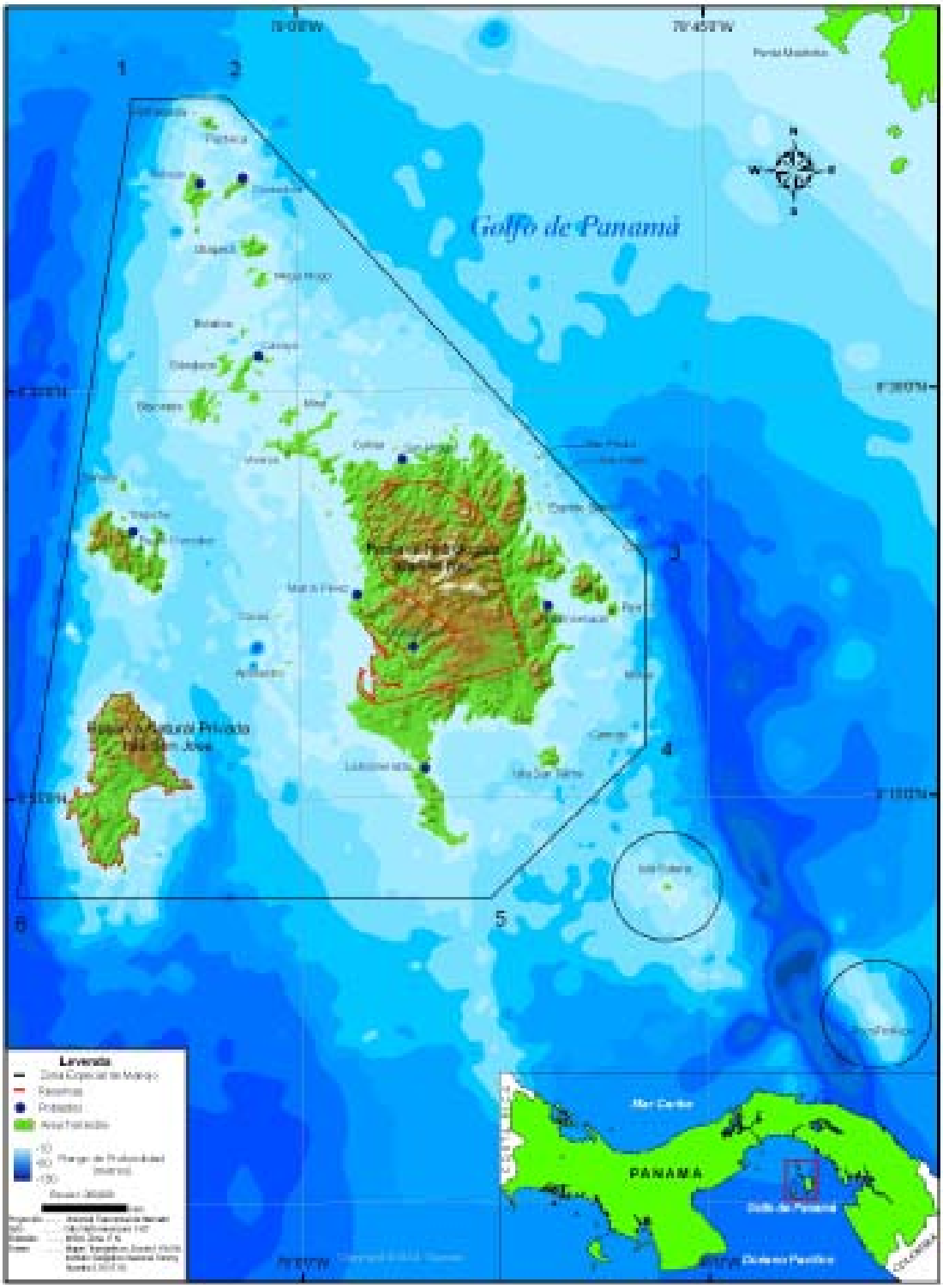
El Secretario General,

Carlos José Smith S.

ÓRGANO EJECUTIVO NACIONAL. PRESIDENCIA DE LA REPÚBLICA.
PANAMÁ, REPÚBLICA DE PANAMÁ, 31 DE mayo DE 2007.

MARTÍN TORRIJOS ESPINO
Presidente de la República

HÉCTOR E. ALEXANDER H.
Ministro de Economía y Finazas


Golfo de Panamá
1
2
3
4
5
6
Leyenda
Mar Caribe
PANAMA
Golfo de Panamá
Océano Pacífico

Appendix III – GenBank Microsatellite Information

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  ORGANISM  Stegastes partitus
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Euteleostomi;
            Actinopterygii; Neopterygii; Teleostei; Euteleostei;
Neoteleostei;
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            Labroidei; Pomacentridae; Stegastes.
REFERENCE   1  (bases 1 to 201)
  AUTHORS   Williams,D.A., Purcell,J.F.H., Hughes,C.R. and Cowen,R.K.
  TITLE     Polymorphic microsatellite loci for population studies of
the
            bicolor damselfish, Stegastes partitus (Pomacentridae)
  JOURNAL   Mol. Ecol. Notes 3 (4), 547-549 (2003)
REFERENCE   2  (bases 1 to 201)
  AUTHORS   Williams,D.A., Purcell,J.F.H., Cowen,R.K. and Hughes,C.R.
  TITLE     Direct Submission
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acgtttaggt
      121 gggaaatatc gcacaacacg ccctcgtttg ttcccagaga tgctcagcga
tgtggcctga
      181 ggaggtttta gttaaatgat c
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SpGATA16

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KEYWORDS    .
SOURCE      Stegastes partitus (bicolour damselfish)
  ORGANISM  Stegastes partitus
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Euteleostomi;
            Actinopterygii; Neopterygii; Teleostei; Euteleostei;
Neoteleostei;
            Acanthomorpha; Acanthopterygii; Percomorpha; Perciformes;
            Labroidei; Pomacentridae; Stegastes.
REFERENCE   1  (bases 1 to 214)
  AUTHORS   Williams,D.A., Purcell,J.F.H., Hughes,C.R. and Cowen,R.K.
  TITLE     Polymorphic microsatellite loci for population studies of
the
            bicolor damselfish, Stegastes partitus (Pomacentridae)
  JOURNAL   Mol. Ecol. Notes 3 (4), 547-549 (2003)
REFERENCE   2  (bases 1 to 214)
  AUTHORS   Williams,D.A., Purcell,J.F.H., Cowen,R.K. and Hughes,C.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-MAR-2003) Biology, University of Miami, 1301
Memorial
            Drive, Coral Gables, FL 33146, USA
FEATURES             Location/Qualifiers
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SpGATA40

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NOV-2003
DEFINITION  Stegastes partitus microsatellite SpGATA40 sequence.
ACCESSION   AY253167
VERSION     AY253167.1  GI:32454086
KEYWORDS    .
SOURCE      Stegastes partitus (bicolor damselfish)
  ORGANISM  Stegastes partitus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata;
Euteleostomi;
            Actinopterygii; Neopterygii; Teleostei; Euteleostei;
Neoteleostei;
            Acanthomorpha; Acanthopterygii; Percomorpha; Perciformes;
            Labroidei; Pomacentridae; Stegastes.
REFERENCE   1  (bases 1 to 263)
  AUTHORS   Williams,D.A., Purcell,J.F.H., Hughes,C.R. and Cowen,R.K.
  TITLE     Polymorphic microsatellite loci for population studies of
the
            bicolor damselfish, Stegastes partitus (Pomacentridae)
  JOURNAL   Mol. Ecol. Notes 3 (4), 547-549 (2003)
REFERENCE   2  (bases 1 to 263)
  AUTHORS   Williams,D.A., Purcell,J.F.H., Cowen,R.K. and Hughes,C.R.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-MAR-2003) Biology, University of Miami, 1301
Memorial
            Drive, Coral Gables, FL 33146, USA
FEATURES             Location/Qualifiers
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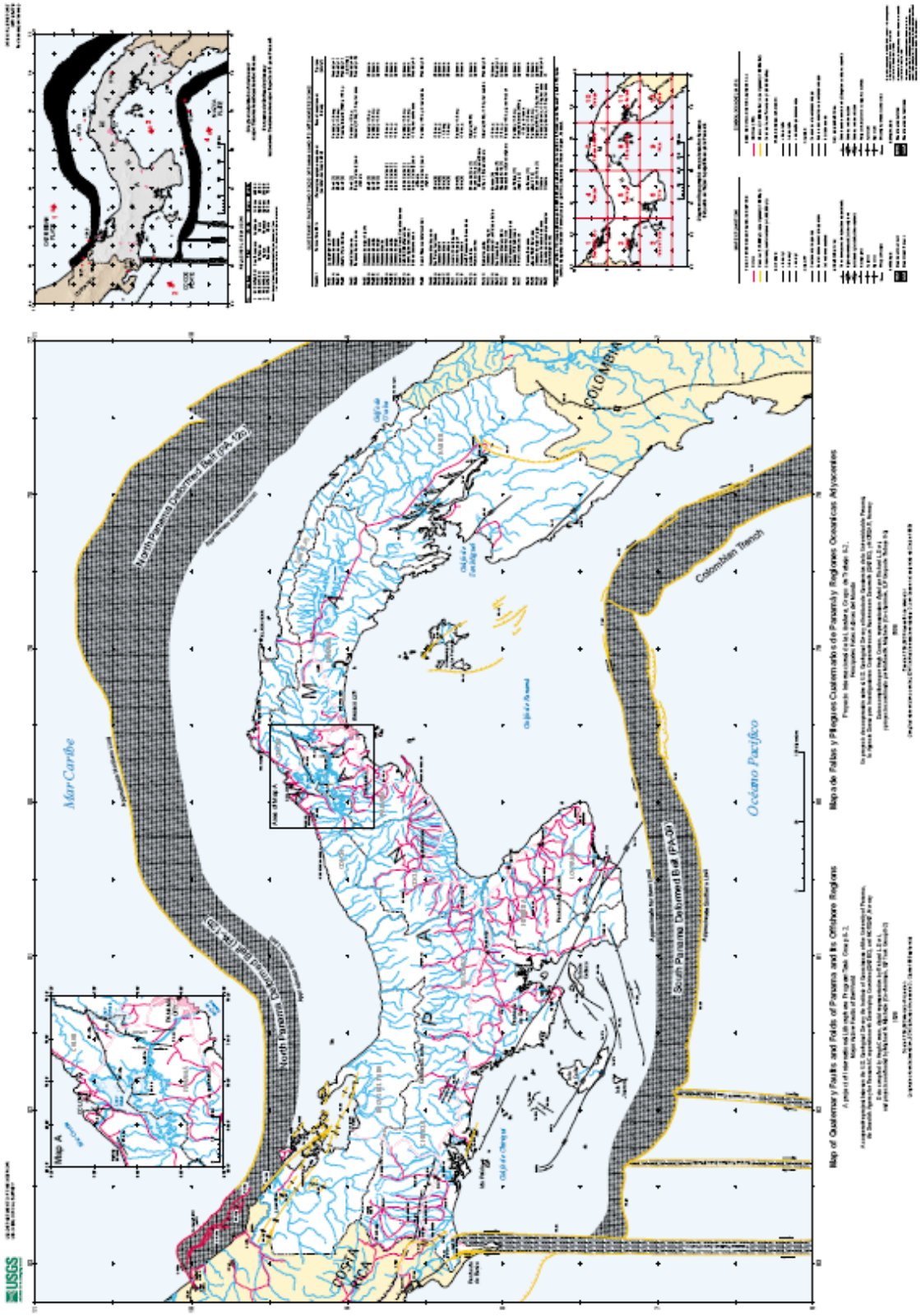
Appendix IV – USGS Survey map of Isthmus of Panama