CONSERVATION OF LIMPET POPULATIONS: A HEAVILY EXPLOITED RESOURCE IN AZORES, NE-ATLANTIC

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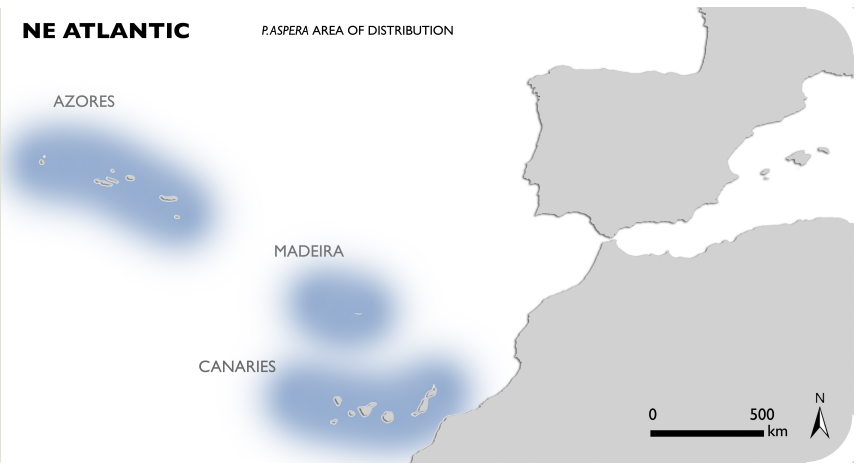
ABSTRACT Limpet harvesting in Azores (NE Atlantic) has been taking place probably since the islands were first colonized in the XV century. Limpet species are highly exploited and populations from most islands have declined steadily bringing about catastrophic effects on coastal communities. Despite their economic importance limpets are also ecologically key species and require prioritizing conservation strategies. Patellid limpets are broadcast spawners which go through a planktonic larval stage in their life cycle. They are benthic as adults and the larva is the only phase during their life-cycle which has the ability to disperse over assumed large spatial distances. However, there is now mounting evidence that gene flow between islands and mainlands can be low, even for species with a relatively long planktonic larval stage. Low levels of larval exchange may thus limit the success of conservation objectives expected upon migration and recruitment. In dispersive isolated oceanic islands such as the Macaronesian Islands, the Azores Archipelago in particular, is not clear whether limpet populations from different islands form a single meta-population or, in contrast, populations on each island are isolated from the rest. Knowledge on this scenario is crucial for the management and conservation of exploited populations of limpets. Here we have developed and described species-specific multiplexed microsatellite markers for the limpet *Patella aspera* using whole genome shotgun 454 sequencing. These genetic tools can allow the study of the population genetic structure and evolutionary history of patellid species in the archipelago of Azores. Genetic studies, alongside with biological, ecological and oceanographic information, represent an important contribution for the understanding of population dynamics by allowing testing hypothesis about larval dispersal patterns, recruitment and life history traits, population connectivity, genetic diversity, and population equilibrium .

MAIN OBJECTIVES

- Develop and describe novel microsatellite markers for *Patella aspera* (Röding, 1798)
- Use genetic, ecological, and oceanographic tools to address the connectivity patterns of limpet populations across the Macaronesian islands (NE-Atlantic)
- Gather information of theoretical and practical importance to be used for conservation strategies aimed at promoting the sustainable exploitation of limpets in these islands.

METHODS

- A total of 865 individuals of *P. aspera* was collected in the archipelagos of Azores (all islands), Madeira (one island) and Canaries (one island).
- Individuals were labelled and preserved in 96% ethanol.
- Genomic DNA was extracted from foot tissue using the E.Z.N.A. Mollusc DNA extraction kit following manufacturer's instructions.



Patella aspera (RÖDING, 1798)

Distribution: Azores, Madeira, Selvagens, the Canaries, and North of Africa.

Habitat: Rocky shores, lower intertidal and sublittoral zones.

Description: The conical shell is light in colour and structurally strong, exhibiting thick and irregular margins. The underside of the muscular foot is yellowish.

Reproduction: *P. aspera* is a protandrous species. Organisms start off as male but many change to female as they age. This means that there are two types of males, permanent males and temporary (protandric) ones.

MICROSATELLITE DEVELOPMENT WORKFLOW

- A DNA admixture from ten individuals (>10 μ g ml^{-1}) was used to develop **microsatellite-enriched libraries** (Genoscreen, France). Fragments from genomic DNA were enriched for SSR (single sequence repeats) content by using magnetic streptavidin beads and labelled TG, TC, AAC, AAG, AGG, ACG, ACAT and ACTC repeat oligonucleotides. High-throughput microsatellite isolation was made through **454 GS-FLX® Titanium pyrosequencing** of enriched libraries. QDD software was used to perform bioinformatic analyses.

Sequences (raw datas)	28992
Sequences containing microsatellites motifs	4754
Bioinformatic validated pairs of primers	**309**
Dinucleotides	88.7%
Trinucleotides	9.3%
Tetranucleotides	2.0%

- **40 pairs of primers** with high and close Tm, compatible allelic ranges and no mispriming, were tested for PCR amplification and annealing temperature. Only perfect motifs with simple repeats were selected (di-, tri- and tetra-nucleotides tandem repeats) and amplicon sizes varied from 90-315 bp.
- Selected primers were fluorescently labelled with **NED, VIC, PET, or 6-FAM**. A total of 20 individuals was used for polymorphism detection. Marker amplification took place on a MyCycler™ thermal cycler (BioRad) using a touchdown PCR protocol with Ta adjusted for each primer pair. Amplified fragments were visualized using an ABI 3730 (Applied Biosystems) automated DNA sequencer. Genotypes were scored using GeneMapper™ v.4.2 (Applied Biosystems).
- **17 polymorphic loci** were combined in three multiplex-PCR reactions and genetic diversity was determined for 152 individuals from 4 different islands of Azores. GENEPOP v4.0 and ARLEQUIN v3.4.1.2 were used to estimate genetic diversities, expected and observed heterozygosities, and to test for linkage disequilibrium and Hardy-Weinberg equilibrium (HWE).

Locus	Flurescent dye	Repeat	N_a	Allelic size range (bp)	H_e	H_o	F_{IS}
MIX_A							
ASP2	NED	aag	12	114-146	0.793	0.690	0.087
ASP3	VIC	tct	10	110-137	0.655	0.232	**0.676**
ASP21	PET	ag	12	166-188	0.777	0.564	0.107
ASP23	FAM	tca	16	166-211	0.847	0.781	0.029
ASP33	FAM	ag	16	233-259	0.779	0.427	**0.398**
ASP34	NED	ga	20	238-320	0.741	0.320	**0.524**
MIX_B							
ASP15	PET	ttc	39	153-234	0.965	0.472	0.505
ASP24	VIC	ga	26	160-214	0.939	0.772	0.218
ASP29	FAM	ct	23	178-254	0.852	0.372	**0.716**
ASP36	NED	ga	16	286-318	0.831	0.321	0.561
ASP38	FAM	ag	17	301-348	0.831	0.470	**0.523**
ASP39	PET	acc	6	330-346	0.521	0.107	**0.789**
MIX_C							
ASP7	PET	atc	11	130-157	0.538	0.475	0.086
ASP17	VIC	ag	16	148-176	0.827	0.581	**0.174**
ASP26	FAM	ag	8	198-214	0.706	0.602	0.191
ASP27	NED	tc	9	203-218	0.776	0.367	0.116
ASP40	VIC	ca	23	302-353	0.772	0.374	**0.336**

N_a = number of observed alleles per locus; sizes of amplified fragments; H_e = expected heterozygosity: H_o = observed heterozygosity. Significant values are in bold, after sequential Bonferroni correction (P<0.05)

- Overall, microsatellite loci were highly polymorphic, the number of detected alleles across all individuals ranged from 6 to 39.
- Mean expected heterozygosity (H_e) was 0.773 ± 0.029 (±SE), ranging from 0.521 to 0.965, and mean observed heterozygosity (H_o) was lower (0.466 ± 0.044).
- Eight loci deviated from HWE due to heterozygote deficiency and linkage disequilibrium was observed between ASP33 – ASP38 and ASP24 – ASP36, respectively.

CONCLUSIONS

- This study shows the usefulness of second-generation sequencing of microsatellite-enriched libraries to develop new microsatellites markers in non-model organisms.
- Conservation strategies in the NE Atlantic region should consider these novel markers to study the population structure of *P. aspera* throughout their distribution area so that future efforts should be focused on **identifying scales of connectivity and preserving stocks that have been severely depleted**.

ONGOING RESEARCH

- This study is part of a research project (www.patelgene.com). Results are expected to provide valuable information for the conservation of limpet species in the Macaronesia.
- A similar approach is also being used for the *P. candei* complex, a species showing a high level of morphological diversity among the Macaronesian archipelagos.
- Spatial and temporal patterns of limpet recruitment are also being examined in parallel by a PhD student.

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Conservation of limpet populations: a heavily exploited resource in Azores, NE-Atlantic

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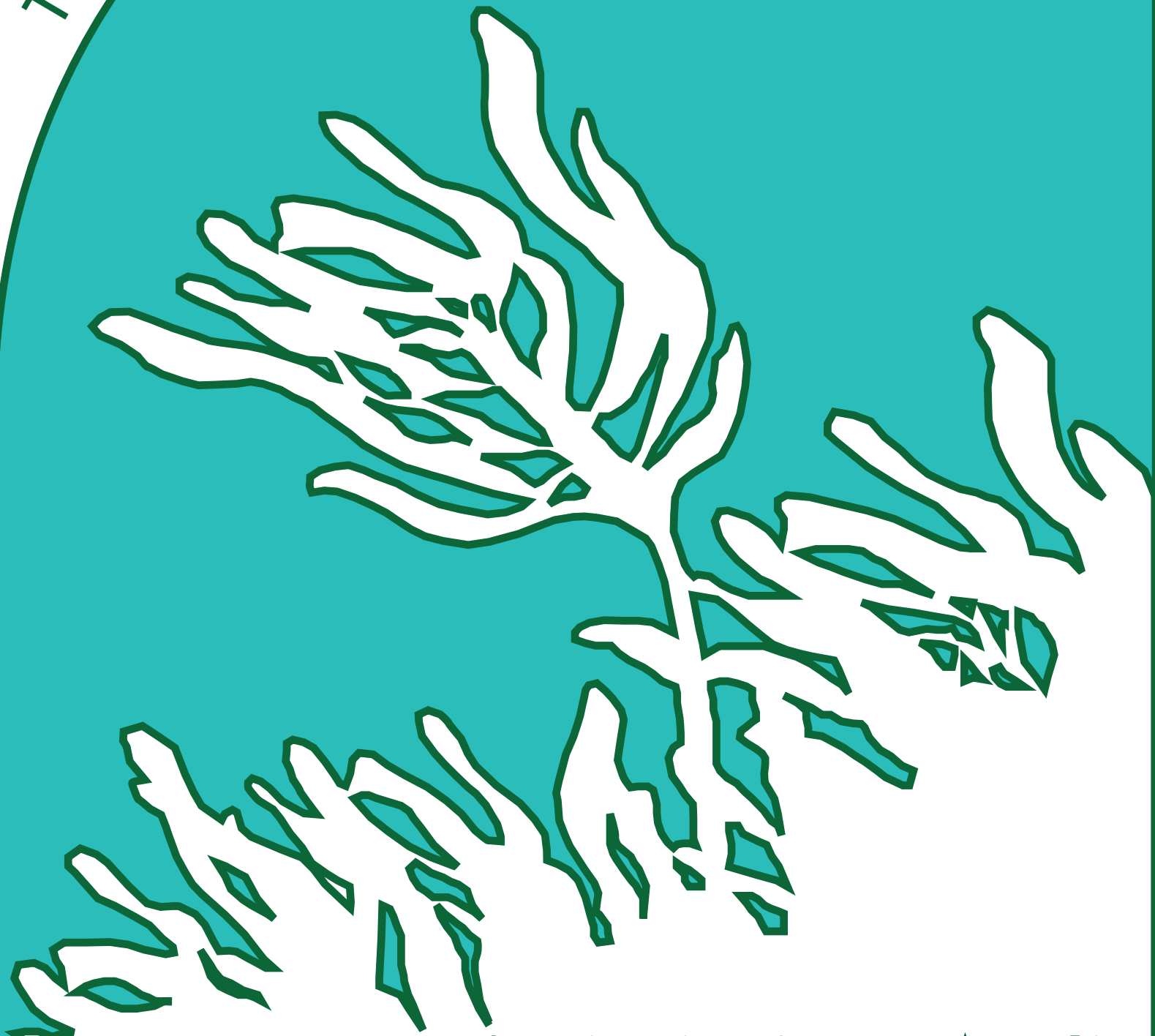
Key words: Gene flow, fisheries overexploitation, insular genetic disruption, marine ecosystems

POSTER PRESENTATIONS: Tuesday 14th January 1600 - 1900	Position
The big picture: imaging and mapping intertidal rocky shores with a remotely piloted aircraft. **Michael Burrows**, Scottish Association for Marine Science, UNITED KINGDOM	1
A low-cost, versatile data logging system for ecological applications. **Miguel Gandra**, CIBIO, Universidade do Porto, PORTUGAL	2
Seasonal variations of primary production and respiration of two rocky-shore communities dominated by canopy-forming algae, Fucus vesiculosus and Fucus serratus. **François Bordeyne**, UPMC - Station Biologique Roscoff, FRANCE	3
Facing the heat: does body orientation reduce desiccation and thermal stress in limpets? **Clarissa Fraser**, The University of Sydney, AUSTRALIA	4
Why live alone: Do sexually selected traits influence the spatial distribution and local population structure of amphipods? **Katherine Heldt**, The University of Adelaide, AUSTRALIA	5
The coupling between phytoplankton production and zooplankton grazing: An experimental study in brackish coastal shallow water areas. **Velda Lauringson**, University of Tartu, ESTONIA	6
Environmental correlates of isotopic variation in primary producers and consumers within a temperate coastal ecosystem. **Andrew Mackey**, Edith Cowan University, AUSTRALIA	7
Foraging movements of the herbivorous starfish Parvulastra exigua. **Aline Sbizera Martinez**, The University of Sydney, AUSTRALIA	8
Role of scavenging on growth, behaviour, and reproductive output of the common periwinkle (Littorina littorea). **Markus Molis**, Alfred-Wegener Institut, GERMANY	9
Mechanisms of nearshore retention and offshore exportation of mussel larvae over the Agulhas Bank, South Africa. **Nicolas Weidberg**, Rhodes University, SOUTH AFRICA	10
Epifaunal colonisation under the Busselton Jetty: Influence of substrate and light on epifaunal community structure. **Fionna Cosgrove**, Murdoch University, AUSTRALIA	11
Biology of the harlequin fish Othos dentex (Serranidae), with particular emphasis on sexual pattern and other reproductive characteristics. **Ben French**, Murdoch University, AUSTRALIA	12
Comparison of sight, sound and scent attractants for pelagic fishes to remote mid-water video stations. **Matthew Rees**, University of Wollongong, AUSTRALIA	13
Conservation of limpet populations: a heavily exploited resource in Azores, NE-Atlantic. **Joao Faria**, CIIMAR, Universidade do Porto, PORTUGAL	14
Influence of natural habitat fragmentation on barnacles and gastropods populations from subtropical rocky shores **Ronaldo Christofoletti,** Universidade de São Paulo, BRAZIL	15

10th International Temperate Reefs Symposium

Abstract Book



The University of Western Australia,
Perth, Australia, 12-17 January 2014

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We are delighted that you could join us in Perth, for what is sure to be another fantastic ITRS, showcasing the best temperate reef science from around the globe. This year's event is very special, as we celebrate the 10th ITRS, since its inception in 1990 and host over 190 delegates from 24 countries, making it one of the biggest ITRS' to date.

The 10th ITRS will be hosted by the University of Western Australia through the Oceans Institute and the School of Plant Biology. The organising committee would like to acknowledge the Traditional Owners of the land hosting our conference, the Nyoongar People. We would like to pay respect to their Elders both past and present and extend that respect to other indigenous Australians.

The overarching theme for the 10th ITRS is *Ecological Transitions*. This theme captures the intention to explore diverse spatial, temporal, environmental and biotic transitions in temperate reef ecosystems in an inclusive way. The theme also recognizes that a key challenge for the future is linking mechanistic ecology with approaches that address global questions.

Inside this book you will find a detailed program of the conference, abstracts from all the oral and poster presentations and contact details of all the delegates.

Please feel free to come and chat to any of the organising committee if you need information on anything throughout the week.

Sincerely,

Thomas Wernberg, Gary Kendrick, Scott Bennett, Thibaut de Bettignies, Paul Lavery, Mat Vanderklift
10th ITRS Organising Committee

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