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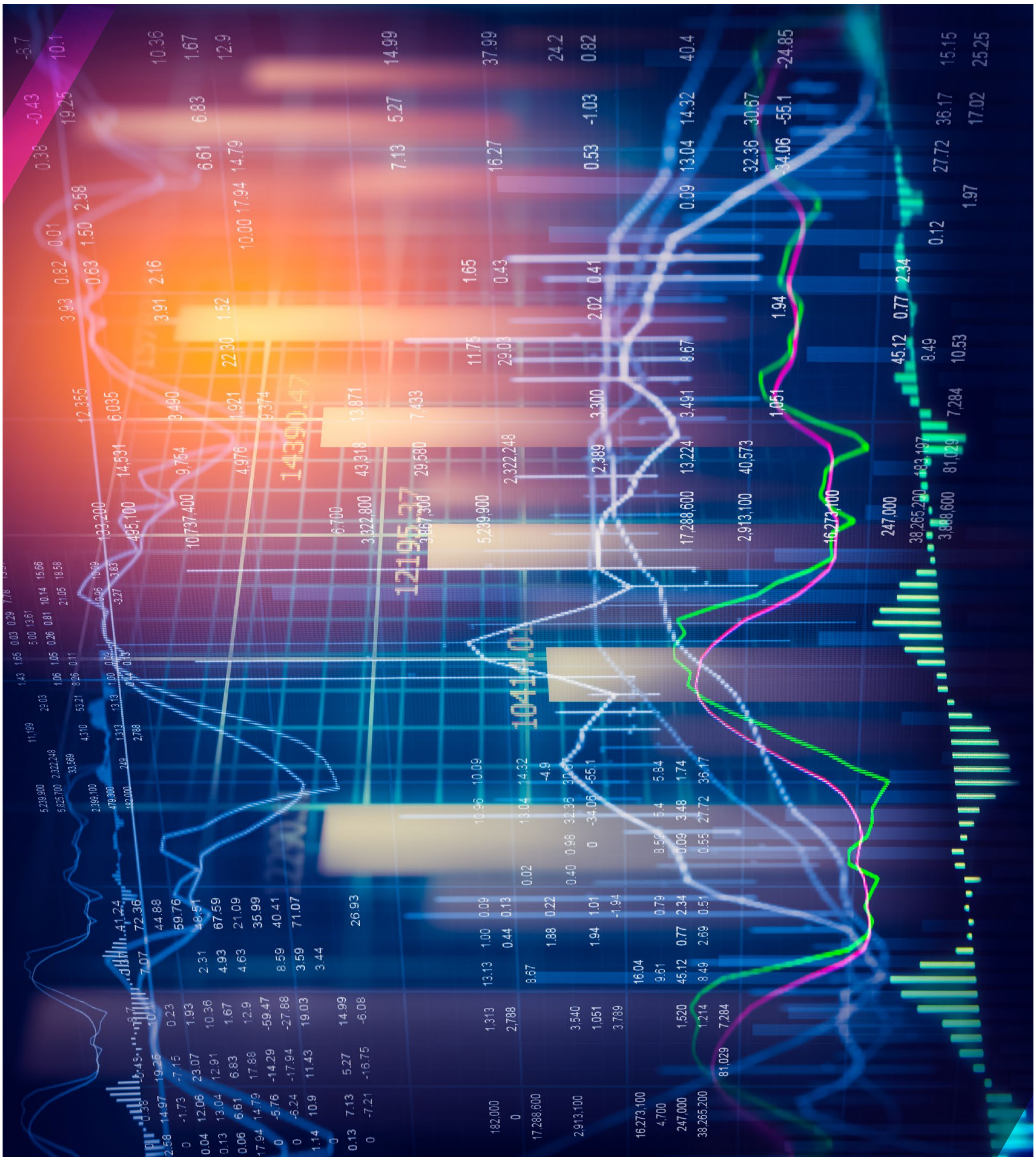

The University of Malta
Research, Innovation & Development Trust

Malta Chamber of Scientists

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Science Journal of the Malta Chamber of Scientists

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Xjenza Online - Science Journal of the Malta Chamber of Scientists
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Xjenza Online Vol. 6 Iss. 1 - 2018

2013–2017
Editor: Giuseppe Di Giovanni
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Xjenza Online Vol. 5 Iss. 2 - 2017
Xjenza Online Vol. 5 SI MNS Proceedings - 2017
Xjenza Online Vol. 5 Iss. 1 - 2017
Xjenza Online Vol. 5 Virtual Issue COST - 2017
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Xjenza Online Vol. 4 Iss. 1 - 2016
Xjenza Online Vol. 3 Iss. 2 - 2015
Associate Editors: *David Magri, Ian Thornton, Ian Cassar, Philip Farrugia, Sebastiano D'Amico, Nicholas Sammut, Joseph Galea, David Mifsud, Sandro Lanfranco, Mario Valentino, Godfrey Baldacchino, Liberato Camilleri*
Xjenza Online Vol. 3 Iss. 1 - August 2015
Xjenza Online Vol. 2 Iss. 2 - October 2014
Xjenza Online Vol. 2 Iss. 1 - March 2014
Xjenza Online Vol. 1 Iss. 2 - October 2013
Xjenza Online Vol. 1 Iss. 1 - March 2013

2003–2007
Editors: Joseph N. Grima and Richard Muscat
Xjenza Vol. 12 - 2007
Xjenza Vol. 11 - 2006
Xjenza Vol. 10 - 2005
Xjenza Vol. 9 - 2004
Xjenza Vol. 8 - 2003

1996–2002
Editor: Angela Xuereb
Associate Editor: *Richard Muscat*
Xjenza Vol. 7 - 2002
Xjenza Vol. 6 - 2001
Associate Editors: *Martin Ebejer and Richard Muscat*
Xjenza Vol. 5 - 2000
Xjenza Vol. 4 Iss. 2 - 1999
Xjenza Vol. 4 Iss. 1 - 1999
Associate Editors: *Martin Ebejer, Richard Muscat, and Christian A. Scerri*
Xjenza Vol. 3 Iss. 2 - 1998
Xjenza Vol. 3 Iss. 1 - 1998
Associate Editors: *Martin Ebejer, Richard Muscat, Christian A. Scerri and Emmanuel Sinagra*
Xjenza Vol. 2 Iss. 2 - 1997
Xjenza Vol. 2 Iss. 1 - 1997
Xjenza Vol. 1 Iss. 2 - 1996
Xjenza Vol. 1 Iss. 1 - 1996

Scope of Journal

Xjenza Online is the Science Journal of the Malta Chamber of Scientists and is published in an electronic format. Xjenza Online is a peer-reviewed, open access international journal. The scope of the journal encompasses research articles, original research reports, reviews, short communications and scientific commentaries in the fields of: mathematics, statistics, geology, engineering, computer science, social sciences, natural and earth sciences, technological sciences, linguistics, industrial, nanotechnology, biology, chemistry, physics, zoology, medical studies, electronics and all other applied and theoretical aspect of science.

The first printed issue of the journal was published in 1996 and the last (Vol. 12) in 2007. The publication of Xjenza was then ceased until 2013 when a new editorial board was formed with internationally recognised scientists, and Xjenza was relaunched as an online journal, with two issues being produced every year. One of the aims of Xjenza, besides highlighting the exciting research being performed nationally and internationally by Maltese scholars, is to provide a launching platform into scientific publishing for a wide scope of potential authors, including students and young researchers, into scientific publishing in a peer-reviewed environment.

Instructions for Authors

Xjenza is the Science Journal of the Malta Chamber of Scientists and is published by the Chamber in electronic format on the website: http://www.mcs.org.mt/index.php/xjenza. Xjenza will consider manuscripts for publication on a wide variety of scientific topics in the following categories

1. Research Articles
2. Communications
3. Review Articles
4. Notes
5. Research Reports
6. Commentaries
7. News and Views
8. Invited Articles and Special Issues
9. Errata

Research Articles form the main category of scientific papers submitted to Xjenza. The same standards of scientific content and quality that applies to Communications also apply to Research Articles.

Communications are short peer-reviewed research articles (limited to three journal pages) that describe new important results meriting urgent publication. These are often followed by a full Research Article.

Review Articles describe work of interest to the wide community of readers of Xjenza. They should provide an in-depth understanding of significant topics in the sciences and a critical discussion of the existing state of knowledge on a topic based on primary literature sources. Review Articles should not normally exceed 6000 words.Authors are strongly advised to contact the Editorial Board before writing a Review.

Notes are fully referenced, peer-reviewed short articles limited to three journal pages that describe new theories, concepts and developments made by the authors in any branch of science and technology. Notes need not contain results from experimental or simulation work.

Research Reports are extended reports describing research of interest to a wide scientific audience characteristic of Xjenza. Please contact the editor to discuss the suitability of topics for Research Reports.

Commentaries: Upon Editor's invitation, commentaries discuss a paper published in a specific issue and should set the problems addressed by the paper in the wider context of the field. Proposals for Commentaries may be submitted; however, in this case authors should only send an outline of the proposed paper for initial consideration. The contents of the commentaries should follow the following set of rules: 3000 words maximum, title 20 words maximum, references 10 maximum (including the article discussed) and figures/tables 2 maximum.

News and Views: The News section provides a space for articles up to three journal pages in length describing leading developments in any field of science and technology or for reporting items such as conference reports. The Editor reserves the right to modify or reject articles for consideration as News.

Invited Articles and Special Issues: Xjenza regularly publishes Invited Articles and Special Issues that consist of articles written at the invitation of the Editor or another member of the editorial board.

Errata: Xjenza also publishes errata, in which authors correct significant errors of substance in their published manuscripts. The title should read: Erratum: "Original title" by ***, Xjenza, vol. *** (year). Errata should be short and consistent for clarity.

Submission of Manuscripts

Manuscripts should be sent according to the guidelines given hereafter to xjenza@mcs.org.mt.

Referees All manuscripts submitted to Xjenza are peer reviewed. Authors are requested to submit with their manuscript the names and addresses of three referees, preferably from overseas. Every effort will be made to use the recommended reviewers; however the editor reserves the right to also consult other competent reviewers.

Conflict of Interest Authors are expected to disclose any commercial or other types of associations that may pose a conflict of interest in connection to with the submitted manuscript. All funding sources supporting the work, and institutional or corporate affiliations of the authors, should be acknowledged on the title page or at the end of the article.

Policy and Ethics The work presented in the submitted manuscript must have been carried out in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans (`http://www.wma.net/en/30publications/10policies/b3/index.html`); EU Directive 2010/63/EU for animal experiments (`http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm`); Uniform Requirements for manuscripts submitted to Biomedical journals (`http://www.icmje.org`). This must be stated at an appropriate point in the article.

Submission, Declaration and Verification Author(s) must only submit work that has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis), that is not under consideration for publication elsewhere, that has been approved for publication by all authors, and tacitly or explicitly, by the responsible authorities where the work was carried out, and that, if accepted, will not be published elsewhere in the same form, in English or in any other language, including electronically, without the written consent of the copyright-holder.

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Preparation of Manuscripts

Xjenza accepts submissions in MS Word, Libre Office Writer and LaTeX, the latter being the preferred option. Anyone submitting in LaTeX should use the journal template, the latest version of which can be found at `http://github.com/hicklin/Xjenza-Journal-Template`. All the necessary files to run the LaTeX document should be supplied together with the rendered PDF.

If a word processor is used the styling should be kept to a minimum. Bold face and italic fonts, as well as subscript and superscript text may be used as required by the context. Text should be in single-column format and the word processor options should not be used in order to justify text or hyphenate words. Alongside the native format of the word processer, a PDF file, generated by the word processor, must be provided. Furthermore, artwork should be in accordance with the artwork guidelines given below and must be submitted separately from the word processor file. Similarly, the bibliographic data of the cited material should be submitted separately as an Endnote (*.xml), Research Information Systems (*.ris), Zotero Library (zotero.splite) or a BibTeX (*.bib) file.

Article Structure

A manuscript for publication in Xjenza will typicall have the following components: Title page, Abstract, Keywords, Abbreviations, Introduction, Materials and Methods, Results, Discussion, Conclusions, Appendices and References.

The manuscript will be divided into clearly defined and numbered sections. Each numbered subsection should have a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when cross-referencing text, i.e. refer to the subsection by the section number.

Title page

- The title should be concise yet informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.
- Author names and affiliations. Indicate the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript number immediately after each author's name and in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address.
- Corresponding author. Clearly indicate who will handle correspondence at all stages of refereeing and publication, including post-publication. Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and complete postal address. Contact details must be kept up to date by the corresponding author.
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Abstract A concise and factual abstract is required of up to about 250 words. The abstract should state briefly the background and purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, references and non-standard abbreviations should be avoided. If essential, these must be defined at first mention in the abstract itself.

Abbreviations Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention as well as in the footnote and should be used consistently throughout the text.

Introduction State the objectives of the work and provide an adequate background, avoid a detailed literature survey or a summary of the results.

Materials and Methods Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

Results Results should be clear and concise. Numbered/tabulated information and/or figures should also be included.

Discussion This should explore the significance of the results of the work, yet not repeat them. Avoid extensive citations and discussion of published literature. A combined section of Results and Discussion is often appropriate.

Conclusions The main conclusions based on results of the study may be presented in a short Conclusions section. This may stand alone or form a subsection of a Discussion or Results and Discussion section.

Appendices Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

Acknowledgements Collate acknowledgements in a separate section at the end of the article before the references. Do not include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided assistance during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Units Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI. Anyone using LaTeX should use the package siunitx in all cases.

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Table Footnotes Indicate each footnote in a table with a superscript lower case letter.

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Citations and References Reference and citation styles for manuscripts submitted to Xjenza should be in accordance to the APA v6 style.

Citation in text References to cited literature in the text should be given in the form of an author's surname and the year of publication of the paper with the addition of a letter for references to several publications of the author in the same year. For further information regarding multiple authors consult the APA v6 guidelines. Citations may be made directly

> Kramer et al. (2010) have recently shown ...

or parenthetically

> as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999).

Groups of references should be listed first alphabetically, then chronologically. When writing in LaTeX use `\textcite{}` and `\parencite{}` for the respective cases mentioned.

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McCreadie, C. and Tinker, A. (2005). The acceptability of assistive technology to older people. *Ageing Soc.*, 25(1):91–110.

Reference to a Book:

Brownsell, B. (2003). *Assistive Technology and Telecare: Forging Solutions for Independent Living.* Policy Press, Bristol.

Fisk, M. J. (2003). *Social Alarms to Telecare: Older People's Services in Transition.* Policy Press, Bristol, 1st edition.

Reference to a Chapter in an Edited Book:

Brownsell, S. and Bradley, D. (2003). New Generations of Telecare Equipment. In *Assist. Technol. Telecare Forg. Solut. Indep. Living*, pages 39–50.

Web references The full URL should be given together with the date the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately or can be included in the reference list.

References in a Special Issue Please ensure that the words 'this issue' are added to any references in the list (and any citations in the text) to other articles in the same Special Issue.

Journal Abbreviations Journal names should be abbreviated according to:

-Index Medicus journal abbreviations: `http://www.nlm.nih.gov/tsd/serials/lji.html`;

-List of title word abbreviations: `http://www.issn.org/2-22661-LTWA-online.php`;

-CAS (Chemical Abstracts Service): `http://www.cas.org/sent.html`.

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The following list will be useful during the final checking of a manuscript prior to sending it to the journal for review. Please consult the Author Guidelines for further details of any item.

- One author has been designated as the corresponding author with contact details:
  - E-mail address.
  - Full postal address.
  - Telephone and fax numbers.
- All necessary files have been sent, and contain:
  - All figures are given separately in PDF, SVG, JPEG of PNG format.
  - Caption for figures is included at the end of the text.
  - All tables (including title, description, footnotes) are included in the text and large tables have been given separately as CSV.
  - The reference list has been given in XML, RIS, zotero.splite or BIB file format.
- Further considerations

- Abstract does not exceed about 250 words.
- Manuscript has been ‘spell-checked’ and ‘grammar-checked’.
- References are in the required format.
- All references mentioned in the reference list are cited in the text, and vice versa.
- Bibliographic data for all cited material has been provided.
- Permission has been obtained for use of copyrighted material from other sources (including the Web).
- A PDF document generated from the word processor used is submitted.

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Sebu, C. (2018). *Xjenza Online*, 6:68–69.

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Editorial

Cristiana Sebu*
Department of Mathematics, University of Malta, Msida, Malta

Dear readers of Xjenza Online, as Editor, I am pleased to see continuous progress in the journal's quality, visibility, availability and readership. Over the years, the journal has evolved alongside the research community it serves. Since the first printed issue of the Xjenza in 1996, the journal has witnessed the progress of scientific effort across many disciplines. The Maltese islands are now the home to a rich and exciting scientific research as highlighted in this current issue.

As today's research is a global endeavour, so is our focus, both in the original research we publish and in our news coverage.

The issue opens with an article co-authored by Ian P. Cassar, one of the most active and enthusiastic promoters of Xjenza Online and a reliable Associate Editor since the relaunch of the journal in 2013. The authors, Ian P. Cassar (University of Malta) and Noel Rapa (Central Bank of Malta), derive the estimates of industry specific multipliers (i.e. output, value added, income and employment multipliers) using the data on Maltese economy's inter-industry transactions recorded in 2010 and published by the National Statistics Office of Malta in 2016. The study demonstrates that the activities related to accommodation and food services feature in the top 15 in almost all categories of multipliers under consideration, confirming the historic tendency for the tourism sector to feature as a key economic driver in Malta.

The following manuscript by Bonello et al. presents a review of studies investigating the dielectric properties of biological tissues for application in hyperthermia and microwave thermal ablation which are important treating modalities for a number of diseases, most notably tumours.

Next, it is a great honour for Xjenza Online to feature an extended view on an action observation and execution network by Professor Giacomo Rizzolatti and his collaborators Rozzi Stefano and Gerbella Marzio. Professor Giacomo Rizzolatti is Professor Emeritus in Physiology at the University of Parma, an Affiliate Professor at the University of Malta, Dept. of Physiology and Biochemistry and Director of the Social and Motor Cognition Centre at the Italian Institute of Technology. Award winner and world-renowned neuroscientist, Professor Giacomo Rizzolatti received the 'Brain Prize' in 2014, a prestigious prize for brain research bestowed by the Grete Lundbeck European Brain Research Prize Foundation in Copenhagen. The study surprisingly reveals that although traditionally the motor nervous system was not thought to be involved in cognitive functions, there is now strong evidence to support that numerous cognitive functions, such as space perception as well as action and emotion recognition, not only require the activity of the motor system, but are also deeply embedded in it.

The paper by Maria Desira and Gillian M. Martin explores the level of awareness of the Maltese general public in relation to the existence and use of biobanks resources for biomedical and genomic research. The results show that the vast majority of the public does not know what the term 'biobank' means, and does not associate the term with biomedical or genomic research. Thus, there is great scope for increasing science communication and public awareness in relation to this type of research.

Then, Fenech et al. presents the results of a study on the biodiversity and ecology of biofilm and biomat forming organisms growing in different microhabitats around the Maltese islands. The effects of changes in environmental parameters on two of these microorganisms, a cyanobacterium and a microalga, were found to be strain specific. The results suggest that some of the strains of such microorganisms could be used as early indicators of climate change in particular habitats.

The research article by William Gatt from Central Bank of Malta presents the estimates of the Philips

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curve in the Maltese economy using data starting from mid-1960s. The analysis shows that over time the link between inflation and economic activity has weakened in Malta which is consistent with findings from other countries, and that there is an increase in the sensitivity of domestic inflation to import price shocks, owing to the increased openness and trade integration of the Maltese economy. The estimated variance of shocks to inflation was rather high in the 1980s, but has fallen greatly since then, peaking again only during the energy and food price shocks of 2007.

The last article by Bonnici et al. reviews the development of the sympathetic system of the heart and attempts to give insights into the possible explanations of referred pain distribution in clinical cardiology.

The issue concludes with a news article which reports on the success of the 36th European Seismological Commission General Assembly which was hosted by the Department of Geosciences of the University of Malta and took place in Malta in September 2–7, 2018, at the Mediterranean Conference Centre, Valletta.

As you can see from above, Xjenza Online publishes manuscripts in one of many formats including short communications, research articles, review articles and original research reports featuring a wide range of applied and theoretical sciences of both local and international relevance. The Editorial Board is making great efforts to meet the indexing requirements set by internationally leading databases. However, the main goals of the coverage remain the same: to serve the local professional scientific community, to publish high-quality original findings in a peer-reviewed environment, and to help early-career researchers to advance their scientific discourse in the community.

Cassar, I. P. and Rapa, N. (2018). *Xjenza Online*, 6:70–85.

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DOI: 10.7423/XJENZA.2018.2.01

Research Article

Estimates of Input-Output Multipliers for the Maltese Economy Based on the Symmetric Input-Output Table for 2010

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Abstract. The study presents the estimates of industry specific multipliers which are derived utilizing a highly disaggregated symmetric input-output table for the Maltese Economy for 2010, published by the National Statistics Office of Malta in 2016. The aim of this study is to derive and analyse a set of output, value added, income and employment multipliers, which illustrate how an exogenous shock to the final demand of each industry would affect the production activities of the Maltese economy on the basis of the Leontief demand driven model. Both the open, as well as the closed Leontief demand driven model are utilized in order to derive the simple and total multipliers. The study presents a comparative analysis of the direct, indirect and induced multiplier effects in terms of the output, value added, income and employment generation for each industry within the Maltese economy. Although there is a considerable degree of heterogeneity in the results obtained across the various multiplier measures, when considering the complete set of multiplier results, a number of industries do feature as relatively strong performers across all categories of multipliers. The accommodation and food services activities feature in the top 15 in almost all categories of multipliers under consideration, confirming the historic tendency for the tourism sector to feature as a key important economic driver. The sectors associated with the public sector, more specifically the public administration, education and human health sectors, were also found to consistently exhibit relatively strong multiplier effects.

Keywords: Input-output analysis, Leontief demand driven model multipliers, Maltese Economy

1 Introduction

This study presents the derivation and analysis of both simple and total output (production), income (income-output), value added (value added-output) and employment (employment-output) multipliers for the Maltese economy. These estimates are based on the symmetric input-output table (SIOT) for 2010 published by the National Statistics Office (NSO) of Malta in May 2016, which adheres to Eurostat System of National and Regional Accounts 2010 (Eurostat, 2013). The estimates derived in this study will allow for a deeper undertaking of underlying inter-industry linkages, which characterize the production structure and are of aid to policy makers during the process of identifying potential strategies for economic development. These multiplier estimates are underpinned by the interrelations between production sectors, and their strength or weakness strongly influences sectoral growth, which in turn has a significant impact on the overall economic activity of the country (Hirschman, 1958). The analysis conducted within this study is based on the input-output methodology originally put forward by the Nobel prize winning economist Wassily Leontief (Leontief, 1941). Over the years, input-output analysis has grown into one of the most widely accepted methods of economic planning and decision making (Baumol, 2000). It should however be noted that only a limited number of studies have been undertaken which apply input-output analysis to the Maltese economy. These can be subdivided into two main categories. The first category relating to studies which aim to shed light on the production structure of the Maltese economy by analysing inter-industry linkages, either via the derivation of industry specific multipliers (Cassar, 2015; Gravino, 2012), or via the

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application of hypothetical extraction analysis (Cassar, 2017), whilst studies within the second category aim to assess the economic impact or contribution of tourism (Briguglio, 1992; Blake, Sinclair, Sugiyarto & DeHann, 2003; Sinclair, Blake, Gooroochurn & DeHann, 2005; Cassar, Vella & Buttigieg, 2016).

2 Data and Methodology

The application of input-output techniques requires the utilization of Symmetric Input-Output Table (SIOT) as its main dataset. An SIOT is an observed dataset illustrating the inter-industry transactions for a specific geographic region, measured for a particular time period (usually one year), and which is generally recorded in monetary terms. The multipliers derived in this analysis are based on an industry-by-industry SIOT, in million Euro, for the reference year of 2010, published by the NSO (National Statistics Office of Malta (NSO), 2016). The SIOT employed in this study for Malta for the reference year of 2010, kindly provided by the NSO, has a 40 industry level of disaggregation and to a great extent follows the classification according to the European Statistical Classification of Economic Activates (NACE) Rev.2. A SIOT records the economy's inter-industry transactions via the disaggregation of the economic activity into *'n'* sectors or industries representing the various producing sectors of the economy. The core data required to populate the Leontief demand driven model consists of the flow of products from each of the *'n'* producing sectors, to each of the *'n'* sectors purchasing input requirements in order to undertake the production of output. The flow of products amongst the *'n'* producing sectors of the economy is what is referred to as inter-industry flows (or transactions). The input-output table is therefore a data-set which essentially traces the monetary values of the numerous transactions amongst the pairs of sectors (for each sector *'i'* to each sector *'j'*) for a given year.

This section shall proceed with a description of the methodology, pertaining to the derivation of the open Leontief demand driven model, from which it is possible to obtain what are referred to as simple output multipliers. The term simple is employed given that they reflect only direct and indirect effects on production caused by exogenous changes to final demand. This is because they omit the notion that increased production requires more labour input, which in turn increases household income, which further increases demand and consequently production. These so-called induced effects can be internalised through the derivation of the closed Leontief model, which endogenizes household behaviour within the input-output system (Cassar, 2015). The multipliers derived from the closed Leontief demand driven model also capture the inter-relationships between revenue, income, and expenditure flows made by households and the productive sector. Hence, the resultant multipliers, also known as total multipliers, include the induced effects relating to the additional impact on domestic production caused by the demand for goods and services made by households. Such demands are induced through the additional income which is obtained via the production of the new output, originally associated with the initial exogenous shock to final demand.

The open Leontief demand driven model applied within this study follows the methodology presented within Miller and Blair (2009) and employed in Cassar (2015). At the core of the Leontief demand driven model is the concept of technical coefficients denoted by 'a'_{ij}

$$a_{ij} = \frac{z_{ij}}{x_j}, \qquad i, j = 1, \ldots, n. \tag{1}$$

Technical coefficients may thus be derived from the ratio of the inputs produced by sector i purchased by industry j, z_{ij}, to the total input used by sector j, x_j. For each industry in the economy, technical coefficients show the proportional value of inputs purchased from all sectors in the economy (including itself) per monetary unit of output. On the basis of this definition of the representation of technical coefficients within the input-output system we can now derive, utilizing matrix algebra notation, a matrix of technical coefficients as follows:

$$A = Z\hat{x}^{-1}, \tag{2}$$

where Z denotes an $(n \times n)$ matrix of inter-industry flows, x denotes an $(n \times 1)$ column vector of output such that $\hat{x}^{-1}$ represents the inverse of the diagonal matrix obtained from vector x, and A represents the matrix of technical coefficients. The technical coefficients matrix is viewed as a means with which to analyse the direct inter-industry linkages that tie the economy together, since for each individual sector it shows the direct effects on output, in terms of the additional demand for input requirements that is generated by an increase in the value of output by one monetary unit.

The solution to the Leontief demand driven model may be obtained by applying the following equation

$$x = (I - A)^{-1} f = Lf. \tag{3}$$

The solution to the input-output system implies that, for a given Leontief Inverse, the amount of total output x produced in the economy is determined solely by the structure of final demand, f, where f is a column vector $(n \times 1)$ of total final demand absorbed by each of the n sectors in the economy.

$$L = (I - A)^{-1} = [l_{ij}]. \tag{4}$$

The Leontief inverse incorporates the concept that the production process required to produce one unit of output for use by final demand, does not only entail an initial or direct output increase, but also requires the production of output by other industries for use as intermediate inputs. Furthermore, the production of these additional intermediate inputs requires subsequent increased rounds of production, since output has to be produced to satisfy the second round of input requirements. All these rounds of additional increases in output are referred to as the indirect effects of an exogenous increase in final demand on total output production.

The derivation of total output multipliers requires solving the Leontief demand driven model for the household augmented technical coefficients matrix, which would generate a Leontief inverse matrix of dimension $(n+1) \times (n+1)$, in which each element would now capture the direct and indirect, as well as the induced effects in output production caused by an increase in exogenous final demand (Miller & Blair, 2009)

$$\tilde{x} = \left(I_{n+1} - \tilde{A}\right)^{-1} f, \tag{5}$$

such that the corresponding Leontief inverse matrix for the closed Leontief model is specified as

$$\tilde{L} = \left(I_{n+1} - \tilde{A}\right)^{-1} = \left[\tilde{l}_{ij}\right], \tag{6}$$

Following Eq. (7), the simple output multipliers (O^S) which capture solely the direct and indirect effects, can be derived via the summation of the column elements of the Leontief inverse matrix derived in Eq. (4)

$$O^S(j) = \sum_{i=1}^{n} l_{ij}. \tag{7}$$

A simple output multiplier for a given sector *'j'* may be defined as the total value of production in all sectors of the economy that is necessary in order to satisfy a 1 Euro worth of final demand for sector *'j'*s*'* output. In other words, the output multiplier measure captures the total sum of direct and indirect input requirements from all sectors, needed to supply 1 Euro worth of sector *'j'*s*'* output to final demand.

Total output multipliers (O^T) are derived via the summation of the column elements of the truncated household endogenized Leontief inverse matrix derived in Eq. (6). The term truncated refers to the fact that the final row $(n+1)$, as well as the final column $(n+1)$ of the Leontief inverse, are not included within the calculation of the total output (production) multipliers. The total output multipliers which account for the direct, indirect and the induced effects that a 1 Euro increase in final demand of a specific industry will have on overall output production, are derived as follows in Eq. (8)

$$O^T(j) = \sum_{i=1}^{n} \tilde{l}_{ij}. \tag{8}$$

Output multipliers illustrate where increases in final demand could have the largest impact in terms of Euro of output production generated throughout the economy. However, policy makers may be more concerned with the impact that additional spending may have in terms of increased value added, jobs created, or additional household income generated. This study shall therefore derive and assess the resultant income-output, value added-output and physical employment-output multipliers for the Maltese economy based on the SIOT for the reference year of 2010. These multiplier measures directly convert the total Euro value of new final demand expenditure into the respective additional value added, income generated and new employment created.

The derivation of these multipliers requires the computation of the respective row vectors of value added input coefficients, labour input coefficients and employment output ratios. The elements of these row vectors are obtained following Eqs. (9)–(11)

$$a_{va,i} = \frac{va_j}{x_j}, \tag{9}$$

$$a_{inc,i} = \frac{inc_j}{x_j}, \tag{10}$$

$$a_{emp,i} = \frac{emp_j}{x_j}. \tag{11}$$

The value added input coefficients and the labour input coefficients reflect the amount of value added, or income generated per euro of output (production) for each sector in the economy, whilst the employment-output ratio illustrates the number of (average) jobs (for the reference year of 2010) per million Euro of output produced for each sector in the economy. The computed three coefficients/ratios, which are presented in Appendix A, reflect the direct effects in terms of the additional amount of value added, as well as the income and jobs created by each sector in response to a marginal increase in production.

The simple and total value added, income and employment multipliers for each sector in the economy are thereafter derived respectively following Eqs. (12)–(14)

$$VA^S(j) = \sum_{i=1}^{n} a_{va,i} l_{ij}, \qquad VA^T(j) = \sum_{i=1}^{n} a_{va,i} \tilde{l}_{ij}, \tag{12}$$

$$INC^S(j) = \sum_{i=1}^{n} a_{inc,i} l_{ij}, \qquad INC^T(j) = \sum_{i=1}^{n} a_{inc,i} \tilde{l}_{ij}, \tag{13}$$

$$EMP^S(j) = \sum_{i=1}^{n} a_{emp,i} l_{ij}, \quad EMP^T(j) = \sum_{i=1}^{n} a_{emp,i} \tilde{l}_{ij}. \tag{14}$$

The simple value added, income and employment multipliers are obtained by multiplying the respective vectors of the derived value added input coefficients, labour input coefficients and employment output ratios, with the Leontief inverse obtained from the open Leontief demand driven model. The simple value added and income multipliers illustrate the increase, in terms of additional value added and income generated throughout the economy, in response to a one Euro increase in final demand for the output of sector 'j', taking into account all of the direct and indirect effects on the production. $VA^S(j)$ and $INC^S(j)$ represent the simple value added multiplier and simple income multiplier for sector 'j', respectively. The simple employment multiplier for each section 'j', denoted by $EMP^S(j)$, measures the additional physical employment that is generated by the direct and indirect effects on production, due to an additional million Euro worth of final demand for sector 'j'.

The total value added, income and employment multipliers are similarly derived by multiplying the respective vectors of the derived value added input coefficients, labour input coefficients and employment output ratios with the truncated Leontief inverse, obtained from the closed Leontief demand driven model. The total value added and total income multipliers denoted by $VA^T(j)$ and $INC^T(j)$, for sector 'j', illustrate the increase, in terms of additional value added and additional income generated throughout the economy, in response to an additional Euro of final demand for the output of sector 'j' taking into account not only the direct and indirect effects, but also the additional induced effects on production which are caused by the increase in the demand for goods and services made by households, induced through the additional income generated as a result of the various rounds of production activities across the economy needed to satisfy the increase in the final demand for sector 'j'. Similarly, the total employment multiplier for a sector 'j', denoted by $EMP^T(j)$, measures the additional physical employment that is generated by the direct, indirect, as well as the induced effects on production, due to an additional million Euro worth of final demand for sector 'j'.

It is also possible to decompose the direct, indirect and induced effects for each of the three total multipliers just discussed, namely, the value added, income and employment total multipliers. Since the value added input coefficient, labour input coefficient and employment output ratio for each sector 'j' denote the direct effects generated in response to a one Euro increase in final demand for the sector, the separate indirect effects may be obtained as the difference between the simple multiplier, incorporating both the direct and indirect effects, with the corresponding coefficient/ratio. Similarly, the separate induced effects, caused by the added household endogeneity, may be derived as the difference between the simple and the total multipliers.

3 Results

Table 1 lists the 15 highest ranking industries in terms of their output simple multipliers. The main factor affecting the magnitude of simple multipliers is the relative share of primary inputs in the total output of each sector. The higher the share of imports, labour compensation and gross operating surplus for each sector, the higher the leakages from the domestic inter-industry system, implying a lower simple multiplier. With a multiplier of 2.09, the industry with the largest simple multiplier is (26): Other professional, scientific and technical activities, followed by (12): Electricity, gas, steam and air conditioning supply, water collection, treatment and supply, with a multiplier of 1.95. The highest ranking manufacturing sector is (7): Manufacture of other non-metallic products, with a multiplier of 1.79. This is followed by industry (13): Mining, quarrying and construction, while sector (18) Accommodation and food services activities ranks 8th, with a simple output multiplier of 1.90. Sector (14): Wholesale and retail trade ranks 13th, with an output multiplier of 1.53.

Fig. 1[1] portrays the 15 highest ranking industries in terms of total output multipliers, as well as the disaggregation between direct, indirect and induced effects. The highest ranking industry is (28): Employment activities, with a total output multiplier of 2.91. Thus, a one Euro exogenous increase in the final demand of employment activities generates an average total production of Euro 2.91, out of which only Euro 0.17 are direct effects, Euro 1.07 are indirect effects and Euro 1.67 are due to induced effects generated through the consumption patterns of households. The high ranking of this relatively small industry is in line with results published in Cassar (2015) using symmetric input output tables for 2008 consistent with ESA 1995 data.

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[1] The simple and total output multipliers for all 40 sectors are presented in Appendix B.

Table 1: Top 15 simple output multipliers.

No.	Industry	Simple Output Multiplier
26	Other professional, scientific and technical activities...	2.09
12	Electricity, gas, steam and air conditioning supply, ...	1.95
29	Travel agency, tour operator reservation service...	1.81
36	Sports activities and amusement and recreation activities	1.79
7	Manufacture of other non-metallic mineral products	1.79
13	Mining and quarrying and construction	1.75
25	Architectural and engineering activities; ...	1.73
18	Accommodation and food service activities	1.70
17	Land transport and transport via pipelines, ...	1.63
1	Crop and animal production, hunting and related service...	1.58
27	Rental and leasing activities	1.54
3	Manufacture of food products, beverages and tobacco...	1.53
14	Wholesale and retail trade and repair of motor vehicles...	1.52
16	Retail trade, except of motor vehicles and motorcycles	1.52
39	Other personal service activities	1.51

Source: Authors' own calculations.

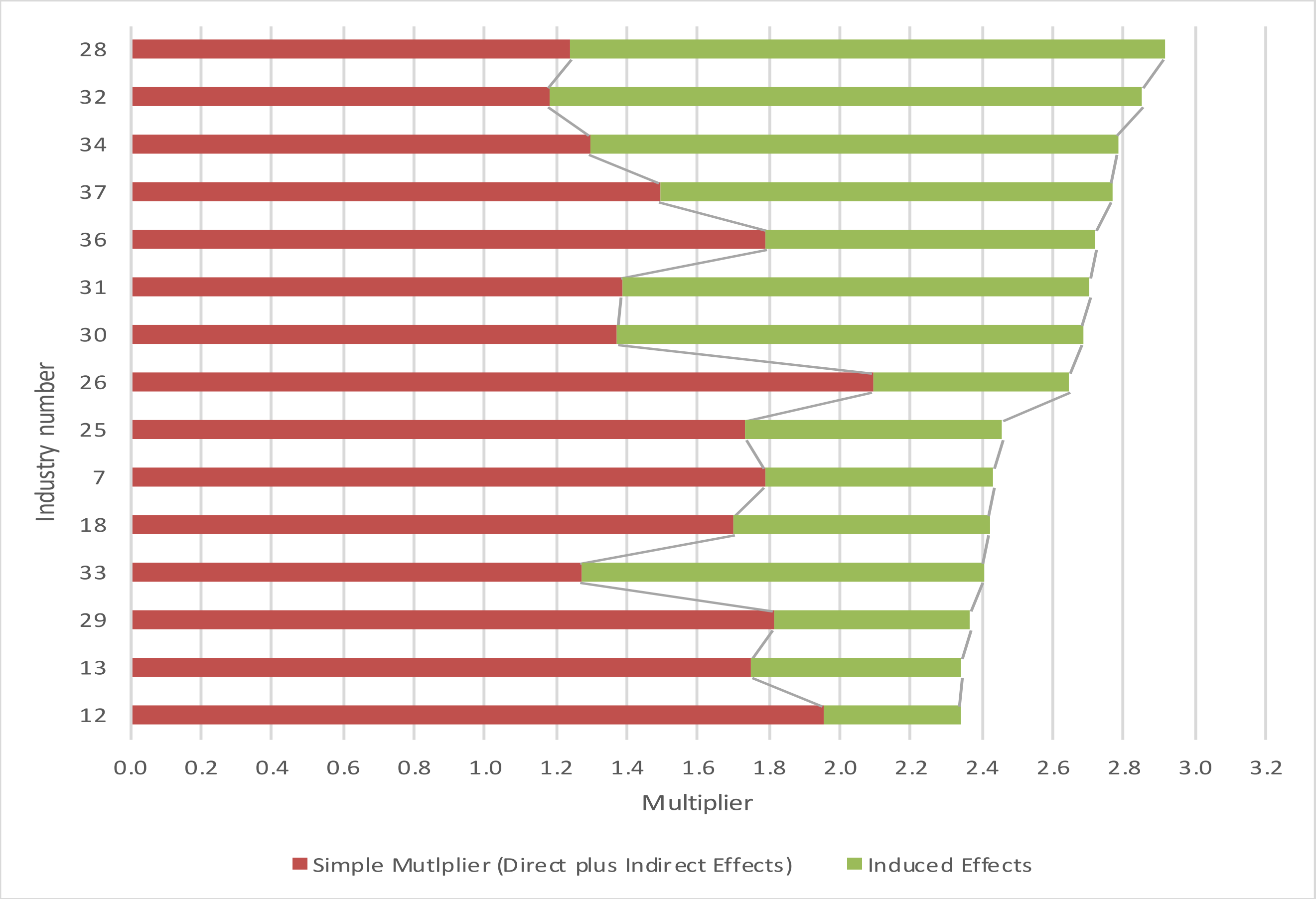

Figure 1: Decomposition of the top 15 total output multipliers. Source: Authors' own calculations.

The second highest ranking industry is (32): Education, followed by (34): Social work activities, with total multipliers of 2.85 and 2.78 respectively. These results are underpinned by the fact that these three sectors exhibit the strongest induced effects out of the industries under consideration.

By comparing the ranks for simple and total output multipliers it can be noted that internalising household consumption patterns leads to significant changes in terms of the industry's rankings. Indeed, out of the top five industries ranked by total multipliers, only one industry, (36): Sports activities and amusement and recreation activities, features in the top five simple multipliers.

While output multipliers are useful when analysing the degree of industry interdependence, they are not particularly relevant when the analyst is interested in measuring the impact of an exogenous change in the final demand of a given industry on Gross Domestic Product (GDP) or on household welfare. Since value added and GDP are conceptually equivalent, value added multipliers are often deemed more relevant than output multipliers. Results in Table 2 show the 15 highest ranking simple value added multipliers; it is immediately noticeable that value added multipliers are significantly smaller than their output counterparts. The industry with the highest simple multiplier is (40): Activities of households as employers, with a multiplier of 0.99. This is followed by (32): Education, and (28): Employment activities, with simple value added multipliers of 0.91 and 0.88 respectively.

Sectors (32): Education, and (28): Employment activities, also score highly in terms of total value added multipliers, ranking 1st and 2nd respectively, followed by (34): Social work activities. Results illustrated in Fig. 2 show that a one Euro exogenous increase in the final demand of these three sectors will lead to an increase of Euro 1.61, Euro 1.59 and Euro 1.45 in Maltese value added, as a result of the sectors' underlying direct, indirect and induced effects.

A comparison of the results for the total and simple value added multipliers, found in Appendix C, show that there have been shifts at the top of the rankings, however, these are not as pronounced as, for example, those seen in the case of output multipliers. It is also interesting to note that that the simple value added multipliers for all sectors are less than one, implying that when only considering direct and indirect effects, an exogenous increase in the final demand for all sectors will translate into a lower increase in value added. This has important policy implications, especially with respect to the fiscal multipliers. A fiscal expansion through, say, a one million Euro increase in public administration expenditure, will create Euro 0.78 million increase in GDP through direct and indirect effects, and Euro 0.56 million increase in GDP through induced effects brought about by household consumption patterns.

The income and employment multipliers, presented in Appendix D and Appendix E respectively, are especially useful for policymakers that seek to trace the impact exogenous changes in sectorial demand might have on household welfare. The income multiplier illustrates the effect on labour income generated from an exogenous increase of one Euro in the final demand for each sector.

Table 2: Top 15 simple value added multipliers.

No.	Industry	Simple Value Added Multiplier
40	Activities of households as employers; ...	0.99
32	Education	0.91
28	Employment activities	0.88
8	Manufacture of basic metals	0.87
23	Real estate activities	0.86
34	Social work activities	0.82
30	Security and investigation activities; services to buildings...	0.82
24	Legal and accounting activities; activities of head offices...	0.81
33	Human health activities	0.81
37	Activities of membership organisations	0.80
31	Public administration and defence; compulsory social security	0.78
16	Retail trade, except of motor vehicles and motorcycles	0.78
15	Wholesale trade, except of motor vehicles and motorcycles	0.77
25	Architectural and engineering activities; ...	0.73
14	Wholesale and retail trade and repair of motor vehicles...	0.72

Source: Authors' own calculations.

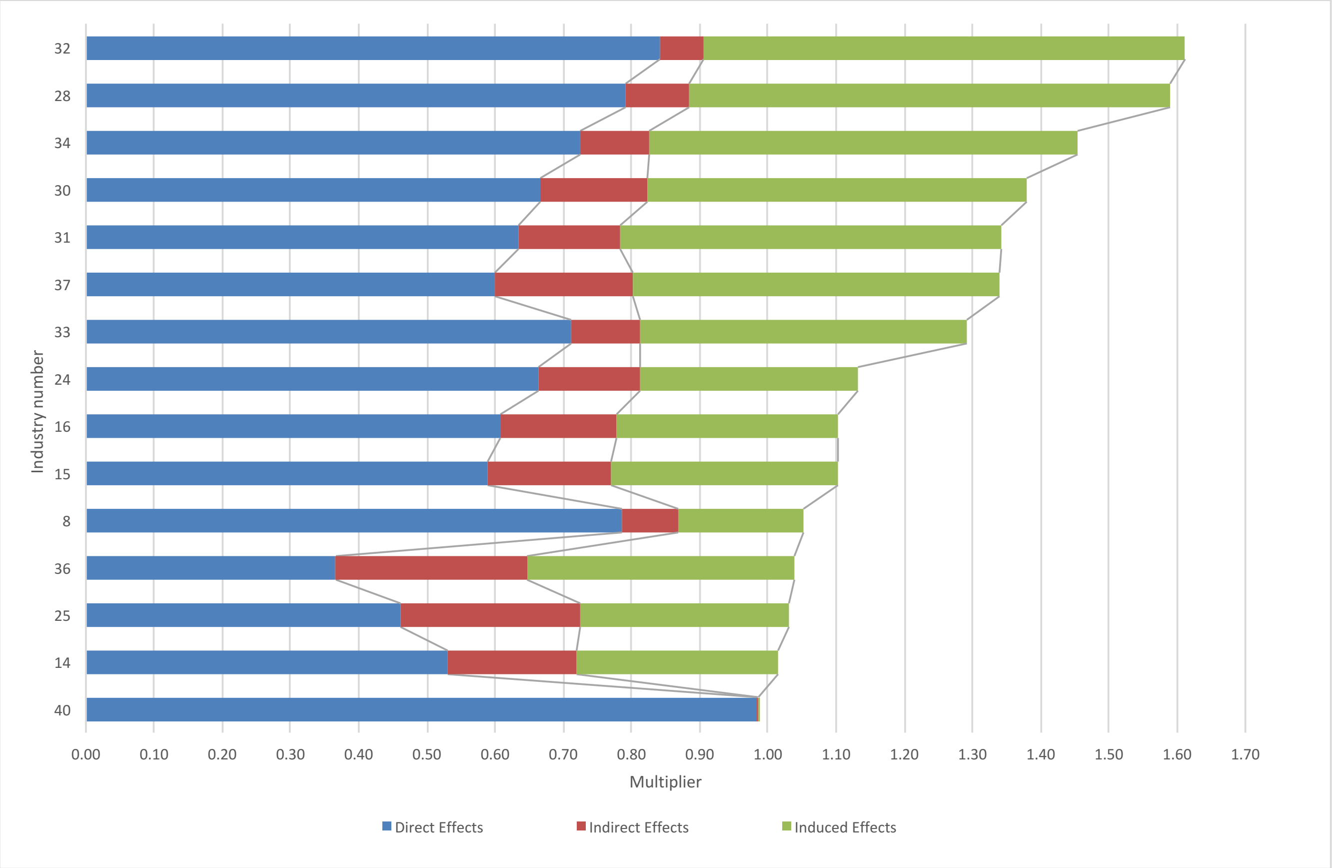

Figure 2: Decomposition of the top 15 total value added multipliers. Source: Authors' own calculations

Table 3: Top 15 simple income multipliers.

No.	Industry	Simple Income Multiplier
28	Employment activities	0.79
32	Education	0.79
34	Social work activities	0.70
31	Public administration and defence; compulsory social security	0.62
30	Security and investigation activities; services to buildings. . .	0.62
37	Activities of membership organisations	0.60
33	Human health activities	0.53
36	Sports activities and amusement and recreation activities	0.44
15	Wholesale trade, except of motor vehicles and motorcycles	0.37
16	Retail trade, except of motor vehicles and motorcycles	0.36
11	Repair and installation of machinery and equipment	0.36
24	Legal and accounting activities; activities of head offices; . . .	0.35
25	Architectural and engineering activities; . . .	0.34
18	Accommodation and food service activities	0.34
14	Wholesale and retail trade and repair of motor vehicles. . .	0.33

Source: Authors' own calculations.

The resulting top 15 simple income multipliers and top 15 total income multipliers are presented in Table 3 and Fig. 3 respectively. As expected, the sectors that intrinsically have a relatively high wage share, such as (28): Employment activities, (32): Education, (34): Social work activities, and (31): Public administration, possess the highest simple and total income multipliers. Conversely, industries which are more capital intensive, such as the manufacturing industries, generate relatively weak income multiplier effects.

Employment multipliers are especially useful for tracing the employment effects of the expansion or contraction of specific industries. Industries with the highest employment output ratios tend to score relatively high employment multipliers. The resulting top 15 simple employment multipliers and top 15 total employment multipliers are presented in Table 4 and Fig. 4 respectively.

In line with the results for income multipliers, (28): Employment activities, (32): Education, (34): Social work activities, and (31): Public administration, rank amongst the top ten industries in terms of their simple and total employment multipliers. Driven by a significantly high employment to output ratio, (37): Activities of membership organisation, has the highest simple and total employment multipliers. Indeed, a one million Euro exogenous increase in the final demand of this sector creates almost 6 new jobs directly within the same industry, and another 90 jobs through indirect effects stemming from other sectors. When taking into consideration the relationship between household income and consumption, the number of new employees created rises to almost 110. Mirroring the results for the income multipliers and driven by relatively low employment to output ratios, manufacturing sectors score quite poorly in terms of employment multipliers.

4 Discussion and Conclusion

By assessing all the individual measures generated in this study it is possible to obtain a very clear picture of the strength of the inter-industry relations. It is also possible to identify how the strength of these relations impacted the Maltese economy in the reference year of 2010 in terms of productive output generated, value added, household income generated, and employment created. These measures can either be used to study the

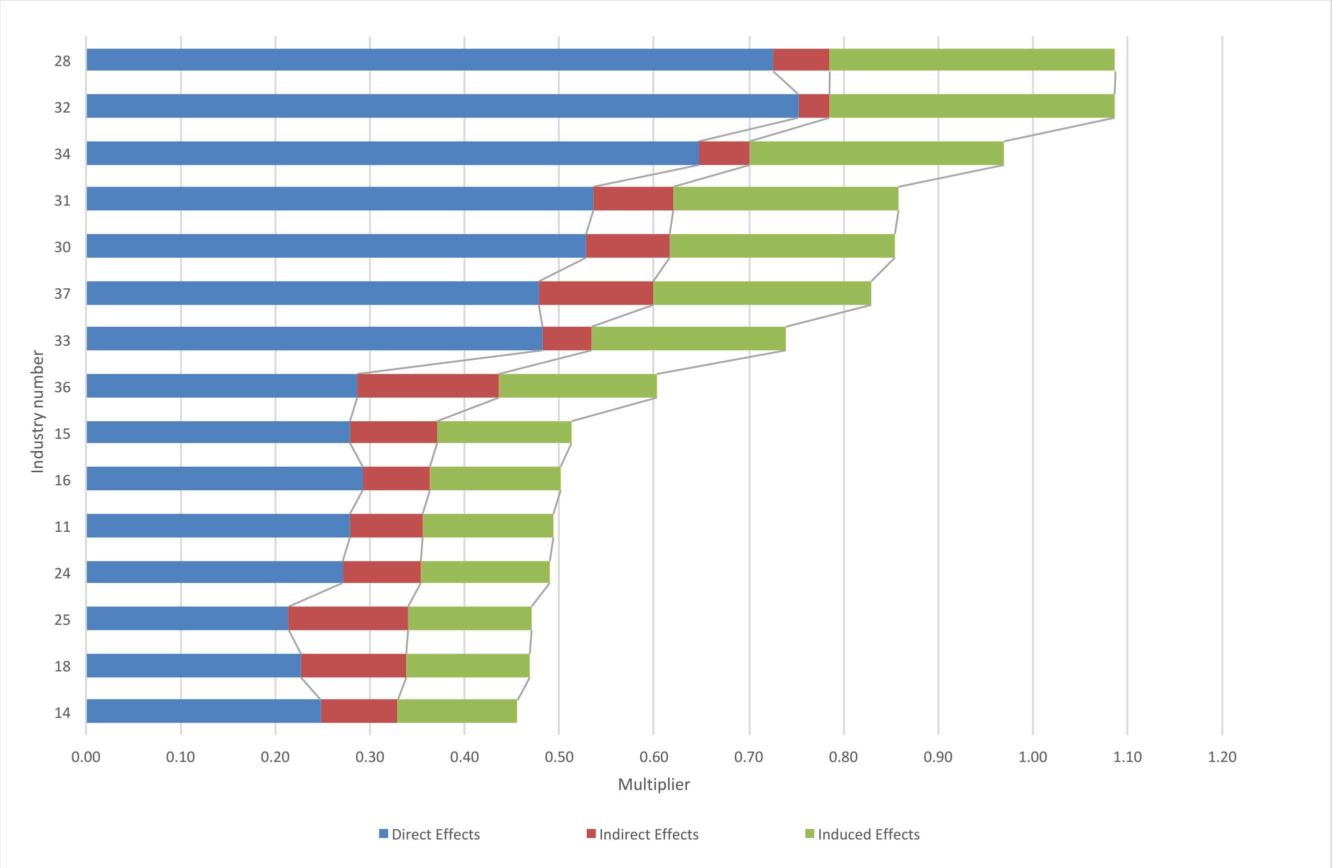

Figure 3: Decomposition of the top 15 total income multipliers. Source: Authors' own calculations.

Table 4: Top 15 simple employment multipliers.

No.	Industry	Simple Employment Multiplier
37	Activities of membership organisations	95.69
28	Employment activities	48.87
30	Security and investigation activities; services to buildings...	42.09
32	Education	38.24
36	Sports activities and amusement and recreation activities	36.93
34	Social work activities	36.36
31	Public administration and defence; compulsory social security	26.42
16	Retail trade, except of motor vehicles and motorcycles	26.04
18	Accommodation and food service activities	24.75
33	Human health activities	22.09
14	Wholesale and retail trade and repair of motor vehicles ...	21.27
15	Wholesale trade, except of motor vehicles and motorcycles	20.10
7	Manufacture of other non-metallic mineral products	19.37
9	Manufacture of fabricated metal products, ...	19.30
25	Architectural and engineering activities; ...	18.11

Source: Authors' own calculations.

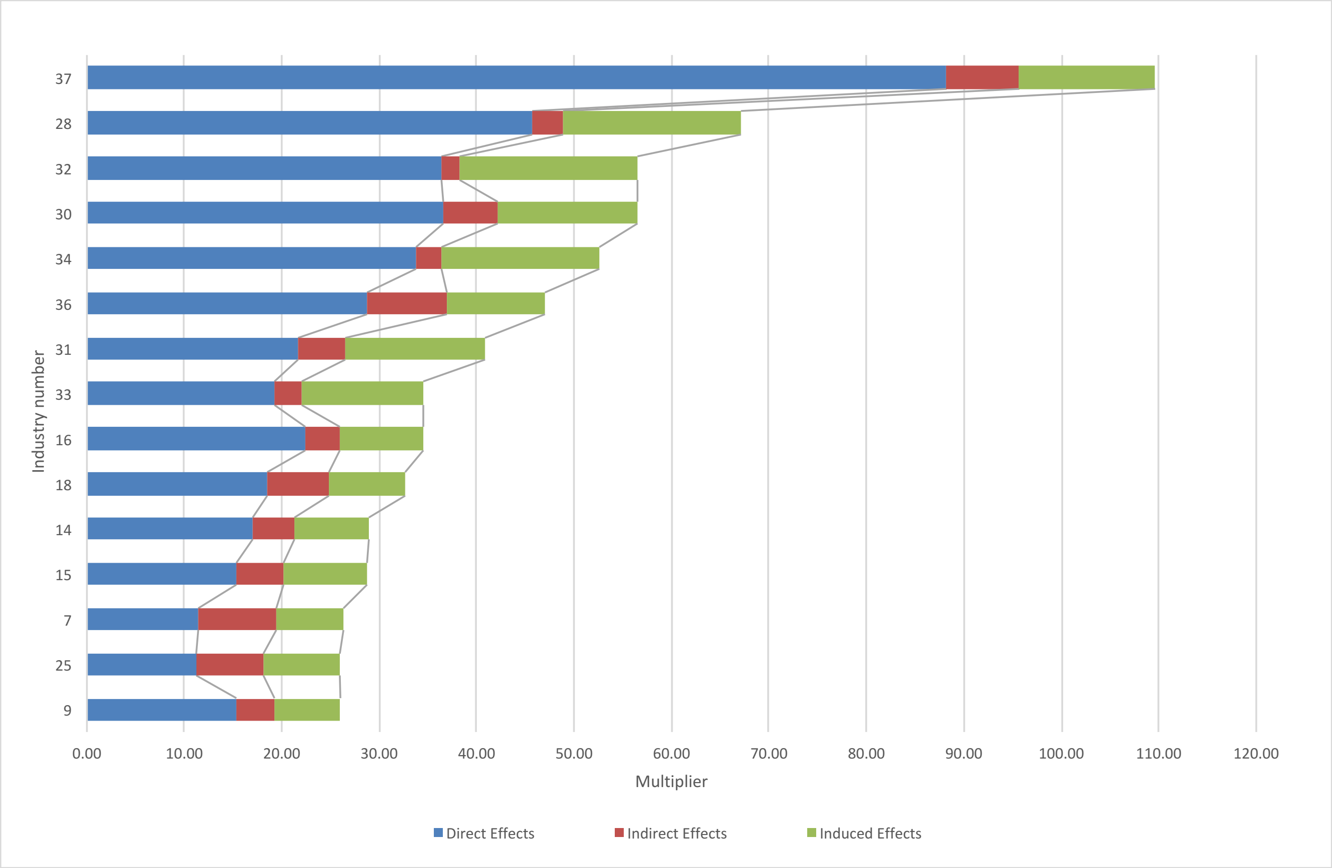

Figure 4: Decomposition of the top 15 total employment multipliers. Source: Authors' own calculations.

characteristics of the structure of production, or crucially can be of aid to policy makers for the identification of industry-specific policies aimed at improving Malta's competitiveness and economic resilience.

Overall there is considerable heterogeneity in the results. Indeed, when considering the complete set of multiplier results, one notes that there are considerable shifts at the top of the rankings, with a number of sectors featuring within the top 15 industries in specific multipliers while ranking relatively low down the list in others. However, a number of industries feature as relatively strong performers across all categories of multipliers. For instance, (18): Accommodation and food services activities, features in the top 15 in almost all categories of multipliers under consideration. This confirms the historic tendency for the tourism sector to feature as an important economic driver, even against the backdrop of the structural changes and the increased diversification that are currently shaping the Maltese economy. Strong results across all types of multipliers are also scored by the public sector, more specifically in (31): Public administration, (32): Education, and (33): Human health sectors. It is also particularly interesting to note that all multipliers pertaining to the financial services sector, including (20): Financial service activities except insurance, (21): Insurance and reinsurance, and (22) Activities auxiliary to financial activities, are amongst the lowest across all industries under consideration. This result contrasts sharply with results of Cassar (2015) obtained from input-output tables for 2008 in ESA 95. Indeed, according to this study, the industries making up the financial sector score considerably higher multipliers. The low multipliers derived for the new input-output tables may be in part driven by the inclusion of Special Purpose Vehicles (SPEs) within ESA 2010 data. Since SPEs contain very high import contents their inclusion reduces the relative magnitude of the local intermediate input requirements of the sector, implicitly weakening the strength of the overall sectoral multiplier effects.

This result highlights that, while industry specific multipliers are very useful, especially to policymakers, their interpretation should take into consideration the limitations of this indicator as well as the assumptions that are at the core of the Leontief demand driven model. Firstly, the analysis presented in this paper is based on modelling multipliers which specifically measure the resultant effect on output, value added, income and employment, due to a marginal change in final demand. Thus, these multipliers do not account for either the relative size of the industry or for the amount of final demand each industry is driving throughout the economy via its multipliers. For this reason, an analysis based solely on these results may give only a partial overview of the importance of each sector. In this light, the analysis can be complemented with accounting multipliers that take into consideration the size of final demand of each industry, together with the magnitude of direct and indirect effects. Secondly, it should be noted that the results obtained from the Leontief demand driven model are based on a number of robust assumptions which have to be taken into account when evaluating the resulting multiplier effects. One key assumption which underpins the Leontief demand driven model is that of fixed prices. The model assumes that an increase in the final demand of one sector (no matter how large) will not be constrained by the supply of labour or by intermediate goods and services (given also the implicit assumption of no resource constraints), nor will it result in a change in relative prices. Furthermore, the assumption of constant returns to scale in the Leontief production function neglects the possibility of substitution of one input for another in the production process. It is assumed the purchases of input requirements by each industry are determined solely by the desired level of output, implying that there has been no consideration into how changes in input prices (which are implicitly assumed to be fixed), changes in technology and efficiency gains through economies of scale, may or may not affect the underlying production processes of sectors in the economy. Therefore, interpreting multiplier estimates in the context of modelling marginal changes in activity (impact analysis), will implicitly invoke various assumptions about how the economy behaves in response to changes in demand, since these measures, would effectively be estimating the resulting impacts in an economic scenario which may differ from that of the given base year of 2010.

The industry specific multipliers derived in this study should be evaluated with caution by policy makers, given the assumptions which are invoked by the Leontief input-output modelling framework. However, they nonetheless still provide an analytically derived first cut estimate of the possible impacts that could be generated throughout the economy, as a result of an exogenous increase in final demand. Oosterhaven, Piek and Stedler (1986) assert that a realistic estimate of the true impact that an increase in final demand would have on the generation of output, value added, income and employment, would be approximately half way between the simple and total multipliers. This statement is based on the observation that simple multipliers probably underestimate economic impacts given that they omit household and factor income activities, whilst total multipliers probably overestimate these impacts due to strict assumptions concerning the behaviour of household income-expenditure patterns.

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Appendix

A Value Added, Income and Employment Ratios

No.	**NACE code**	**Industry**	**Value Added Input Coefficient**		**Income Input Coefficient**		**Employment Output Ratio**	
			Value	**Rank**	**Value**	**Rank**	**Value**	**Rank**
1	**A01-02**	Crop and animal production, hunting...	0.51	17	0.15	29	9.02	26
2	**A03**	Fishing and aquaculture	0.29	34	0.10	35	4.85	34
3	**C10T12**	Manufacture of food products, beverages...	0.28	35	0.16	24	9.37	24
4	**C13T15**	Manufacture of textiles, wearing apparel...	0.45	20	0.18	20	10.68	21
5	**C16**	Manufacture of wood and of products...	0.37	25	0.16	25	11.85	17
6	**C17-22**	Manufacture of paper and paper products, ...	0.42	22	0.19	18	9.06	25
7	**C23**	Manufacture of other non-metallic mineral...	0.26	37	0.17	21	11.44	18
8	**C24**	Manufacture of basic metals	0.79	4	0.15	26	12.30	15
9	**C25**	Manufacture of fabricated metal products, ...	0.35	28	0.22	15	15.26	13
10	**C26-32**	Manufacture of computer, electronic...	0.25	38	0.11	34	5.61	32
11	**C33**	Repair and installation of machinery...	0.30	32	0.28	11	11.04	20
12	**D35-E39**	Electricity, gas, steam and air conditioning...	0.18	39	0.08	36	3.74	36
13	**B+F**	Mining and quarrying and construction	0.33	29	0.15	27	9.55	23
14	**G45**	Wholesale and retail trade and repair...	0.53	15	0.25	13	17.03	11
15	**G46**	Wholesale trade, except of motor vehicles...	0.59	13	0.28	10	15.37	12
16	**G47**	Retail trade, except of motor vehicles...	0.61	11	0.29	8	22.47	7
17	**H49-53**	Land transport and transport via pipelines, ...	0.35	27	0.18	19	7.64	27
18	**I**	Accommodation and food service activities	0.38	23	0.23	14	18.50	10
19	**J58-63**	Publishing activities, motion picture, video...	0.43	21	0.17	23	6.66	31
20	**K64**	Financial service activities, except insurance...	0.07	40	0.04	38	1.38	38
21	**K65**	Insurance, reinsurance and pension funding...	0.52	16	0.15	28	4.82	35
22	**K66**	Activities auxiliary to financial services...	0.29	33	0.14	30	6.78	30
23	**L68**	Real estate activities	0.75	5	0.01	39	0.74	39
24	**M69+70**	Legal and accounting activities; ...	0.66	9	0.27	12	12.83	14
25	**M71+M72**	Architectural and engineering activities; ...	0.46	19	0.21	16	11.22	19
26	**M73-75**	Other professional, scientific and technical...	0.27	36	0.11	33	5.59	33
27	**N77**	Rental and leasing activities	0.37	24	0.11	32	7.50	29
28	**N78**	Employment activities	0.79	3	0.73	2	45.74	2
29	**N79**	Travel agency, tour operator...	0.31	30	0.14	31	7.62	28
30	**N80-82**	Security and investigation activities; ...	0.67	8	0.53	5	36.58	3
31	**O84**	Public administration and defence...	0.63	10	0.54	4	21.60	8
32	**P85**	Education	0.84	2	0.75	1	36.46	4
33	**Q86**	Human health activities	0.71	7	0.48	6	19.17	9
34	**Q87_88**	Social work activities	0.72	6	0.65	3	33.70	5
35	**R90T92**	Creative, arts and entertainment activities; ...	0.30	31	0.05	37	1.80	37
36	**R93**	Sports activities and amusement...	0.37	26	0.29	9	28.68	6
37	**S94**	Activities of membership organisations	0.60	12	0.48	7	88.21	1
38	**S95**	Repair of computers and personal...	0.55	14	0.20	17	10.66	22
39	**S96**	Other personal service activities	0.50	18	0.17	22	12.00	16
40	**TU**	Activities of households as employers; ...	0.98	1	0.00	40	0.00	40

Source: NSO.

B Output Multipliers

No.	NACE code	Industry	Simple Output Multiplier		Induced Effects		Total Output Multiplier	
			Value	**Rank**	**Value**	**Rank**	**Value**	**Rank**
1	**A01-02**	Crop and animal production, hunting…	1.58	10	0.52	25	2.10	22
2	**A03**	Fishing and aquaculture	1.30	27	0.32	35	1.62	34
3	**C10T12**	Manufacture of food products, beverages…	1.53	12	0.53	24	2.07	23
4	**C13T15**	Manufacture of textiles, wearing apparel…	1.38	21	0.50	26	1.89	29
5	**C16**	Manufacture of wood and of products…	1.36	24	0.48	29	1.84	30
6	**C17-22**	Manufacture of paper and paper products, …	1.24	33	0.49	28	1.73	31
7	**C23**	Manufacture of other non-metallic mineral…	1.79	5	0.64	16	2.43	10
8	**C24**	Manufacture of basic metals	1.22	34	0.44	30	1.66	32
9	**C25**	Manufacture of fabricated metal products, …	1.35	25	0.61	18	1.96	26
10	**C26-32**	Manufacture of computer, electronic…	1.21	35	0.30	36	1.51	35
11	**C33**	Repair and installation of machinery…	1.47	18	0.76	11	2.23	19
12	**D35-E39**	Electricity, gas, steam and air conditioning…	1.95	2	0.38	33	2.34	15
13	**B+F**	Mining and quarrying and construction	1.75	6	0.59	19	2.34	14
14	**G45**	Wholesale and retail trade and repair…	1.52	13	0.70	15	2.22	20
15	**G46**	Wholesale trade, except of motor vehicles…	1.50	16	0.79	9	2.29	17
16	**G47**	Retail trade, except of motor vehicles…	1.52	14	0.77	10	2.29	16
17	**H49-53**	Land transport and transport via pipelines, …	1.63	9	0.62	17	2.25	18
18	**I**	Accommodation and food service activities	1.70	8	0.72	14	2.42	11
19	**J58-63**	Publishing activities, motion picture, video…	1.42	19	0.50	27	1.92	27
20	**K64**	Financial service activities, except insurance…	1.04	39	0.10	39	1.13	39
21	**K65**	Insurance, reinsurance and pension funding…	1.14	37	0.35	34	1.50	36
22	**K66**	Activities auxiliary to financial services…	1.26	31	0.39	32	1.65	33
23	**L68**	Real estate activities	1.29	29	0.12	38	1.41	37
24	**M69+70**	Legal and accounting activities; …	1.38	22	0.75	12	2.14	21
25	**M71+M72**	Architectural and engineering activities; …	1.73	7	0.72	13	2.46	9
26	**M73-75**	Other professional, scientific and technical…	2.09	1	0.56	22	2.65	8
27	**N77**	Rental and leasing activities	1.54	11	0.43	31	1.98	25
28	**N78**	Employment activities	1.24	32	1.67	1	2.91	1
29	**N79**	Travel agency, tour operator…	1.81	3	0.56	21	2.37	13
30	**N80-82**	Security and investigation activities; …	1.37	23	1.31	5	2.68	7
31	**O84**	Public administration and defence…	1.39	20	1.32	4	2.70	6
32	**P85**	Education	1.18	36	1.67	2	2.85	2
33	**Q86**	Human health activities	1.27	30	1.14	7	2.40	12
34	**Q87_88**	Social work activities	1.29	28	1.49	3	2.78	3
35	**R90T92**	Creative, arts and entertainment activities; …	1.12	38	0.15	37	1.27	38
36	**R93**	Sports activities and amusement …	1.79	4	0.93	8	2.72	5
37	**S94**	Activities of membership organisations	1.49	17	1.27	6	2.76	4
38	**S95**	Repair of computers and personal …	1.34	26	0.56	20	1.90	28
39	**S96**	Other personal service activities	1.51	15	0.55	23	2.06	24
40	**TU**	Activities of households as employers; …	1.02	40	0.00	40	1.02	40

Source: Authors' own calculations.

C Value Added Multipliers

No.	**NACE code**	**Industry**	**Simple Value Added Multiplier**		**Induced Effects**		**Total Value Added Multiplier**	
			Value	**Rank**	**Value**	**Rank**	**Value**	**Rank**
1	**A01-02**	Crop and animal production, hunting…	0.70	16	0.22	25	0.92	18
2	**A03**	Fishing and aquaculture	0.39	35	0.13	35	0.53	36
3	**C10T12**	Manufacture of food products, beverages…	0.49	33	0.23	24	0.71	31
4	**C13T15**	Manufacture of textiles, wearing apparel…	0.56	26	0.21	26	0.77	28
5	**C16**	Manufacture of wood and of products…	0.51	30	0.20	29	0.71	30
6	**C17-22**	Manufacture of paper and paper products, …	0.50	31	0.21	28	0.71	33
7	**C23**	Manufacture of other non-metallic mineral…	0.53	28	0.27	16	0.81	25
8	**C24**	Manufacture of basic metals	0.87	4	0.18	30	1.05	11
9	**C25**	Manufacture of fabricated metal products, …	0.50	32	0.26	18	0.76	29
10	**C26-32**	Manufacture of computer, electronic…	0.32	39	0.13	36	0.45	38
11	**C33**	Repair and installation of machinery…	0.46	34	0.32	11	0.78	27
12	**D35-E39**	Electricity, gas, steam and air conditioning…	0.39	36	0.16	33	0.55	35
13	**B+F**	Mining and quarrying and construction	0.59	22	0.25	19	0.84	21
14	**G45**	Wholesale and retail trade and repair…	0.72	15	0.30	15	1.02	14
15	**G46**	Wholesale trade, except of motor vehicles…	0.77	13	0.33	9	1.10	10
16	**G47**	Retail trade, except of motor vehicles…	0.78	12	0.33	10	1.10	9
17	**H49-53**	Land transport and transport via pipelines, …	0.56	25	0.26	17	0.83	23
18	**I**	Accommodation and food service activities	0.63	20	0.30	14	0.94	16
19	**J58-63**	Publishing activities, motion picture, video…	0.58	23	0.21	27	0.79	26
20	**K64**	Financial service activities, except insurance…	0.08	40	0.04	39	0.12	40
21	**K65**	Insurance, reinsurance and pension funding…	0.55	27	0.15	34	0.70	34
22	**K66**	Activities auxiliary to financial services…	0.36	37	0.16	32	0.53	37
23	**L68**	Real estate activities	0.86	5	0.05	38	0.90	20
24	**M69+70**	Legal and accounting activities; …	0.81	8	0.32	12	1.13	8
25	**M71+M72**	Architectural and engineering activities; …	0.73	14	0.31	13	1.03	13
26	**M73-75**	Other professional, scientific and technical…	0.60	21	0.23	22	0.83	22
27	**N77**	Rental and leasing activities	0.53	29	0.18	31	0.71	32
28	**N78**	Employment activities	0.88	3	0.71	1	1.59	2
29	**N79**	Travel agency, tour operator…	0.57	24	0.23	21	0.81	24
30	**N80-82**	Security and investigation activities; …	0.82	7	0.55	5	1.38	4
31	**O84**	Public administration and defence…	0.78	11	0.56	4	1.34	5
32	**P85**	Education	0.91	2	0.71	2	1.61	1
33	**Q86**	Human health activities	0.81	9	0.48	7	1.29	7
34	**Q87_88**	Social work activities	0.82	6	0.63	3	1.45	3
35	**R90T92**	Creative, arts and entertainment activities; …	0.34	38	0.06	37	0.40	39
36	**R93**	Sports activities and amusement…	0.65	19	0.39	8	1.04	12
37	**S94**	Activities of membership organisations	0.80	10	0.54	6	1.34	6
38	**S95**	Repair of computers and personal…	0.68	18	0.24	20	0.91	19
39	**S96**	Other personal service activities	0.69	17	0.23	23	0.93	17
40	**TU**	Activities of households as employers; …	0.99	1	0.00	40	0.99	15

Source: Authors' own calculations.

D Income Multipliers

No.	NACE code	Industry	Simple Income Multiplier		Induced Effects		Total Income Multiplier	
			Value	Rank	Value	Rank	Value	Rank
1	**A01-02**	Crop and animal production, hunting...	0.24	25	0.09	25	0.34	25
2	**A03**	Fishing and aquaculture	0.15	35	0.06	35	0.21	35
3	**C10T12**	Manufacture of food products, beverages...	0.25	24	0.10	24	0.35	24
4	**C13T15**	Manufacture of textiles, wearing apparel...	0.24	26	0.09	26	0.33	26
5	**C16**	Manufacture of wood and of products...	0.23	29	0.09	29	0.31	29
6	**C17-22**	Manufacture of paper and paper products, ...	0.23	28	0.09	28	0.32	28
7	**C23**	Manufacture of other non-metallic mineral...	0.30	16	0.12	16	0.42	16
8	**C24**	Manufacture of basic metals	0.21	30	0.08	30	0.28	30
9	**C25**	Manufacture of fabricated metal products, ...	0.29	18	0.11	18	0.40	18
10	**C26-32**	Manufacture of computer, electronic...	0.14	36	0.05	36	0.19	36
11	**C33**	Repair and installation of machinery...	0.36	11	0.14	11	0.49	11
12	**D35-E39**	Electricity, gas, steam and air conditioning...	0.18	33	0.07	33	0.25	33
13	**B+F**	Mining and quarrying and construction	0.28	19	0.11	19	0.39	19
14	**G45**	Wholesale and retail trade and repair...	0.33	15	0.13	15	0.46	15
15	**G46**	Wholesale trade, except of motor vehicles...	0.37	9	0.14	9	0.51	9
16	**G47**	Retail trade, except of motor vehicles...	0.36	10	0.14	10	0.50	10
17	**H49-53**	Land transport and transport via pipelines, ...	0.29	17	0.11	17	0.40	17
18	**I**	Accommodation and food service activities	0.34	14	0.13	14	0.47	14
19	**J58-63**	Publishing activities, motion picture, video...	0.23	27	0.09	27	0.32	27
20	**K64**	Financial service activities, except insurance...	0.04	39	0.02	39	0.06	39
21	**K65**	Insurance, reinsurance and pension funding...	0.17	34	0.06	34	0.23	34
22	**K66**	Activities auxiliary to financial services...	0.18	32	0.07	32	0.25	32
23	**L68**	Real estate activities	0.05	38	0.02	38	0.08	38
24	**M69+70**	Legal and accounting activities; ...	0.35	12	0.14	12	0.49	12
25	**M71+M72**	Architectural and engineering activities; ...	0.34	13	0.13	13	0.47	13
26	**M73-75**	Other professional, scientific and technical...	0.26	22	0.10	22	0.36	22
27	**N77**	Rental and leasing activities	0.20	31	0.08	31	0.28	31
28	**N78**	Employment activities	0.79	1	0.30	1	1.09	1
29	**N79**	Travel agency, tour operator...	0.26	21	0.10	21	0.36	21
30	**N80-82**	Security and investigation activities; ...	0.62	5	0.24	5	0.85	5
31	**O84**	Public administration and defence...	0.62	4	0.24	4	0.86	4
32	**P85**	Education	0.79	2	0.30	2	1.09	2
33	**Q86**	Human health activities	0.53	7	0.21	7	0.74	7
34	**Q87_88**	Social work activities	0.70	3	0.27	3	0.97	3
35	**R90T92**	Creative, arts and entertainment activities; ...	0.07	37	0.03	37	0.10	37
36	**R93**	Sports activities and amusement...	0.44	8	0.17	8	0.60	8
37	**S94**	Activities of membership organisations	0.60	6	0.23	6	0.83	6
38	**S95**	Repair of computers and personal...	0.26	20	0.10	20	0.37	20
39	**S96**	Other personal service activities	0.26	23	0.10	23	0.36	23
40	**TU**	Activities of households as employers; ...	0.00	40	0.00	40	0.00	40

Source: Authors' own calculations.

E Employment Multipliers

No.	NACE code	Industry	Simple Employment Multiplier		Induced Effects		Total Employment Multiplier	
			Value	Rank	Value	Rank	Value	Rank
1	**A01-02**	Crop and animal production, hunting…	14.34	24	5.68	25	20.02	24
2	**A03**	Fishing and aquaculture	7.74	34	3.50	35	11.24	34
3	**C10T12**	Manufacture of food products, beverages…	14.60	23	5.84	24	20.43	22
4	**C13T15**	Manufacture of textiles, wearing apparel…	13.35	26	5.51	26	18.86	28
5	**C16**	Manufacture of wood and of products…	15.97	19	5.25	29	21.21	20
6	**C17-22**	Manufacture of paper and paper products, …	11.06	30	5.38	28	16.44	30
7	**C23**	Manufacture of other non-metallic mineral …	19.37	13	7.04	16	26.41	13
8	**C24**	Manufacture of basic metals	14.98	21	4.79	30	19.77	25
9	**C25**	Manufacture of fabricated metal products, …	19.30	14	6.71	18	26.01	15
10	**C26-32**	Manufacture of computer, electronic…	7.45	35	3.25	36	10.70	35
11	**C33**	Repair and installation of machinery…	15.13	20	8.29	11	23.42	18
12	**D35-E39**	Electricity, gas, steam and air conditioning …	8.51	33	4.20	33	12.71	33
13	**B+F**	Mining and quarrying and construction	17.28	17	6.50	19	23.78	17
14	**G45**	Wholesale and retail trade and repair …	21.27	11	7.67	15	28.93	11
15	**G46**	Wholesale trade, except of motor vehicles…	20.10	12	8.62	9	28.72	12
16	**G47**	Retail trade, except of motor vehicles…	26.04	8	8.44	10	34.48	9
17	**H49-53**	Land transport and transport via pipelines, …	12.81	28	6.78	17	19.59	26
18	**I**	Accommodation and food service activities	24.75	9	7.87	14	32.62	10
19	**J58-63**	Publishing activities, motion picture, video…	9.97	31	5.45	27	15.42	31
20	**K64**	Financial service activities, except insurance…	1.73	39	1.04	39	2.77	39
21	**K65**	Insurance, reinsurance and pension funding…	5.79	36	3.88	34	9.67	36
22	**K66**	Activities auxiliary to financial services…	8.82	32	4.24	32	13.05	32
23	**L68**	Real estate activities	3.13	37	1.27	38	4.39	37
24	**M69+70**	Legal and accounting activities; …	17.43	16	8.25	12	25.68	16
25	**M71+M72**	Architectural and engineering activities; …	18.11	15	7.92	13	26.03	14
26	**M73-75**	Other professional, scientific and technical…	13.03	27	6.08	22	19.11	27
27	**N77**	Rental and leasing activities	12.35	29	4.72	31	17.07	29
28	**N78**	Employment activities	48.87	2	18.28	1	67.16	2
29	**N79**	Travel agency, tour operator…	14.66	22	6.08	21	20.74	21
30	**N80-82**	Security and investigation activities; …	42.09	3	14.37	5	56.46	4
31	**O84**	Public administration and defence…	26.42	7	14.44	4	40.85	7
32	**P85**	Education	38.24	4	18.28	2	56.52	3
33	**Q86**	Human health activities	22.09	10	12.43	7	34.52	8
34	**Q87_88**	Social work activities	36.36	6	16.29	3	52.65	5
35	**R90T92**	Creative, arts and entertainment activities; …	2.60	38	1.65	37	4.26	38
36	**R93**	Sports activities and amusement…	36.93	5	10.14	8	47.07	6
37	**S94**	Activities of membership organisations	95.69	1	13.94	6	109.63	1
38	**S95**	Repair of computers and personal…	14.00	25	6.17	20	20.17	23
39	**S96**	Other personal service activities	17.13	18	6.06	23	23.18	19
40	**TU**	Activities of households as employers; …	0.03	40	0.02	40	0.04	40

Source: Authors' own calculations.

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Review Article

A Review of Studies Investigating the Dielectric Properties of Biological Tissues for Application in Hyperthermia and Microwave Thermal Ablation

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Abstract. Heating of biological tissues beyond 40 °C has become an established method of treating a number of diseases, most notably tumours, where hyperthermia and thermal ablation are important modalities. In some interventions, tissue temperatures reached can even go beyond 100 °C, and demand precise knowledge of tissue dielectric properties and how these vary with frequency and temperature in order to facilitate accurate computational simulations for preclinical planning. This paper reviews the available literature concerning dielectric properties of biological tissues and their temperature dependence, focusing on the frequencies of 915 MHz and 2.45 GHz, at which most of the studies reviewed investigate predominantly liver tissue. In this review a comparative analysis of the results obtained by different research groups are presented in the different studies is also made, indicating possible limiting factors in the different studies. These studies propose a number of different models which could be used to describe temperature dependence. Due to the prevalence of liver investigations, it would be ideal to conduct further studies on different biological tissues.

Keywords: Dielectric properties, biological samples, temperature variation, hyperthermia, ablation

1 Introduction

Nowadays a number of medical conditions are treated through the use of localised heating beyond normal body temperatures. Two distinct temperature ranges are employed: between 40 and 45 °C for hyperthermia (Velazquez-Ahumada, Freire & Marques, 2011; Nguyen, Abbosh & Crozier, 2015; Strohbehn, 1983; Horsman & Overgaard, 2007), and between 50 and 100 °C for thermal ablation (Lopresto, Pinto, Farina & Cavagnaro, 2017; Cavagnaro, Pinto & Lopresto, 2015; Lopresto, Pinto, Lodato, Lovisolo & Cavagnaro, 2012; Rossmann & Haemmerich, 2014). In hyperthermic processes, the increased tissue temperature increases cellular metabolism, oxygenation and blood perfusion. In an ablative process, the high temperature destroys or modifies the tissue in an irreversible manner.

Hyperthermic and ablative procedures can be used to treat cancer, varicose veins, joint laxity, hyperopia, hyperplasia and other medical conditions (Rossmann & Haemmerich, 2014; Subwongcharoen, Praditphol & Chitwiset, 2009; Brace, 2010). These procedures can be performed during open surgery, laparoscopically, percutaneously or transcutaneously (Brace, 2010). Higher temperatures affect a number of tissue properties, such as electrical, mechanical, thermal and the rate of perfusion. These changes affect the rate at which the tissue absorbs heat.

The frequencies commonly used for medical procedures are 915 MHz and 2.45 GHz (Brace, 2010). The other frequency ranges used for medical applications include 433 MHz, and broad band pulses with the greatest spectral energy density between 1 GHz and 10 GHz.

The electrical interaction between electromagnetic fields and materials is best described by the permittivity and the permeability. In the case of biological tissues at 915 MHz and 2.45 GHz, the permeability is comparable to that of free space, and is not normally considered.

Knowledge of the dielectric properties and how these change with temperature is crucial for modelling the interaction of microwaves with the body tissues. The medical industry, specialists and patients would greatly benefit from patient-specific field simulation software for treatment planning prior to medical interventions. The

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effective implementation of this software relies on accurate knowledge of the dielectric properties of the target tissues at the treatment frequency and intended temperature range.

This review presents a detailed report on the dependence of the dielectric properties of biological tissues on frequency and temperature, with specific focus on the frequencies of 915 MHz and 2.45 GHz, these being at present the most common frequencies employed in the medical field.

2 Electromagnetic Interactions with Biological Tissues

Over the frequency range of interest for current medical applications, the main effect of an electromagnetic wave interacting with biological tissue is an increase in temperature. To date, the change in temperature of the biological tissue is best modelled using Pennes' Bioheat equation (Pennes, 1949)

$$\rho c_t \frac{\partial T}{\partial t} = k\nabla^2 T + Q - \rho_{bl} c_{bl} \left(T - T_{bl}\right), \qquad (1)$$

where, ρ is the tissue density $\left(\mathrm{kg\,m^{-3}}\right)$, c_t is the specific heat capacity $\left(\mathrm{J\,kg^{-1}\,K^{-1}}\right)$, k is the thermal conductivity $\left(\mathrm{W\,m^{-1}\,K^{-1}}\right)$, and Q is the heat supplied $\left(\mathrm{W\,m^{-1}}\right)$ from the heating modality (RF or MW energy). The quantity $\rho_{bl} c_{bl} \left(T - T_{bl}\right)$ is the term used to describe the heat being lost through blood perfusion.

Many biological tissues, including cancerous have a relatively high-water content (Allen, Krzywicki & Roberts, 1959). When the incident electromagnetic field interacts with the water molecules' dipole moments, it causes them to realign periodically with the incident time-dependent field. This constant realignment of the dipoles increases the thermal energy, as a result of which the tissue temperature increases.

The microwave heat source can be modelled by

$$Q = \frac{\sigma}{2}|E|^2. \qquad (2)$$

In Eq. (2), σ is the effective conductivity $\left(\mathrm{S\,m^{-1}}\right)$ and $|E|$ is the applied electric field intensity $\left(\mathrm{V\,m^{-1}}\right)$.

In biological tissue, the heat generated by absorption of electromagnetic waves is determined by the material dielectric properties, which are described by the complex (relative) permittivity

$$\varepsilon^\star = \varepsilon' - j\varepsilon'', \qquad (3)$$

where ε' represents the real part of the relative permittivity of the material and ε'' represents the imaginary part of the permittivity. ε'' represents both the ionic conduction as well as the dipole rotation. The imaginary part of the permittivity is sometimes represented using the effective conductivity (σ), in the rest of the paper this is referred to as conductivity, as this is how it has been referred to in cited papers. Both ε' and ε'' depend on frequency, temperature, tissue type and water content.

To date many studies on a range of tissue types (see for example Farrugia, Wismayer, Mangion & Sammut, 2016; Gabriel, Lau & Gabriel, 1996; Lazebnik et al., 2007) have been carried out over the frequency range 10 kHz to 10 GHz. However, the temperature variation of dielectric properties still leaves a substantial gap in knowledge. The subsequent paragraphs provide an overview of the pertinent available literature on the subject.

In two separate studies performed by Chin and Sherar (2001, 2004), they report on the temperature coefficients of dielectric properties bovine liver and rat prostate. In both works measurements, were conducted at 915 MHz using the open-ended coaxial probe technique, and the permittivity calculated using a two-capacitor model. The samples were heated using a water bath and the temperature ranged from room temperature to 75 °C.

In the first study (bovine liver), Chin and Sherar (2001) report that during the heating process, there is an initial decrease of 5% in ε', followed by a 10% increase, eventually resulting in a 5% overall increase in ε' after 40 minutes. When examining σ, Chin and Sherar report a sharp initial increase which then stabilises to about twice the initial value after about 30 minutes (Fig. 1). The temperature coefficients reported for ε' and σ were $-0.13\,\%\,^\circ\mathrm{C}^{-1}$ and $1.82\,\%\,^\circ\mathrm{C}^{-1}$, respectively.

In the second study by Chin and Sherar (2004), measurements were conducted a day after excision. The tissues were stored in phosphate-buffered saline, which is shown to minimally affect the native ionic content. Despite stating that the effect of the phosphate-buffered saline was minimal, the study does not quantify this effect. This study on the temperature variation of tissue dielectric properties revealed an increase in the tissue conductivity with temperature, while the relative permittivity decreased with temperature. The study goes on to point out that, as the temperature increased, the tissue shrunk in dimensions. This would have been due to desiccation of the tissue while it was being heated, but the change in size was identified as a possible reason for the increase in measurement uncertainty at high temperatures (Chin & Sherar, 2001, 2004). The dielectric temperature coefficients for rat prostate were reported to be $-0.31\,^\circ\mathrm{C}^{-1}$ for ε' and $1.10\,\%\,^\circ\mathrm{C}^{-1}$ for σ.

Chin and Sherar (2004) report that, as opposed to rat prostate, the dielectric properties of bovine liver do not return to their native values on cooling. For bovine liver, Chin distinguishes between reversible and irreversible changes occurring with tissue temperature increase, where the initial decrease observed in the relative permittivity for bovine liver occurs due to reversible changes, and the increase in ε' occur due irreversible structural changes. Chin and Sherar (2004) state that "liver exhibits a more organized architecture compared with prostate" and that this is a possible explanation for the different behaviours of liver and prostate with temperature.

On comparing the two studies, it is evident that different tissues react differently to an increase in temperature. Therefore, there is a need to measure dielectric properties of different tissues at different temperatures and to see how they behave in order to construct reliable models of different tissue types for medical intervention planning.

In another study carried out by Stauffer, Rossetto, Prakash, Neuman and Lee (2003), the dielectric properties of liver tissue were studied. The samples were obtained from four different species - human, bovine, canine and porcine. The temperature variation aspect of this study is however focused on porcine and bovine liver. In the case of porcine liver, the freshly excised tissue was allowed to cool down from body to ambient temperature prior to measurements, which were carried out between 15 and 37 °C. Bovine liver measurements were conducted between 10 and 90 °C. The temperature coefficient for ε_r was $-0.2\,\%\,°\mathrm{C}^{-1}$ for porcine liver and $-0.04\,\%\,°\mathrm{C}^{-1}$ for bovine liver while σ increased steadily at approximately $1.1\,\%\,°\mathrm{C}^{-1}$ (porcine liver) and $2\,\%\,°\mathrm{C}^{-1}$ (bovine liver), as shown in Fig. 2. This study focused on a number of other aspects and as a result did not analyse the reasons for the differences in the measured increase in σ of bovine and porcine liver.

Lazebnik, Converse, Booske and Hagness (2006) measured the dielectric properties of porcine and bovine liver over the frequency range 0.5 to 20.0 GHz while

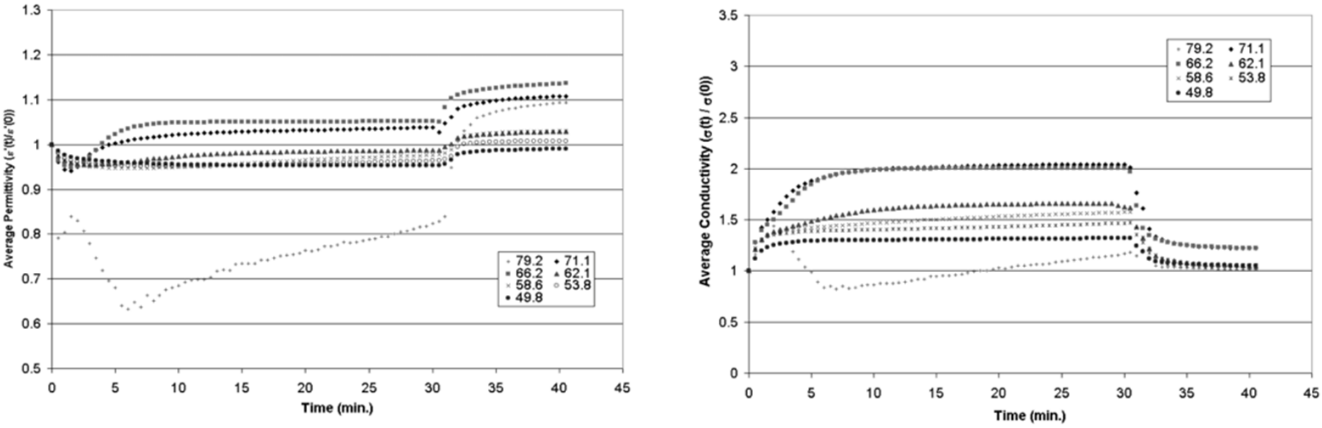

Figure 1: Changes in normalized bovine liver permittivity and conductivity, as a function of heating time for a range of target temperatures (49.8–79.2 °C). Plots obtained from Chin and Sherar (2001).

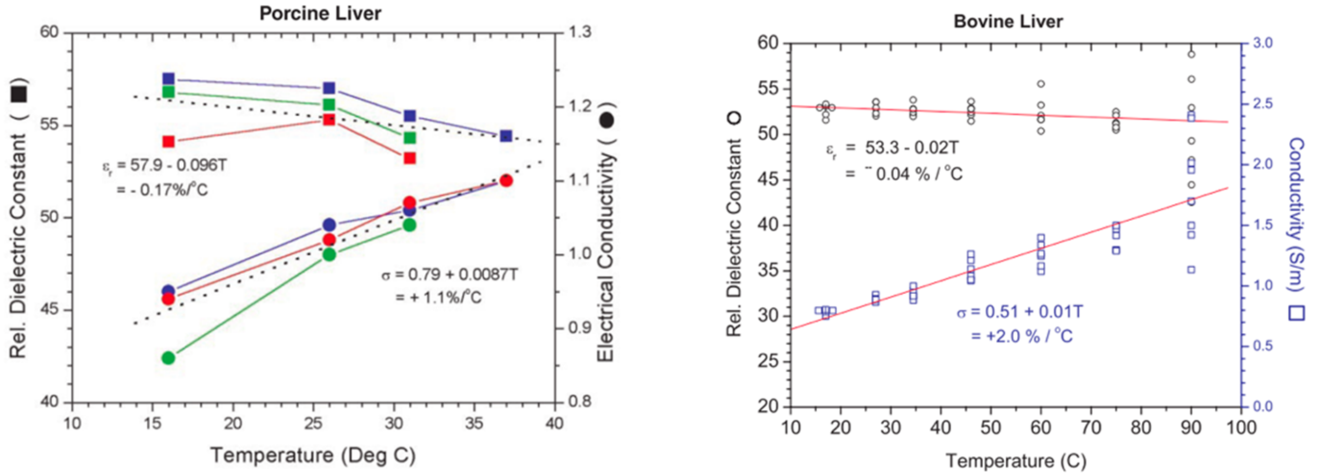

Figure 2: Dielectric properties of liver tissue at 915 MHz as a function of temperature. Plots taken from Stauffer, Rossetto, Prakash, Neuman and Lee (2003).

warming the tissue samples in an oven up to a temperature of 60 °C. They observed that the variation of the dielectric constant and conductivity are also frequency dependant, with a cross-over point at around 4 GHz, observed for the dielectric constant. There were two cross-over points for the conductivity at 3 and 15 GHz (Fig. 3). The temperature coefficients obtained at 915 MHz are $-0.20\,\%\,°\text{C}^{-1}$ and $1.33\,\%\,°\text{C}^{-1}$ for ε and σ, respectively during heating, and $-0.13\,\%\,°\text{C}^{-1}$ and $1.16\,\%\,°\text{C}^{-1}$ during cooling. At 2.45 GHz the temperature coefficients obtained were $-0.17\,\%\,°\text{C}^{-1}$ and $0.20\,\%\,°\text{C}^{-1}$ for ε and σ, respectively during heating, and $-0.090\,\%\,°\text{C}^{-1}$ and $0.008\,\%\,°\text{C}^{-1}$ during cooling.

Also highlighted in this study are the differences observed between the measurements recorded during the heating and cooling cycles. The heating cycle showed repeatable anomalies when compared to the cooling cycle. The reason given for these anomalies are non-equilibrium effects. This was consistent with Chin and Sherar's work (2001, 2004) where it was also stated that during the heating cycle there are irreversible changes taking place in the tissues. Lazebnik et al. (2006) unfortunately do not explain the phenomena which occur during the heating cycle, which would be of particular interest to medical practitioners. In Lazebnik et al. (2006) also reports non-linear dielectric coefficients, especially notable for the conductivity at 2.45 GHz. This result is similar to that reported by Sipahioglu, Barringer and Bircan (2003). Lazebnik et al. (2006) also present four quadratic equations to model the variation of dielectric properties of the material under study with temperature and frequency.

Another relevant study carried out by Brace (2008), where *ex-vivo* bovine liver tissue samples were ablated using a commercial multi-probe RF ablation system operating at 500 kHz, allowing dielectric measurements to be carried out by the open-ended coaxial probe reflection technique over the frequency range 500 MHz to 5 GHz while the tissue was being heated. The temperature coefficients obtained in this study are $-0.22\,\%\,°\text{C}^{-1}$ for ε_r and $1.29\,\%\,°\text{C}^{-1}$ for σ at 915 MHz. The variations in conductivity at 915 MHz and 2.45 GHz are shown in Fig. 4. At 2.45 GHz, the coefficients obtained were $-0.18\,\%\,°\text{C}^{-1}$ for ε_r and $-0.2\,\%\,°\text{C}^{-1}$ for σ, in contrast with the positive coefficient obtained by Lazebnik et al. (2006). Brace explains that the heating method could be a possible reason for this difference, as in this study, on the contrary to previous studies, there was no attempt at limiting water loss from the samples under test. Consequently, tissue properties were observed to vary rapidly and irreversibly when approaching 100 °C. It would prove useful to carry out further measurements on similar tissues in order to obtain a more reliable data set.

Ji and Brace (2011) investigated the dielectric properties of fresh liver in an effort to establish an empirical model to improve simulations at high temperatures. In this study, Ji and Brace used a microwave ablator operating at 2.45 GHz to heat the tissue and measured the dielectric properties with the open-ended coaxial probe technique over the frequency range 500 MHz to 6 GHz. Although the liver samples were placed in containers with normal saline, Ji and Brace do not comment on the effect that this solution had on the measured dielectric properties. The experimental outcome of this study below 70 °C was similar to that of previous studies by Lazebnik et al. (2006) and Stauffer et al. (2003).

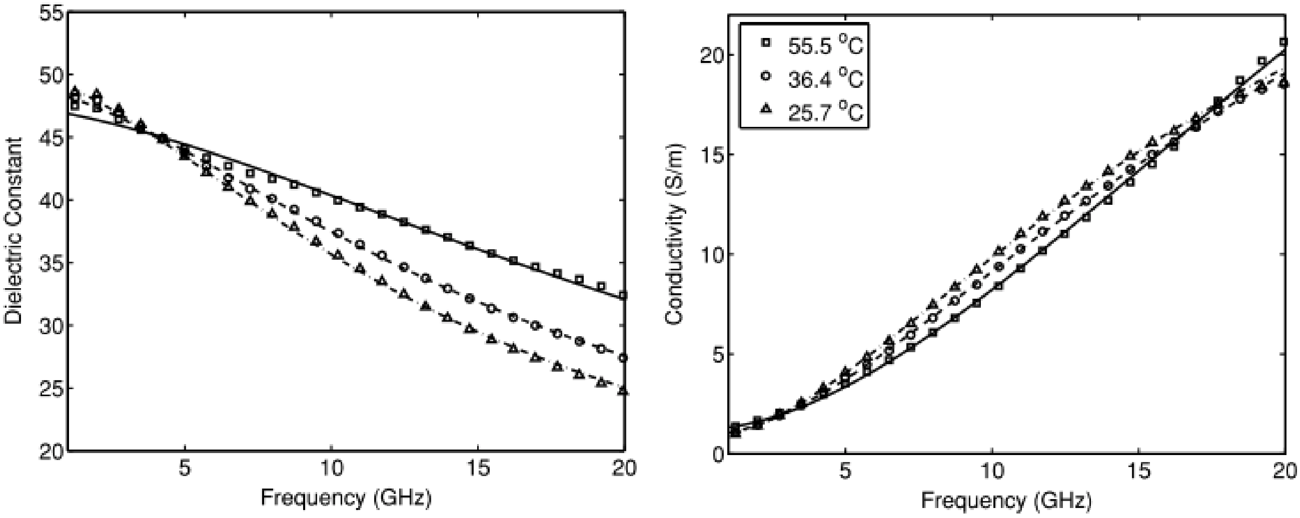

Figure 3: Example of dielectric constant and conductivity of liver tissue as a function of frequency at three distinct temperatures. Reproduced from Lazebnik, Converse, Booske and Hagness (2006).

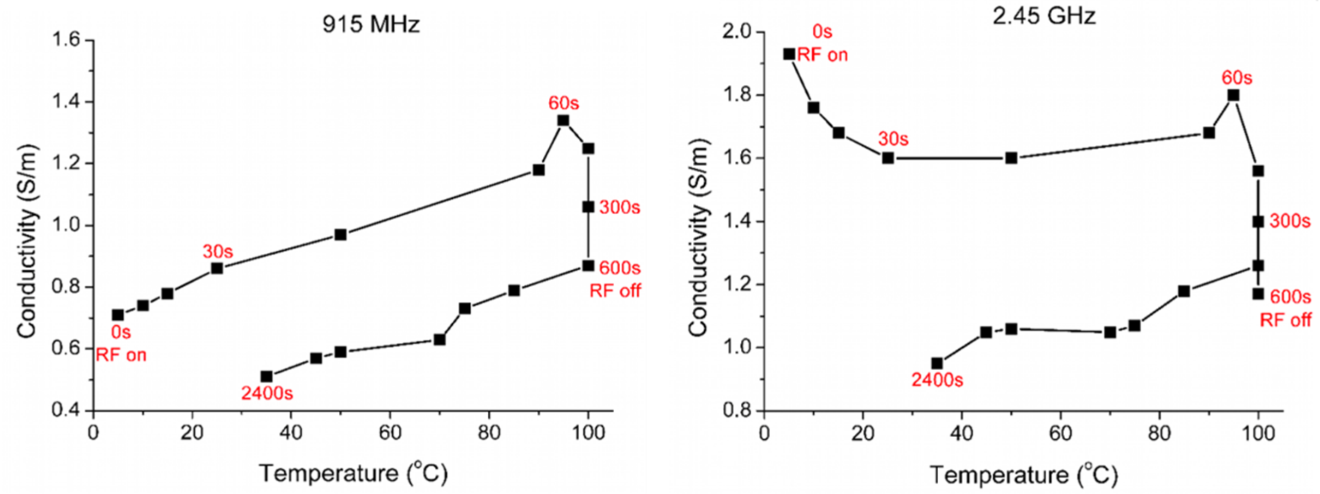

Figure 4: Plots showing changes obtained at 915 MHz and 2.45 GHz in measured conductivity of *ex-vivo* bovine liver with temperature. Reproduced from Brace (2008).

In a study performed by Ji and Brace (2011), it was established that once the 70 °C mark is surpassed, both the relative permittivity and the conductivity decrease significantly as seen from Fig. 5. In addition to the experimental results, this work also performs numerical simulations. Ji and Brace also modelled a time-temperature curve for both the relative permittivity and effective conductivity, and compare their models to others, concluding that sigmodal models better model the temperature-dependence of tissue dielectric properties. Finally, Ji and Brace note that the simulations slightly underestimated the temperatures reached when compared to experiment but these are more accurately determined with sigmodal models.

Lopresto, Pinto, Lovisolo and Cavagnaro (2012) also used a microwave ablator to heat excised bovine liver and determined the dielectric properties at 2.45 GHz. The difference between this study and that of Brace (2008) is that in Lopresto, Pinto, Lovisolo and Cavagnaro (2012) the operating frequency of the ablator was in the frequency range being investigated, and the ablator and the dielectric measurement probe were positioned at 90 °C to each other to avoid electromagnetic coupling between the two (Lopresto, Pinto, Lovisolo & Cavagnaro, 2012; Lopresto et al., 2011). Lopresto et al. compared dielectric measurements when heating the sample tissue with the ablator and in a water bath, and concluded that the heating method was not responsible for any significant differences in the measured values. The final part of their study incorporated computer simulations that compared reasonably well with the measurements.

A further contribution of Lopresto, Pinto, Lovisolo and Cavagnaro (2012) was that they reported on the dependence of the variation of dielectric properties with temperature on the size of the thermal lesion caused by the ablator. The importance of being able to predict the extent of the thermal lesion cannot be overstated in any planned treatment, as the goal is always to extend this to just beyond the tumour region, in order to ensure the destruction of the entire tumour region, while restricting as much as possible the thermal damage to the surrounding healthy tissue. This work also confirms the findings of Brace (2008) that there is a decrease

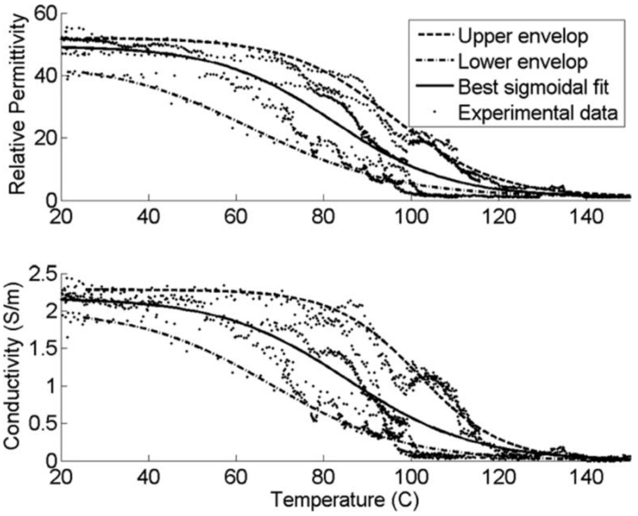

Figure 5: Experimental results (dots) of relative permittivity and conductivity at 2.45 GHz versus temperature during microwave ablation. Also shown are the best-fit sigmoidal curves (solid lines), along with the upper and lower envelops (dashed lines) used for numerical simulation. Plots taken from Ji and Brace (2011).

in the tissue permittivity and conductivity with temperature, as opposed to what was reported by Stauffer et al. (2003). The differences could be due to the fact that Stauffer et al. report on measurements at 915 MHz, whereas in the work of Lazebnik et al. (2006) a turning point is reported between 915 MHz and 2.45 GHz.

A study reported by Jaspard, Nadi and Rouane (2003) investigated the temperature dependence of the dielectric properties of blood, measured with an electrical impedance analyser in the frequency range from 1 MHz to 1 GHz. The conductivity is reported to be sensitive to tissue temperature increases at a rate of $1\,\%\,°C^{-1}$. In contrast, the variations measured for the permittivity were reported to be around $0.3\,\%\,°C^{-1}$ at 1 MHz and $-0.3\,\%\,°C^{-1}$ at 1 GHz, with a change in gradient at 50 MHz. The permittivity increased with temperature till 50 MHz and then decreased with temperature.

The frequency ranges used in interventions related to magnetic resonance image-guided focused ultrasound surgery (MRgFUS) are from 42.58 MHz to 468 MHz. The work reported by Fu, Xin and Chen (2014) measured the dielectric properties of porcine uterus, liver, kidney, urinary bladder, skeletal muscle and fat from 36 °C to 60 °C in this frequency range. They reported the highest positive temperature gradients at 42.6 and 64 MHz for all tissues except fat.

Zhuang, Nelson, Trabelsi and Savage (2007) measured the dielectric properties of chicken breast over the temperature range of 5 °C to 85 °C from 10 MHz to 1.8 GHz. They conclude that below 100 MHz the increase in both the dielectric constant and loss factor are constant with temperature. Zhuang et al. (2007) also show that the change in dielectric properties with temperature also depends on the type of muscle under study and note that there is no difference in both the loss factor and dielectric constant resulting from time following deboning.

Some other studies which examined the variation of dielectric properties with temperature were those of Macchi, Gallati, Braschi and Persi (2014), Ryan, Platt, Dadd and Humphries (1997) and Zurbuchen et al. (2010). However, all these studies were conducted at or below 0.5 MHz and hence fall outside the remit of interest of this review.

From the studies at 915 MHz, tissue dependence can be noted in temperature coefficients of dielectric properties. Chin and Sherar report a $-0.13\,\%\,°C^{-1}$ for bovine liver (Chin & Sherar, 2001) and $-0.31\,\%\,°C^{-1}$ for rat prostate (Chin & Sherar, 2004). The temperature gradients also depend on temperature range, as indicated by Stauffer et al. (2003), who obtained a temperature gradient of $-0.04\,\%\,°C^{-1}$ in ε', which is lower than the values reported in Stauffer et al. (2003) over a wider temperature range, and those reported in Chin and Sherar (2004) and Lazebnik et al. (2006).

At 2.45 GHz, studies by Brace (2008) and Lopresto, Pinto, Lovisolo and Cavagnaro (2012), Lopresto et al. (2011) report a negative temperature gradient of ε' while Lazebnik temperature gradient reported is positive. The main difference between the studies was the tissue heating, as in Lazebnik et al. (2006) the tissue was heated in an oven while in Brace (2008), Lopresto, Pinto, Lovisolo and Cavagnaro (2012) and Lopresto et al. (2011), tissue samples were either heated with a thermal ablator or in a water bath. A decrease in dielectric properties with temperature is most likely due to decreasing tissue water content with temperature, as water content is the major factor affecting dielectric properties in biological tissue. These observations are confirmed by measurements we conducted on freshly excised tissues which in the process for publication.

3 Conclusion

This work reports on studies of the variation of dielectric properties of biological tissue at super physiological temperatures. It is established that the dielectric properties of biological tissues of all types vary with temperature. There is consensus that tissues heated to temperatures exceeding 60 °C undergo irreversible physiological changes. In experiments carried out by our research group resulted in negative temperature gradients of tissue permittivity and conductivity, in line with those reported by other groups. This continues to strengthen the view that tissue desiccation during heating is the main reason for the observed changes.

Finally, we note that the majority of published studies focus on the dielectric properties of liver, which is an organ with a high-water content. It would be useful for further studies of the behaviour of dielectric properties with temperature to be conducted on different tissues.

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Invited Research Article

Action Observation and Execution Network: An Extended View

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Abstract. The mirror mechanism is a basic mechanism that transforms sensory representations of others' behaviours into one's own motor or visceromotor representations concerning that behaviour. In this review, we examine the different functions of the mirror mechanism according to its location in the brain, with particular emphasis on recent data concerning the prefrontal cortex and the emotional centres.

Keywords: mirror mechanism, action understanding, emotion, prefrontal cortex

1 Introduction

For many years, the action execution-observation network has been considered mostly formed by reciprocally connected premotor and parietal areas. In particular, in the monkey, the parietal area PFG and the premotor area F5 were reupted the crucial areas of this network. Functionally, the basic operation of the neurons belonging to these areas was thought to involve a transformation of visual representations of actions into motor representations of the same actions. This transformation was named the Mirror Mechanism. The role attributed to this transformation was that to provide a detailed understanding of others' actions.

In the last few years, evidence was provided that indicates that the action observation network is not limited to the aforementioned areas, but is much more extended and involves additional parietal areas, such as the anterior intraparietal area (AIP), second somatosensory area, primary motor cortex, mesial premotor area F6 and ventral prefrontal areas 46 and 12.

The aim of this review is to describe these new nodes of the circuit, with particular emphasis to their functional role in action execution and observation. We will first review the anatomy and physiology of the action execution and observation system in the monkey, and then we will compare the anatomical and functional properties of these nodes with those of the human action execution and observation circuit. Finally, we will discuss some emotional centres that have recently been thought to comprise of neurons described endowed with the mirror mechanism.

2 Anatomical Organization of the Action Execution-Observation Network in the Monkey

2.1 The Anatomy of the Premotor Nodes of the Action Execution-Observation Network

Fig. 1 shows the architectonic parcellation of the monkey frontal cortex. Among the areas constituting the agranular frontal cortex, the crucial node of the action execution-observation network is area F5. Luppino and co-workers showed that this area is architectonically not homogeneous, but consists of three sub-areas (Belmalih et al., 2009): F5 anterior (F5a), F5 posterior (F5p) and F5 convexity (F5c). Their location is shown in Fig. 1. F5p and F5a are located on the posterior bank of the inferior arcuate sulcus whilst F5c lies on the cortical convexity. Of these sub-areas, F5c and F5p have an agranular structure, while F5a appears to be a transition area towards the prefrontal cortex (Belmalih et al., 2009).

Connectional studies support this architectonic subdivision. In fact, F5c and F5p are strongly connected with the inferior parietal lobule (IPL), and in particular with areas AIP, PF, PFG and the SII-complex, as well as with the primary motor cortex. Area F5 – especially F5p – projects to the spinal cord. Although F5a, F5c and F5p all have connections with IPL, F5a is the only F5 sub-area connected with the ventrolateral prefrontal

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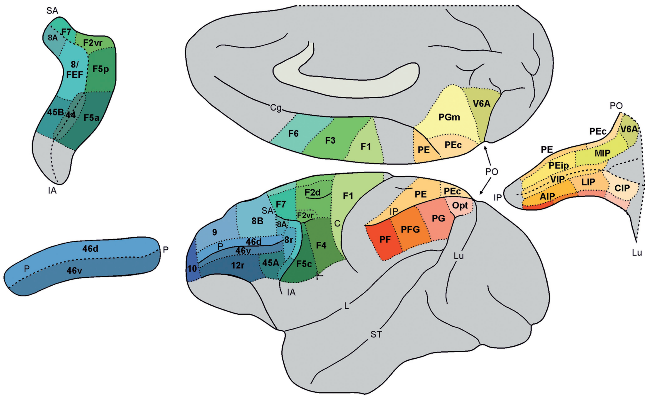

Figure 1: Lateral and mesial views of the macaque brain showing parcellation of the frontal and posterior parietal cortex. The prefrontal cortex is subdivided according to Carmichael and Price (1994), except for its caudo-ventral part (Gerbella, Belmalih, Borra, Rozzi & Luppino, 2007). Agranular frontal areas are classified according to Matelli, Luppino and Rizzolatti (1991) and Belmalih et al. (2009). The parietal areas are named according to Pandya and Seltzer (1982). For the denomination of other areas see the text. The areas located within the arcuate and the principal sulci are shown in an unfolded view of the sulci in the left part of the figure, and the areas located within the intraparietal sulcus in the right part of the figure. Dashed lines indicate the architectonic borders. C: central sulcus; Cg: cingulate sulcus; IA: inferior arcuate; L: lateral fissure; Lu: lunate sulcus; P: principal sulcus; PO: parieto-occipital sulcus; SA: superior arcuate; ST: superior temporal sulcus; DLPF: dorsolateral prefrontal cortex; AI: inferior arcuate sulcus; AS: superior arcuate sulcus; C: central sulcus; Ca: calcarine fissure; Cg: cingulated sulcus; DLPF: dorsolateral prefrontal cortex; IO: inferior occipital sulcus; IP: intraparietal sulcus; L: lateral fissure; Lu: lunate sulcus; P: principal sulcus; PO: parieto-occipital sulcus; ST superior temporal sulcus; VLPF: ventrolateral prefrontal cortex (Modified from Matelli, Luppino & Rizzolatti, 1991).

cortex (areas 46v and 12r) and the frontal operculum (Matelli, Camarda, Glickstein & Rizzolatti, 1986; Gerbella, Belmalih, Borra, Rozzi & Luppino, 2011). Furthermore, whilst essentially F5a is neither connected with the primary motor cortex nor the spinal cord, it is richly linked with the other premotor areas. In particular, it has important connections with mesial premotor area F6 (Luppino, Matelli, Camarda & Rizzolatti, 1993; Gerbella et al., 2011).

Area F6 (preSMA) belongs to the agranular frontal cortex. However, if compared to the adjacent area F3 (SMA), it shows some features granularity proper to the prefrontal areas (Matelli, Luppino & Rizzolatti, 1991). Furthermore, it lacks direct connections with the primary motor cortex and the spinal cord (Luppino et al., 1993; Dum & Strick, 2002). F6 is also linked with the cingulate cortex (including area 24c) and with the prefrontal cortex, in particular with the intermediate part of both the ventral and dorsal portions of area 46. In the parietal lobe, it is connected with areas PG and PFG, in addition to forming weaker connections with AIP and V6A (Luppino et al., 1993; Gamberini et al., 2009).

2.2 The Anatomy of the Parietal Nodes of the Action Observation-Execution Network

The parietal area considered crucial for action observation and execution is area PFG (Gallese, Fadiga, Fogassi & Rizzolatti, 2002; Fogassi et al., 2005; Rozzi, Ferrari, Bonini, G & Fogassi, 2008). PFG is strongly connected with neighbouring inferior parietal areas (PF, PG, AIP), with the parietal operculum (PGop and SII), with the insula and with ventral premotor areas, especially area F5 and prefrontal area 46 (Rozzi et al., 2006; Bruni et al., 2018).

Another area belonging to the action execution and observation network is AIP. This area is strongly connected with other inferior parietal areas (PF, PFG, PG, LIP, VIP), and with the parietal operculum (PGop and SII). Further strong connections of AIP have been described with a large sector of the inferior temporal cortex, the insula, the ventral premotor area F5, and the ventral prefrontal cortex (Area 12r and 46v).

2.3 The Anatomy of the Prefrontal Nodes of the Action Execution-Observation Network

It is well known that a specific sector of the ventrolateral prefrontal cortex is connected with the premotor and parietal areas containing mirror neurons. This sector includes part of areas 12r and 46v (see Fig. 1). In the monkey, area 46v occupies almost the entire rostrocaudal extent of the ventral bank of the principal sulcus. The rostral two-thirds of the convexity cortex, adjacent to area 46v, is occupied by area 12r. Recently, Luppino and co-workers, on the basis of connectional studies, showed that areas 12r and 46v could be subdivided into three caudo-rostrally located vertical strips (Fig. 2). The caudal most strip is mainly connected with cortical areas and subcortical centres involved in the control of eye movements (Gerbella, Belmalih, Borra, Rozzi & Luppino, 2010, 2013; Borra, Gerbella, Rozzi & Luppino, 2015). More specifically, it is connected with the parietal area LIP, frontal areas FEF and SEF, and the intermediate and superficial layers of the superior colliculus. The rostral most strip essentially shows intrinsic prefrontal connections, including the frontal pole and the orbital prefrontal areas (Borra, Gerbella, Rozzi & Luppino, 2011; Gerbella et al., 2013; Saleem, Miller & Price, 2014). Finally, the intermediate strip is connected with parietal and premotor areas, as well as with subcortical structures involved in forelimb action execution and observation (Borra et al., 2011, 2014; Gerbella et al., 2013, 2016). This latter strip has been recently proposed as an additional prefrontal node of the action execution-observation network (Bruni et al., 2018).

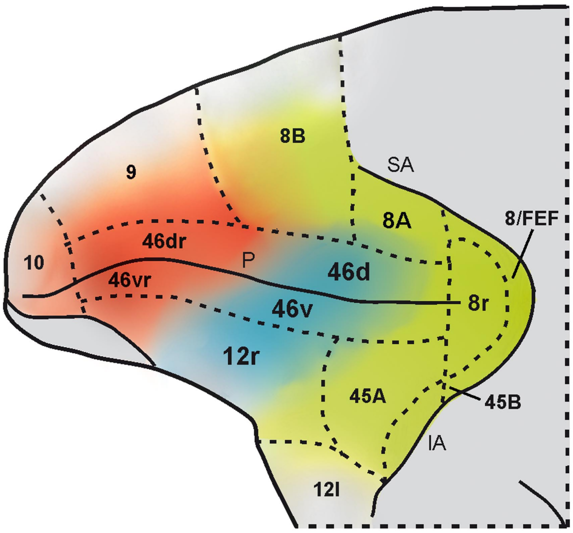

Figure 2: Lateral view of the monkey prefrontal cortex. Dashed lines indicate the architectonic borders. The coloured shadings delimit three groups of areas. Green: areas mostly connected to oculomotor cortical and subcortical centers (Gerbella, Belmalih, Borra, Rozzi & Luppino, 2010, 2013; Borra, Gerbella, Rozzi & Luppino, 2015; Borra, Ferroni et al., 2017); blue: areas mostly connected to skeletomotor cortical and subcortical centers (Borra, Gerbella, Rozzi & Luppino, 2011, 2014; Gerbella, Borra, Tonelli, Rozzi & Luppino, 2013, 2016; Borra, Ferroni et al., 2017); red: areas mostly showing intrinsic prefrontal connections (Borra, Gerbella, Rozzi & Luppino, 2011; Gerbella, Borra, Tonelli, Rozzi & Luppino, 2013; Borra, Ferroni et al., 2017).

Albeit, the intermediate strip of areas 12r and 46v is connected to areas forming the classically described action execution-observation network, each of them showing some specificity in their connections. Area 12r is connected with areas AIP and F5, particularly with F5a. In addition, it is also linked with the SII-complex and with the anterior part of the ventral bank of STS (Borra et al., 2011), possibly corresponding to area LB2, an area active during observation of hand grasping actions (Nelissen et al., 2011). Additional connections were also observed in the middle part of the insula. The adjacent area 46v shows a pattern of connections similar to that of area 12r, displaying connections with areas F5a and AIP, as well as with SII and the insula. In addition, it is strongly connected with area PFG, and to a lesser extent with area F6, cingulate motor area 24 and with the temporal lobe (Gerbella et al., 2013). Note that the connections of area 12r and 46v with the temporal lobe increase ventrally, suggesting a stronger role of ventral prefrontal areas in processing of object visual features. Conversely, the connections with the parietal and premotor areas increase dorsally, suggesting a major role for area 46v in controlling motor behaviour.

In conclusion, the prefrontal information reaches premotor action execution-observation areas through two main gateways: F5a, ventrally, and F6, dorsally.

3 Coding Motor Goals: The Vocabulary of Motor Acts

Single neuron studies revealed that most of F5 neurons code specific motor acts, rather than individual movements (Rizzolatti et al., 1988). F5 neurons were subdivided into various classes based on the effective motor act as the classification criterion. Neurons discharging for a specific motor act typically do not discharge during the execution of similar movements aimed at a different goal. For example, a neuron that discharges during finger movements for grasping an object does not discharge during similar movements aimed at scratching. On the other hand, F5 neurons typically discharge when the same goal is achieved by using different effectors (e.g. the right hand, the left hand or the mouth), and thus requiring completely different movements (Fig. 3A).

On this basis it has been proposed that F5 contains a “vocabulary” of motor acts (Rizzolatti et al., 1988). A further demonstration that F5 neurons encode motor acts has been provided by a study (Umiltà et al., 2008) in which the same motor goal (taking possession of food) was achieved by means of opposite movements. Monkeys grasped objects using “normal pliers”, which require hand closure in order to take possession of the object, and “reverse pliers”, that require hand opening to achieve the same goal (Fig. 3B). In both cases, the neural discharge encoded food grasping, regardless of whether it was achieved by closing the hand or by opening it. Similar to area F5, PFG neurons also typically code the goal of the motor acts rather than movements (Gallese et al., 2002; Fogassi et al., 2005; Rozzi et al., 2008).

Subsequent studies addressed the issue of how the intention of an action, i.e., the overarching goal of a series of motor acts, is encoded in both PFG and F5 neurons. With this aim, grasping neurons were recorded in two conditions: in one, the monkey grasped a piece of food and brought it to the mouth for eating, whilst in the other, it grasped an object or a piece of food to place it into a container (Fogassi et al., 2005; Bonini et al., 2010). Some neurons discharged stronger when the monkey grasped food to bring it to its mouth, while a weaker or absent response was observed when it grasped food to put it into a container (Fig. 3C). Other cells had an opposite behaviour (Fig. 3C). Interestingly, the difference in response in the two conditions was already present during grasping that was executed in exactly the same way. The differential activation of these neurons according to the overarching goal, represents the neural substrate of the acting individual intention.

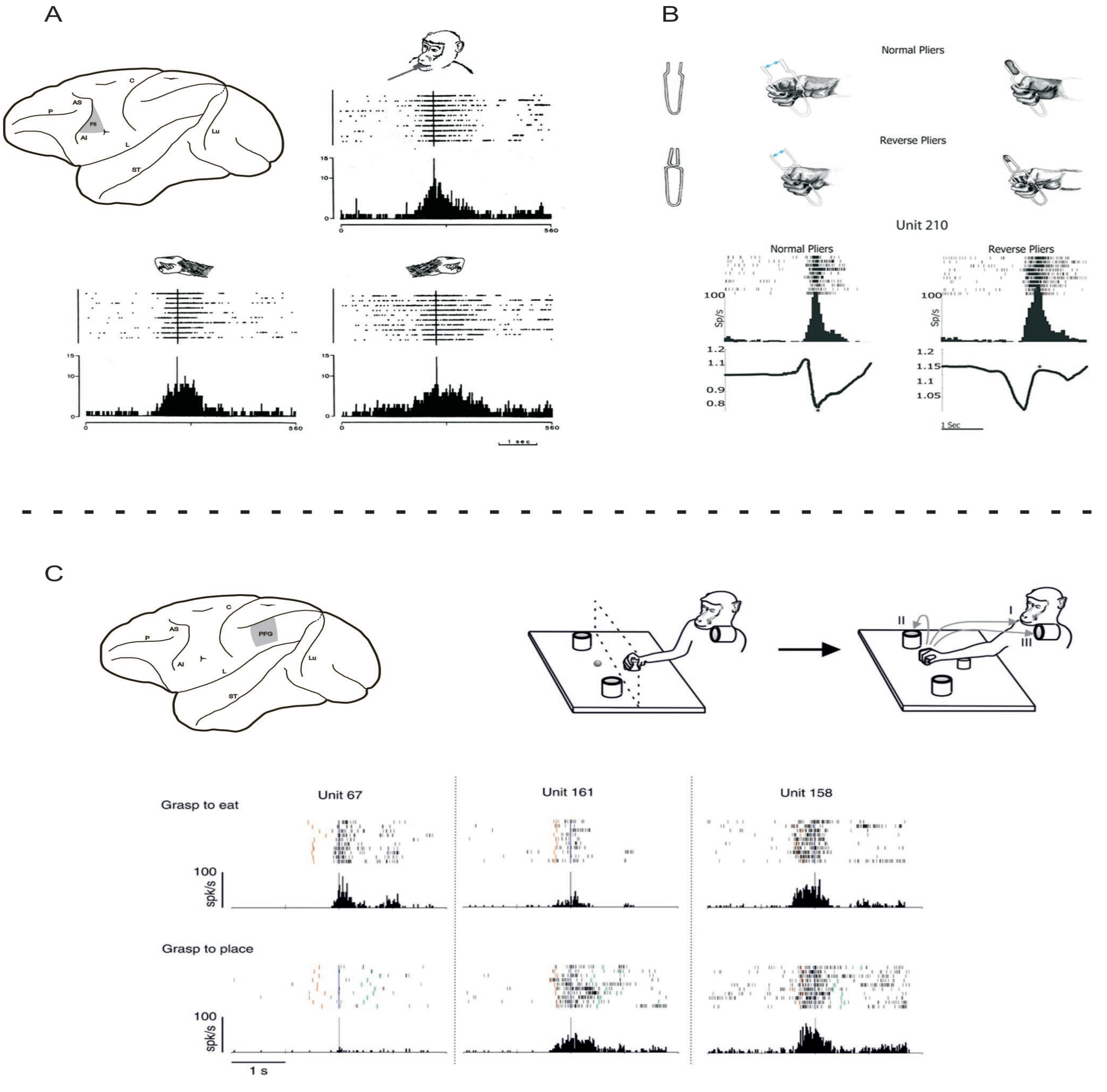

Figure 3: Goal and intention encoding in areas F5 and PFG. **A:** Upper part, left: lateral view of the monkey brain showing the location of area F5, right and lower part: discharge of an F5 neuron active during grasping with the mouth, the right hand and the left hand. Conventions as in Fig. 6A. **B:** Example of an F5 neuron discharging during grasping with normal and reverse pliers. Upper part: Pliers and hand movements necessary for grasping with the two types of pliers. Lower part: Rasters and histograms of the neurons' discharge during grasping with pliers. The alignments are with the end of the grasping closure phase (asterisks). The traces below each histogram indicate the hand position, recorded with a potentiometer, expressed as function of the distance between the plier's handles. When the trace goes down, the hand closes, when it goes up, it opens. The values on the vertical axes indicate the voltage change measured with the potentiometer. Other conventions as in Fig. 6A. **C:** Example of a motor neuron in PFG modulated by action intention. Upper part left: lateral view of the monkey brain showing area PFG. Upper part right: paradigm used for the motor task. The monkey, starting from a fixed position, reaches and grasps a piece of food or an object, then it brings the food to the mouth and eats it (I, grasp-to-eat), or places it into a container (II/III, grasp-to-place). Lower part left: Activity of three IPL neurons during grasping in the two actions. Rasters and histograms are aligned with the moment when the monkey touched the object to be grasped. Red bars: monkey releases the hand from the starting position. Green bars: monkey touches the container. Modified from Rizzolatti et al. (1988) (**A**), Umiltà et al. (2008) (**B**) and Fogassi et al. (2005) (**C**).

4 Mirror Neurons and Action Understanding

Areas F5 and PFG contain a peculiar class of visuomotor neurons, the mirror neurons. These neurons discharge, both when the monkey performs a goal-directed motor act and when it observes the same, or a similar, motor act performed by another individual (Fig. 4A; di Pellegrino, Fadiga, Fogassi, Gallese & Rizzolatti, 1992; Gallese, Fadiga, Fogassi & Rizzolatti, 1996; Rizzolatti, Fadiga, Gallese & Fogassi, 1996). Mirror neurons do not respond to object presentation. The observed hand motor acts more effective in eliciting mirror neurons discharge are grasping, manipulating and holding. The visual response of many mirror neurons is invariant with respect to visual aspects of the observed action. However, other mirror neurons show specificity for the direction of the hand movement (left or right), the space sector in which the observed motor act is presented (close or far), or the hand used by the observed agent (left or right) (Caggiano, Fogassi, Rizzolatti & Thier, 2009; Gallese et al., 1996). In terms of the location of the motor act presentation, it was found that half of mirror neurons sensitive to this parameter discharged stronger when the motor act was performed within the monkey's peripersonal space, while the other half responded better when the same motor act was performed in the extra-personal space. Interestingly, when the monkey's peripersonal space (defined as the space within which grasping is possible) was reduced by introducing a transparent barrier, and thus the space was no longer reachable, a set of extra-personal neurons started to discharge within the previously peripersonal space (Caggiano et al., 2009).

It has been proposed that the observation of a motor act done by others determines an automatic retrieval of a potential motor act from the "vocabulary" of the observer, thus enabling mirror neurons to encode the visual description of a goal directed act in motor terms, allowing the observer to understand what another individual is doing. The hypothesis that mirror neurons have an important role in the understanding of others' motor acts has been supported by various studies. In one study, it was shown that grasping mirror neurons discharge not only when the monkey observes a grasping motor act (effective visual stimulus), but also when it sees the agent's hand moving towards the target hidden by an opaque screen (Umiltà et al., 2001). The discharge was absent when the monkey knew that there was no object behind the screen. This finding suggests that mirror neurons use prior information to retrieve the motor representation of the observed motor act.

In another study, sensory information concerning the motor act was presented to the monkey in an acoustic and/or a visual format (Kohler et al., 2002). It was found that a subset of mirror neurons, called "audio-visual mirror neurons", discharged not only during execution and observation of a motor act (e.g. breaking a peanut), but also by listening to the typical sound produced by that act. This indicates that a motor act is understood regardless of how the information reaches the mirror neurons.

Besides mirror neurons encoding hand motor acts, mouth mirror neurons have been also described. These neurons are mostly found in the lateral part of area F5. The majority of them respond to the observation and execution of ingestive motor acts such as biting, sucking and licking (Ferrari, Gallese, Rizzolatti & Fogassi, 2003; Ferrari, Gerbella, Coudé & Rozzi, 2017). They do not respond to object presentation or to mouth mimed motor acts.

In the previous sections we showed that PFG grasping neurons discharge is modulated by the overarching action intention (Fogassi et al., 2005). For this purpose their activity was recorded while the monkey executed a motor task in which the same motor act (grasping) was embedded into two different actions (grasping to eat and grasping to place). The neurons were also tested with the monkey observing the same task, performed by an experimenter. Similar to the motor task outcome, in the visual task most mirror neurons also discharged differently during grasping, depending on overarching goal of the actions (Fig. 4B). Since in this case grasping was performed by the observed agent, it was suggested that the neuronal selectivity for the action goal during grasping observation activated the chain of motor neurons corresponding to a specific intention. Similar results were also obtained in area F5, where the same paradigm was applied (Bonini et al., 2010).

5 Mirror Neurons in Additional Nodes of the Action-Observation Network

5.1 Primary Motor Cortex

It has been recently addressed the issue of whether the output of premotor and motor cortex contains neurons endowed with mirror properties. Kraskov and collaborators (Kraskov, Dancause, Quallo, Shepherd & Lemon, 2009) have recently investigated the activity of corticospinal neurons (PTNs) located in both area F5 and F1, in order to address the issue regarding whether the output of premotor and motor cortex contains neurons endowed with mirror properties.

They found that about half of F5 cortico-spinal neurons responded to action observation. Interestingly, about 25% of these PTNs showed a suppression of their discharge when the monkey observed the experimenter grasping an object. The authors suggested that the suppression of the PTNs during grasping observation may play a role in inhibiting the movement of the observer

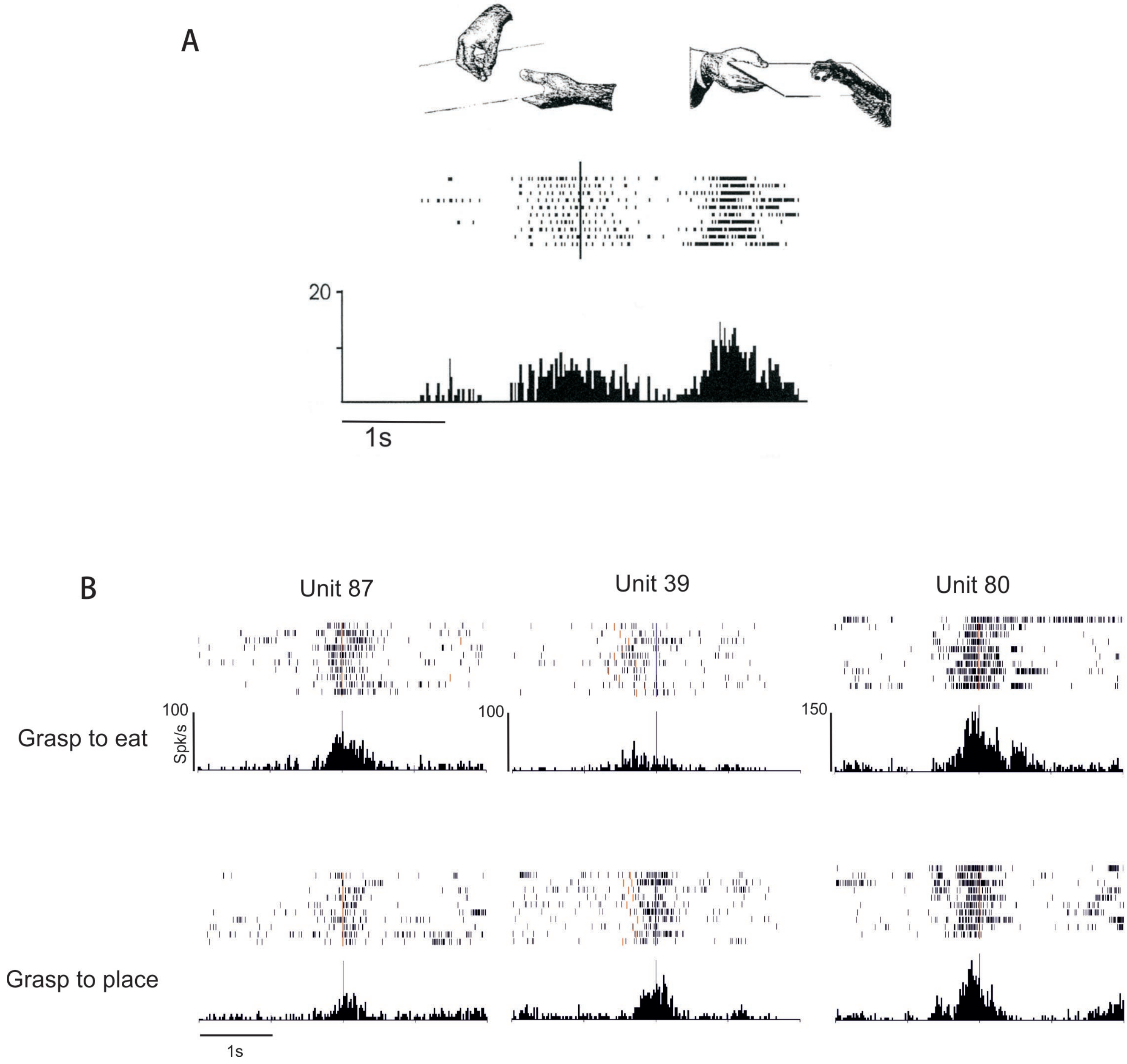

Figure 4: Mirror neurons. **A:** Example of an F5 mirror neuron. Left: Grasping observation. Right: Grasping execution. **B:** Examples of parietal mirror neurons modulated by action motor intention. Activity of three IPL neurons during grasping observation of two actions: grasp-to-eat, and grasp-to place. Rasters and histograms are aligned with the moment when the experimenter touched the object to be grasped. Red bars: experimenter grasps the object. Unit 87 is selective for observation of grasping to eat, while unit 39 is selective for observation of grasping to place. Unit 80 is not selective. Conventions as in Fig. 3. Modified from di Pellegrino, Fadiga, Fogassi, Gallese and Rizzolatti (1992) (**A**) and Fogassi et al. (2005) (**B**).

triggered by the observed action.

The same paradigm was applied to F1 PTNs (Kraskov et al., 2009). As in F5, about half of these neurons were modulated during action observation. The majority increased their discharge during action observation ("facilitation-type" mirror neurons), while some reduced or stopped their firing ("suppression-type" mirror neurons). A comparison of the properties of F1 and F5 PTNs mirror neurons showed that the visual response in F1 was much weaker than in F5. Taken together these data indicate that the understanding of motor goals is not only the function of F5 mirror neurons, but rather of the activation of a complex motor pattern that involves corticospinal tract neurons, including those originating in F1.

5.2 Area F6 (Pre-SMA)

Several physiological investigations indicated that area F6 has a role in forwarding prefrontal signals to posterior premotor areas, allowing the transformation of potential actions into actual movements, by determining movement onset based on contextual and motivational information (Gerbella, Rozzi & Rizzolatti, 2017; Borra, Gerbella, Rozzi & Luppino, 2017). This idea is in line with the presence of strong F6 connections with prefrontal, cingulate and premotor areas (Luppino et al., 1993; Morecraft et al., 2012). Recent studies show that F6 also plays a role in social behaviour, forming part of a brain network dedicated to the processing of social interactions (Sliwa & Freiwald, 2017), as well as hosting neurons encoding others' actions (Yoshida, Saito, Iriki & Isoda, 2011, 2012; Livi et al., submitted). In particular, Isoda and co-workers (Yoshida et al., 2011) trained two monkeys to monitor each other's actions for adaptive behavioural planning. In each trial, one monkey was assigned the role of the actor and the other the role of the observer, inverting the role every two trials. They found that F6 and the adjacent cingulate motor cortex, besides containing neurons that discharge during monkey actions, also host neurons that fire during action execution and observation ("mirror" neurons), as well as neurons that fire during the observation of other's actions exclusively ("partner" neurons). In a subsequent experiment, Yoshida et al. (2012) used the same paradigm to analyse the neural activity during actor's error trials. They identified a set of neurons that showed a significant activity increase associated with errors of the monkey actor. Nearly half of these neurons showed activity changes consistent with general reward-omission signals, whereas the remaining neurons specifically responded to another's erroneous actions. These findings are in line with the recent demonstration of "other-predictive neurons" in the cingulate motor cortex (Haroush & Williams, 2015).

Similarly, a recent study (Livi et al., submitted) showed that area F6 hosts neurons selectively encoding objects when they are the target of monkey's grasping action (self-type), another agent's action (other-type), or both (mirror neurons or self-and-other type). This type of code strongly depends on the position of the object in the peripersonal space of both agent and observer. These findings suggests a novel, non-inferential "object-mirroring" mechanism, through which observers could accurately predict another's impending action by recruiting the same motor representation they would activate if they were to act upon the same object in the same context.

5.3 Area AIP

As described above, IPL mirror neurons were originally discovered in PFG (Fogassi et al., 2005; Gallese et al., 2002; Rozzi et al., 2008), but were more recently also found in AIP and in SII (Maeda, Ishida, Nakajima, Inase & Murata, 2015; Pani, Theys, Romero & Janssen, 2014; Hihara, Taoka, Tanaka & Iriki, 2015; Lanzilotto et al., submitted).

Although AIP is classically considered to be involved in the visuomotor transformations for grasping, recent data showed that it also contains mirror neurons.

In recent years, three independent studies described the presence of neurons in AIP that are sensitive to action observation (Maeda et al., 2015; Pani et al., 2014; Lanzilotto et al., submitted). In particular, they showed that a set of AIP neurons that are active during grasping execution, is also activated by the observation of a video of the same grasping action seen from a first-person perspective (Maeda et al., 2015; Pani et al., 2014). Interestingly, neural activation was also observed when the hand movement is visible, while object target of the action is not. Lanzilotto et al. (submitted) showed that AIP grasping neurons also activate during observation of other hand manipulative actions observed from a third-person perspective. Altogether, these data indicate that AIP mirror neurons play a crucial role in the visual control of own action and in action understanding.

5.4 Area SII

Classical neurophysiological studies performed in macaque monkeys have shown that the secondary somatosensory cortex (SII) is essentially engaged in the processing of somatosensory information. In contrast, subsequent human brain-imaging investigations have revealed the effects of visual and auditory stimuli on SII activity, suggesting the presence of multisensory integration in this region (Keysers et al., 2004; Gazzola & Keysers, 2009). Accordingly, recent deoxyglucose and electrophysiological experiments in monkeys showed that this region is active during manual action execution and observation, particularly when the viewpoint is a first-person perspective (Raos & Savaki, 2016; Ishida, Fornia, Grandi, Umiltà & Gallese, 2013; Hihara et al., 2015). In particular, Hihara and co-workers demonstrated that 30% of SII neurons respond to visual stimuli. About $^1/_3$ of visual neurons respond to the observation of human action. These data and the anatomical connections of SII with many nodes of action observation and execution network (Gerbella, Rozzi & Rizzolatti, 2017; Borra, Gerbella, Rozzi & Luppino, 2017; Bruni et al., 2018) suggest that this region is involved in coding an observer-centred, haptic description of actions.

5.5 Ventrolateral Prefrontal Cortex

Social interactions deeply rely on the understanding of others' actions and the predictions of their outcomes. Thus, if the prefrontal cortex has a role in this type of interaction, one could predict the presence of neurons sensitive to others' actions. In recent years, it has been shown that this may be the case for arm actions. Nelissen, Luppino, Vanduffel, Rizzolatti and Orban (2005), in an fMRI monkey experiment, identified a VLPF activation during the observation of grasping actions contrasted with the observation of static controls or scrambled stimuli. The activated areas included areas 46, 45A, and 45B.

Concerning single-neuron studies, Tsunada and Sawaguchi (2012) recorded monkey VLPF neurons selectively activated during the observation of videos showing conspecifics grooming or mounting another monkey and not when the monkeys observed videos in which several conspecifics were present but not interacting. In a recent study, monkeys were trained to observe videos showing arm and hand movements and object motion during a VLPF neuronal activity recording (Simone, Bimbi, Rodà, Fogassi & Rozzi, 2017). Arm and hand movements included goal-directed actions performed by monkeys or humans (e.g., reaching-grasping of food/objects) and non-goal-directed movements performed by humans (e.g., extending an arm or pantomiming the grasping action). The main result was that in areas 12, 46 and 45A, there are neurons responding to the observation of arm movements. The observation of reaching-grasping actions was the most effective stimulus (Fig. 5A). To assess whether the neural discharge to action observation was dependent on visual information, a control task was carried out in which different parts of the actions, including the final goal, were obscured. The response of most of the tested neurons was unaffected by the obscuration (Fig. 5B). Thus, the interpretation of this effect was that these neurons code the goal of the action. This type coding is similar to that demonstrated for premotor mirror neurons. Interestingly, a portion of prefrontal action observation neurons, are mirror neurons, since they also discharged when the monkey performs a grasping action (Simone, Rozzi, Bimbi & Fogassi, 2015).

In addition, this study demonstrated that VLPF neurons can code other features of the observed action, such as the agent (human or monkey) or the perspective from which the action was observed (first- or third-person perspective). In fact, the most effective observed actions were those performed by a monkey, especially from the first-person perspective. A preference for this perspective was found also in mirror neurons of the ventral premotor area F5, which were recorded during monkeys' observation of videos depicting grasping actions performed by another monkey from three different perspectives (Caggiano et al., 2011).

6 Further Nodes of the Action Execution-Observation Network: Indirect Evidence

In a recent study, neural tracers were injected in F5, in PFG/AIP or in both these regions hosting mirror neurons (Bruni et al., 2018). This study allowed the identification of all nodes of the network, including those in which mirror neurons has not been yet described (Fig. 6). For example, projections to both F5 and PFG/AIP sectors containing mirror neurons originate from limbic structures. In particular, connections were found with a portion of the insula where long-train microstimulation evokes forelimb movements (Jezzini, Caruana, Stoianov, Gallese & Rizzolatti, 2012). These projections may provide the action execution and observation network with information related to the internal states underlying executed and observed actions and their affective significance (vitality forms; Di Cesare, Di Gio, Marchi & Rizzolatti, 2015).

In addition, we identified subcortical connections with specific polysensory and sensory-motor thalamic nuclei (Central lateral, Mediodorsal, and Pulvinar) and with the mid-dorsal claustrum. Furthermore, different areas of the action execution-observation network (F5, PFG/AIP and 12r/46v) project to the same portion of the basal ganglia (Gerbella et al., 2016). The role in the mirror mechanism of these cortical and subcortical nodes has still to be elucidated.

7 The Mirror Mechanism in Humans

7.1 Goal-Directed Arm, Hand and Mouth Actions

The existence of the mirror mechanism in humans has been demonstrated by a large number of neurophysiological (EEG, MEG and TMS) and neuroimaging (PET and fMRI) studies (Rizzolatti & Craighero, 2004; Caspers, Zilles, Laird & Eickhoff, 2010; Grosbras, Beaton & Eickhoff, 2012; Molenberghs, Cunnington & Mattingley, 2012). These studies have shown that the mirror network mainly includes two regions: the dorsal part of the inferior parietal lobule, comprising the cortex located inside the intraparietal sulcus and the ventral premotor cortex, plus the caudal part of the inferior frontal gyrus (area 44). Additional areas, such as the dorsal premotor cortex and the superior parietal lobule, were also found to be active during action observation (Di Dio et al., 2013; Filimon, Nelson, Hagler & Sereno, 2007).

Similar to monkeys, there is clear evidence from fMRI studies that human mirror sectors encode the goal of motor acts. Gazzola and colleagues (Gazzola, Rizzo-

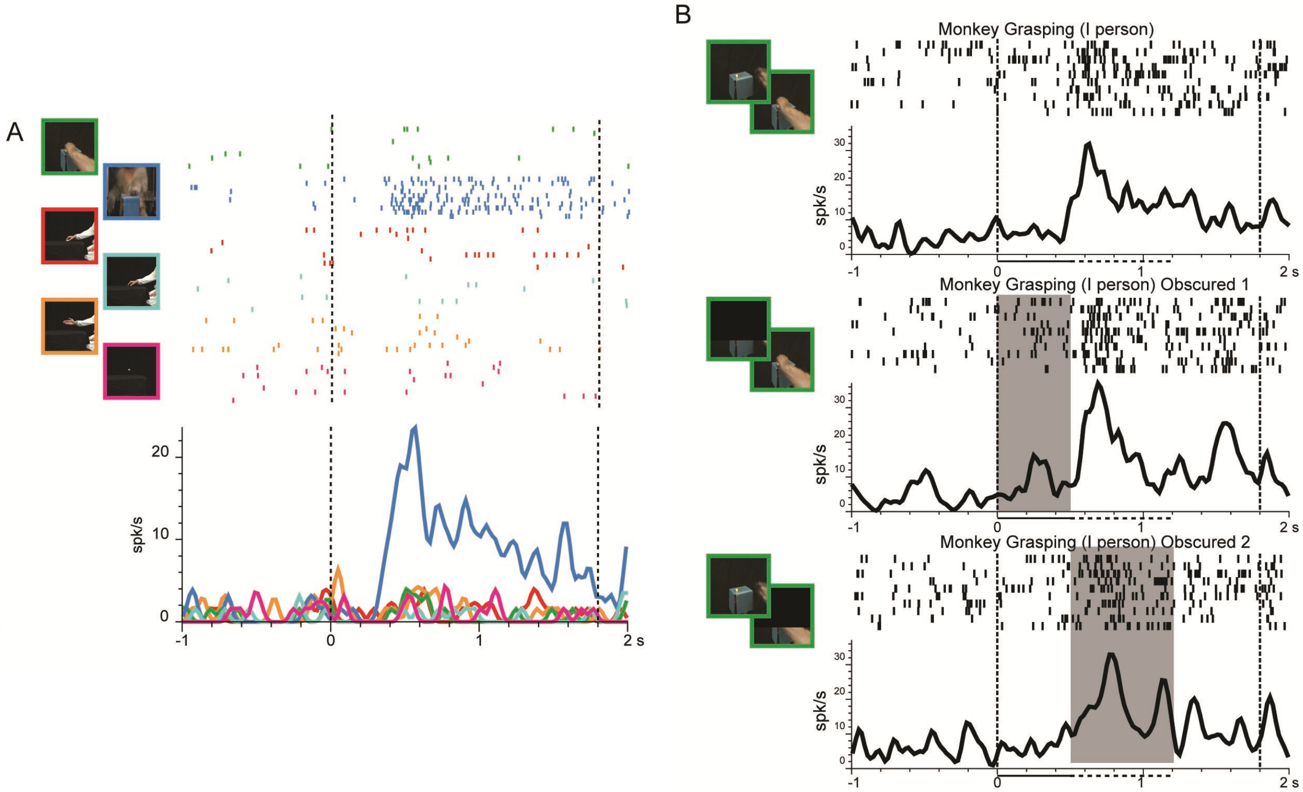

Figure 5: Prefrontal neurons responding to action observation. **A:** Neuron responding exclusively during the observation of a monkey grasping a piece of food from a third person perspective. **B:** Example of a neuron discharging during the observation of a monkey grasping an object from a first person perspective (Video Epoch 2), that, with respect to when the video is fully visible (top), does not change its discharge when either the first (middle) or second (bottom) video epoch is obscured. The activity is aligned on the beginning of the video presentation. Abscissae: time (s); Ordinates: firing rate (spikes/s). The shaded areas on rasters and histograms in **B** indicate the timing of obscuration. The horizontal lines under the x-axis indicate the duration of the first (continuous line) and second (dashed line) Video Epochs considered for statistics. Modified from Simone, Bimbi, Rodà, Fogassi and Rozzi (2017).

latti, Wicker & Keysers, 2007) presented volunteers with video-clips depicting either a human or a robot arm grasping objects. The results showed that the parieto-frontal mirror circuit was activated by both these types of stimuli. This observation was extended by Peeters and colleagues (Peeters et al., 2009). They investigated the cortical activations in response to the observation of motor acts performed by a human hand, a robot hand and a variety of tools in both humans and monkeys. Regardless of the type of effector used, the mirror circuit was activated in humans, as well as in monkeys. In humans, the observation of tool motor acts also activated a rostral sector of the left anterior supramarginal gyrus. Such activation was absent in monkeys, even when they observed actions made with the tools they have learned to use.

A series of experiments addressed the issue of the somatotopic organization of the areas endowed with the mirror mechanism (Buccino, Binkofski & Fink, 2001; Wheaton, Thompson, Syngeniotis, Abbott & Puce, 2004; Wilson, Saygin, Sereno & Iacoboni, 2004; Sakreida, Schubotz, Wolfensteller & von Cramon, 2005; Shmuelof & Zohary, 2005; Ulloa & Pineda, 2007). These studies showed that the observed motor acts are encoded in the precentral gyrus according to a rough somatotopic organization similar to that of the classical motor physiology. A somatotopic organization was found to be also present in the inferior parietal lobule along to and within the intraparietal sulcus. The mouth is located rostrally, the hand in an intermediate position and the leg caudally (Buccino et al., 2001). A recent study by Jastorff, Begliomini, Fabbri-Destro, Rizzolatti and Orban (2010) tried to better define the general principles underlying the somatotopic organization in the parietal and frontal cortex. Four motor acts (grasping, dragging, dropping and pushing) performed with the mouth, hand and foot were presented to volunteers. The results confirmed the data from previous authors concerning the premotor cortex. As for the parietal lobe, they showed that different sectors of IPL were activated

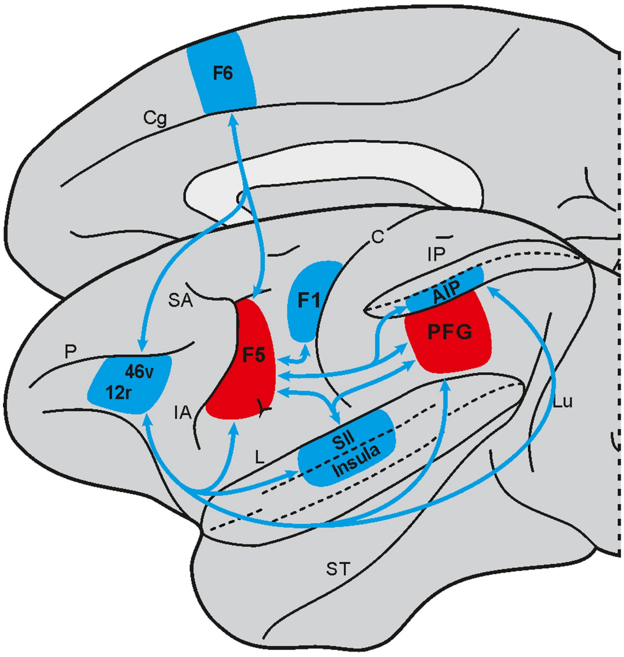

Figure 6: Lateral and mesial views of the macaque brain showing the connections between the two main nodes (red) and the additional ones (blue) forming the extended object-grasping network. Abbreviations as in Fig. 1.

by the observation of motor acts having the same behavioural valence, independent of the observed effector. More specifically, there was a subdivision between the localization of self-directed (grasping and dragging) and outward-directed motor acts (dropping and pushing). Therefore, it appears that, while in the premotor cortex motor acts executed with the same effector tends to cluster together, in the parietal cortex the encoding is biased by the action valence.

A few studies also showed that in humans the mirror network is involved in intention understanding (Iacoboni et al., 2005; Ortigue, Sinigaglia, Rizzolatti & Grafton, 2010). In an fMRI experiment Iacoboni and co-workers (Iacoboni et al., 2005) tested volunteers in three conditions: a) “context”; b) “action”; c) “intention”. In the context condition individuals were presented with a scene showing either a “ready breakfast” or a “finished breakfast”; in the action condition, they saw pictures of a hand grasping a mug, without context; in the intention condition, the individuals saw the same hand grasping the mug within one of the two contexts. The context represented the clue that allowed the participants to understand the agent’s intention. The comparison between conditions showed that intention understanding determined the strongest increase in the activity of the mirror system, in particular of its frontal node.

7.2 Vitality Forms

Actions might take different forms, for example a hand shaking can be delicate or vigorous. Attention to the ”forms” of the observed gestures provides information about the agent’s affective states, moods and attitudes. Stern coined the term “vitality affect” or “vitality form”, in order to highlight that they are routed in our more basic social interactions, thus deeply shaping our experience of ourselves or others (Stern, 1985, 2010).

Vitality form attracted very little attention from neurophysiologists. Recent human functional imaging studies (Di Cesare et al., 2015) have provided evidence that the middle dorsal insula is modulated by the affective aspects of the movements, during both execution and observation of actions. Based on this finding, it has been suggested that during the execution of an action, the dorso-central insula modulates how it is performed according to the affective state of the agent, whereas during action observation it allows the observer to recognize the affective state of the agent. An interesting question points to discovery of the anatomical substrate allowing the insula to modulate the parieto-frontal circuit for action execution and observation. Recent diffusion tensor imaging evidence demonstrated that the dorso-central insula is connected with all the parietal, premotor and prefrontal nodes of the action execution and observation network. The results of this study provided the anatomical pathways through which the insula influences the expression of the affective state of the agent and/or allows recognizing those of the others (Di Cesare et al., 2018).

8 Emotions in Humans and Monkeys

8.1 Disgust

In contrast to the dorso-central part of the insula, its anterior part is involved with oro-alimentary behaviours in both humans and monkeys. In particular, an extensive mapping of the monkey insula using electrical stimulation showed that its anterior sector determines positive or negative ingestive behaviours (Jezzini et al., 2012). In particular, the stimulation of the dorsal part produces a series of mouth and tongue movements typical of food ingestive behaviours; in contrast the stimulation of the ventral sector determines typical disgust behaviours, such as grimaces, spiting out the food, etc. Similar results were found in experiments carried out in drug-resistant epileptic human patients (Krolak-Salmon et al., 2003).

The anterior insula became activated, not only following electrical stimulation, but also after the administration of natural disgusting stimuli, such as unpleasant odors (Wicker et al., 2003). Most interestingly, this fMRI study showed that the anterior insula mediates both the experience of disgust determined by natural stimuli, and also the observation of someone else expressing disgust (Wicker et al., 2003). More specifically, in some runs of this study participants were exposed to disgusting or pleasant odorants, whereas in other runs they observed short movie clips of other individuals displaying a facial expression of disgust or pleasure. The main finding was that the observation of others' disgust activated the same neuronal substrate within the anterior insula that was activated by first-person exposure to the disgusting odorants. Further evidence supporting the role of the anterior insula in disgust observation and experience comes from lesion studies showing a selective impairment in recognizing disgust from others' faces following insula damage (Calder, Lawrence & Young, 2001). In addition, the deficit for others' disgust was mirrored by an equivalent deficit in the patient's first-person experience of the same emotion. Of particular interest is an observation by Calder and co-workers of a patient with a lesion involving the insula and adjacent putamen. Compared to controls, the patient was less disgusted, or even indifferent to a disgust provoking scenario (Calder, Keane, Manes, Antoun & Young, 2000). The studies described above suggest that the anterior insula is endowed with the mirror mechanism, and when activated determines a complex visceromotor response. The anatomical basis underpinning this behaviour consists of several subcortical centres (Fig. 7A) modulating autonomic reactions for controlling feeding behaviour and visceromotor responses. These centres include the lateral hypothalamus, the ventral tegmental area, the ventral striatum, and the ventrolateral sector of the periaqueductal gray (An, Bandler, Ongür & Price, 1998; Ongür, An & Price, 1998; Jezzini et al., 2015; Venkatraman, Edlow & Immordino-Yang, 2017).

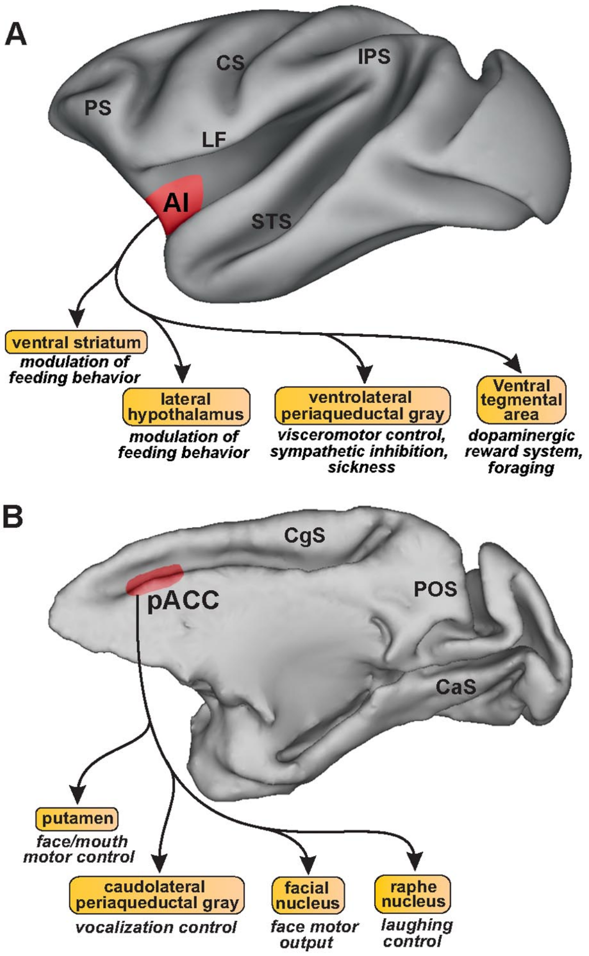

Figure 7: Localization and subcortical projections of anterior insula and pregenual anterior cingulate cortex. **A:** Lateral view of the monkey brain showing the anterior insula field in which electrical stimulation induced disgust-related behaviour (red area), and its subcortical projections. **B:** Mesial view of the monkey brain showing the localization of the pregenual sector of the anterior cingulate cortex (red area), and its subcortical projections. Abbreviations as in Fig. 1. Modified from Gerbella, Caruana and Rizzolatti (2017).

8.2 Laughter

The cingulate cortex is another territory that has recently been thought to comprise of neurons endowed with the mirror mechanism. A study carried out by Caruana et al. (2018) on a large number of drug-resistant epileptic patients, showed that there is a specific part of the anterior cingulate cortex whose stimulation determines smiling and laughter, with or without mirth. This region is located in the pregenual sector of the ACC (pACC). Furthermore, since in most patients mirth and merriment accompanies the production of laughter triggered by pACC stimulation, Caruana and co-workers concluded that this region is involved not only in generating the motor aspects of laughter, but also its emotional content. Evidence showing that pACC is a fundamental node for both the production of mirthful laughter, and for encoding observation of this behaviour, is provided by the finding that the same pACC site from which laughter is evoked by electrical stimulation, is also activated (gamma activity) by the presentation of movies showing laughing individuals. In contrast, movies depicting the same individuals expressing sadness, or showing a neutral face, do not elicit any response (Caruana et al., 2017). This evidence suggests that the pACC is endowed with the mirror mechanism, transforming sensory representation of other's laughter into the motor representation of the same behaviour. In line with this result, a meta-analysis of more than one hundred fMRI studies on emotional face processing showed that, when compared to neutral faces, happy faces selectively activate the ACC (Fusar-Poli et al., 2009).

Although there is not clear evidence in humans of the circuit leaving the pACC and reaching the subocortical centres controlling laughing, some inferences can be drawn from monkey anatomical studies. In the monkey the pACC projects to the caudal raphe nucleus, a brainstem structure whose damage may result in an uncontrolled motor pattern of laughing in humans, the so called "Fou rire prodromique" (Hornung, 2003). Additionally, the pACC involves the face/mouth field of the motor putamen, the vocalization centres of the caudolateral part of the periaqueductal gray, and the facial nerve nuclei (Müller-Preuss & Jürgens, 1976; Porrino & Goldman-Rakic, 1982; Devinsky, Morrell & Vogt, 1995; An et al., 1998). These latter projections bilaterally reach both the dorsal and intermediate subnuclei of the bulb, thus controlling upper face muscles, which are those characterizing the "true" emotional laughter (Fig. 7B). If these projections are preserved in evolution, one can depict the nervous network enabling human pACC to produce the complex pattern of visceromotor and motor activations which characterize laughing.

9 Conclusion

Traditionally, the motor system was not thought to be involved in cognitive functions, however, the discovery of the mirror mechanism radically changed this view. There is now rich evidence to support that numerous cognitive functions, such as space perception around the body as well as action and emotion recognition, not only require the activity of the motor system, but are also deeply embedded in it. In particular, we showed that both action and emotion understanding rely on potential motor acts that originally evolved for motor behaviour, and subsequently became the substrate for understanding others.

In this review, we discussed evidence indicating that the arm/hand action execution-observation network is not only constituted by classical parieto-frontal areas (F5 and PFG/AIP), but also by additional cortical sectors such as the F6/preSMA and prefrontal areas (12r and 46v). In addition, we described that the mirror mechanism is also present in centres whose primary function is to control emotional behaviour. In particular, we discussed one region controlling a negative emotion, the rostral insula, and one region that controls a positive one, the anterior cingulate cortex.

The data reviewed here could be useful for guiding future studies aimed at discovering other areas endowed with the mirror mechanism, in terms of emotional centres as well as areas involved in motor behaviour without emotional content.

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Research Article

Banking on You? The Level of Public Awareness of Biobanks in Malta

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Abstract. This paper explores the level of awareness of the Maltese general public in relation to the existence and use of biobanks as resources for biomedical and genomic research. Using a quantitative research design, a four question survey was administered face-to-face to a random stratified quota sample ($n = 387$) of the Maltese population. The survey assessed whether the general public understands what a biobank is, and what the people believe/ think a biobank might be. Results show that the overwhelming majority of the public is not aware of the term 'biobank', and when asked to think about what a biobank could be, the majority of these failed to give an accurate answer, with a financial institution being the most frequent suggestion. This said, 26.5% of those who initially claimed that they did not know what a biobank is (or claimed they were not sure) went on to give a legitimate response when asked to speculate about what a biobank could be. Most of these respondents mentioned biobanks which store gametes and/or embryos and biobanks which store blood and organs for the purpose of donation. Whilst gender does not seem to be a significant factor in the outcome as an independant variable, educational achievement did have an influence on the accuracy of the responses. The key finding is that only 2.3% of the Maltese population promptly associated the term 'biobank' with a facility for storing tissue for the purpose of biomedical research, while the vast majority believed it referred to a financial institution.

Keywords: Biobanks, biomedical research, participants, knowledge, perceptions

1 Introduction

A biobank can be defined as a "bank that collects, stores and distributes biological samples" (Schmilden, 2016). Understood in its broadest form, a biobank stores biological matter ranging from human blood and/or other tissue, to tissue of animals and/ or plants (de Souza & Greenspan, 2013). It is, however, the awareness of biobanking related to biomedical and genomic research that is the key focus of this paper.

Large-scale biobanks have become important in this context as they serve as a resource for stored samples and their accompanying data, and a potential hub for sharing and reuse of these samples rooted within stringent ethical proceedures (Holub et al., 2018; Colledge, Elger & Howard, 2013). Using established networks of biobanks as a collaborative infrastructure for resources becomes even more pertinent when dealing with rare genetic diseases where participants are normally sparsley distributed geographically. Biobanking protocols allow researchers to share samples and data, consequently facilitating the research process (Schmilden, 2016). Within the backdrop of the recent key role of genetic studies in understanding the aetiology and development of human diseases, there has been a rise in the number of biobanks around the globe over the past decades (Chen & Pang, 2015).

Malta is no exception to this trend. The Malta Biobank was established in 1989 and forms part of the Centre for Molecular Medicine and Biobanking, University of Malta. It is a founder member of the Biobanking and BioMolecular Resources Research Infrastructure, European Research Infrastructure Consortium (BBMRI-ERIC), aimed at facilitating sample and data sharing (Mayrhofer, Holub, Wutte & Litton, 2016).

The biobank currently holds approximately 26,000 samples of human biological samples in its clinical and population based banks. One of its major collections is the Globin Bank which includes abnormal haemoglobin and thalassemia in the Mediterranean. There are also collections related to research into type 2 dia-

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betes melitis, muscular dystrophy, cystic fibrosis, ALS and colorectal cancer (see also University of Malta, n.d.-a). The Malta Human Genome Project is also based at the Malta Biobank where a Maltese reference draft genome has been drawn up through sequencing a random collection of 400 (i.e. 0.1% of the population) Maltese DNA samples (Borg, 2018, September). The team is now in the process of recruiting participants to build a collection of samples from 1% of the Maltese population. This project is aimed at discovering new DNA variants that cause disease in Malta. Once completed, it will have the potential to positively impact medical practice in Malta, by creating the possibility for clinicians to provide personalised treatments for their patients (University of Malta, n.d.-b).

Clearly, all research depends on the active engagement of participants who provide their tissues/blood and permission to link their biological sample to personal health data. To date, there are no studies assessing the awareness of biobanking activity in Malta, and the perception and understanding of the general public in this respect. Findings from a Europe based study, however, indicate a very low level of awareness. The authors of one particular study raised concerns in their conclusions where they stated that "[t]wo-thirds of Europeans recently surveyed have never heard of biobanks. [...] most Europeans haven't heard of their nation's repositories of human blood and tissue samples. Promote them, or they could fail" (Gaskell & Gottweis, 2011).

The success of a biobank is entirely dependent on active participants who are motivated to participate by donating their biospecimen for research purposes (Gaskell & Gottweis, 2011). This motivation is intrinsically related to issues of transparancy and trustworthiness of biobanking proceedures, clear and unambiguous consenting protocol, and robust ethical governance of data and samples (Locock & Boylan, 2016). Even more fundamental, however, is nurturing frank understanding of the purpose of research oriented biobanks and their potential to improve the health of future generations (Goisauf & Durnová, 2018). On a local level, the 'Connecting for Health' project which is currently ongoing at the Centre for Molecular Medicine and Biobanking within the University of Malta, is building an IT portal to facilitate public engagement. It will act as a communication interface between the research community at the Malta biobank and the general public, with the aim of encouraging members of the public to become research partners by donating their sample for genomic research.

The data presented here are from a preliminary survey related to this project which will contribute to contextualising subsequent phases of planned qualitative research into beliefs and perceptions of biobanking and genomic research in Malta.

2 Methods

A quantitative research design was used to collect statistically generalizable data about the level of awareness and accuracy of understanding of the Maltese general public in relation to biobanks.

The research questions driving the design were:

- Does the Maltese general public know what a biobank is?
- How aware is the Maltese general public of the existence of biobanks?
- What first comes to the mind of the Maltese general public when they hear the word 'biobank'?

2.1 Sample

National statistics office (NSO) data on the Maltese population (450,000 in 2017) were used to set the quotas on a random sample stratified for gender and education. A sample size of 387 participants was used which allows for a ±5% margin of error and a confidence interval of 95%. Quotas were set to stratify the sample for gender, and for education as detailed in Table 1.

2.2 Research Tool

A simple four-point survey was designed to gather data as follows:

Q1: Do you know what a biobank is? **(Skip to Q4 if answer is NO. Continue if answer is YES.)**
Q2: What is a biobank?
Q3: How did you learn about biobanks? **(Stop here)**
Q4: What do you think a biobank is?

Table 1: Sample stratified by education.

SAMPLE		
59.8% of 387 participants	**231 participants**	People who completed primary and/or secondary education - this includes persons who never attended an educational institution.
22.4% of 387 participants	**87 participants**	People who completed post-secondary education.
17.8% of 387 participants	**69 participants**	People who graduated from tertiary education.
100% of 387 participants	**387 participants**	**TOTAL**

2.3 Data Collection

Data were gathered between the 6th of April 2018 and the 26th of April 2018 by inviting individuals to participate at strategic points of public access (Valletta Bus Terminus, Valletta Republic street, Ċirkewwa terminal and University campus canteen and quadrangle). Participants were recruited according to the sample stratification strategy as described above.

The survey was conducted in the language of preference (Maltese or English) of the respondent, with Maltese being the dominant choice. These responses were translated at source and data recorded in English. The word ‘Biobank’ was used in both languages. No suggestions or prompts were given as possible answers to the survey questions, and all data were spontaneous responses from interviewees.

2.4 Ethics

The study was carried out with approval from the University of Malta Research Ethics Committee. All participants were given a verbal explanation of the aims of the study, and the ways in which the data will be used, emphasising the fact that all data will be fully anonymous. No personal data were collected. All participants were given a printed information sheet about the research project.

3 Results

3.1 General Understanding of the Term ‘Biobank’

When asked the initial question of the survey questionnaire (Q1: ‘Do you know what a biobank is?’), a significant 87.1% (337) claimed ‘no’ and another 5.4% (21) said ‘not sure’. Only 7.5% (29) of the sample ($n = 387$) claimed that ‘yes’ they do know what a biobank is (see Fig. 1).

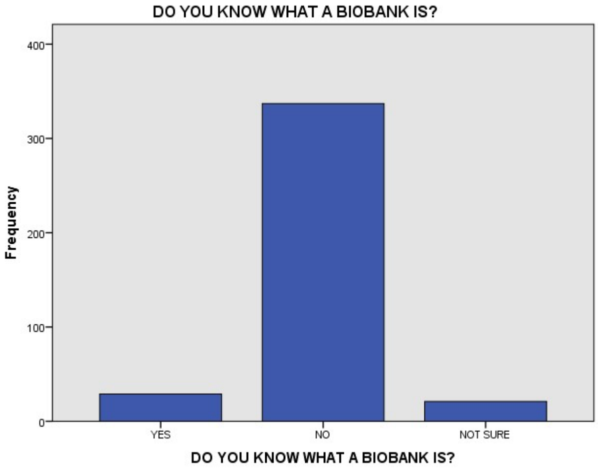

Figure 1: Do you know what a biobank is? ($n = 387$)

It is interesting to note, as will be discussed in more detail below, that not all who claimed ‘yes’ gave a valid answer when asked to explain what a biobank is, in fact 27.6% of those who answered ‘yes’ when initially asked ‘Do you know what a biobank is?’, gave an invalid response when asked to specify (see Fig. 3). Hence, when compared to the number of respondents who claimed to know what a biobank is, the number of respondents actually knowing what a biobank is, and mentioning one type of biobank, dropped to 5.4% from the original 7.5%, as of the 29 respondents who claimed to know what a biobank is, 8 respondents gave an invalid explanation.

3.2 Speculation About Possible Meaning of the Word ‘biobank’

Those respondents who declared that that they did not know what a biobank is were then invited to speculate about the meaning of the word. Fig. 2 illustrates in a categorical manner the responses of these 358 participants (92.5%) when asked to speculate about what a biobank might be after responding with ‘no’ or ‘not sure’ when asked if they knew what a biobank is. The most frequent response by far is that it was some form of bank for money.

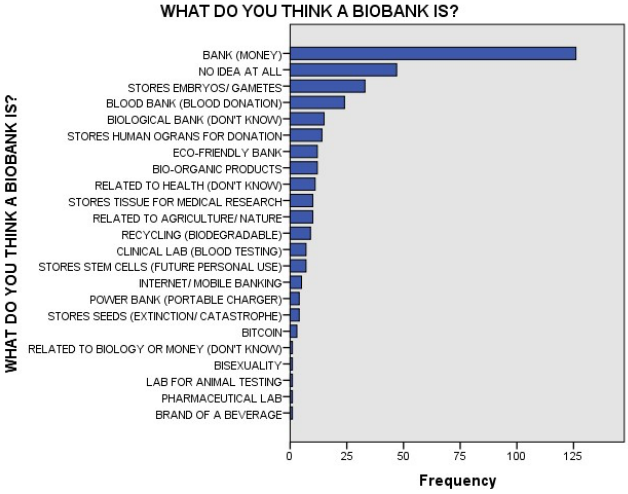

Figure 2: Responses to Q4: What do you think a biobank is? ($n = 358$)

It is clear that the suffix ‘bank’ in ‘biobank’ is dominant in forming perceptions in the Maltese public, with 40% of those who were invited to speculate on the meaning of ‘biobank’ assuming it referred to a financial institution (126 respondents mentioned ‘bank (money)’, 12 respondents mentioned ‘eco-friendly bank’ and another 5 respondents mentioned ‘mobile banking’).

The prefix ‘Bio’ in ‘biobank’ proved to be influential in some responses, with 6.7% suggesting it may be linked to some eco-friendly (12 mentions) or ‘organic’ (12 mentions) institution/product. Another 4.2% (15 respondents) assumed that a ‘biobank’ is a biological bank, however failed to reason out what the function of such a bank might be. Some responses were interesting in virtue of their originality, with ‘powerbank charger’ (4 mentions), ‘bitcoin’ (3 mentions) and ‘brand of beverage’ (one mention) being the most notable.

It is interesting to note that 3.1% said that they believed that a biobank is something which relates to health, but had no idea of its use; and another 2.6% of the respondents mentioned types of scientific labs; namely ‘a clinical lab’ (2%), ‘a pharmaceutical lab’ (0.3%) and ‘a lab for animal testing’ (0.3%).

13.4% of the respondents claimed that they could not think of anything that a biobank could represent and did not want to try and guess, claiming that they had “no idea at all”.

A notable 25.4% (91 respondents) of those who initially claimed that they did not know what a biobank is or claimed they were not sure ($n = 358$) then gave a legitimate response and mentioned a type of biobank when asked to think about what a biobank could be, with the storage of embryos and gametes being the most frequent association in this respect. The percentage breakdown of these data is as follows:

- 9.2% of these mentioned embryo freezing and/or gamete freezing;
- 6.7% mentioned blood banks with the purpose of blood transfusion/ donation;
- 3.9% mentioned biobanks which store organs for donation;
- 2.8% mentioned biobanks which store tissue for the purpose of biomedical research;
- 1.7% mentioned stem cells to be used for future self;
- 1.1% mentioned the storing of seeds to be used in case of extinction/ catastrophe.

When analysing these data in association with level of education, 38.5% of the respondents had completed their education at either primary or secondary level. 29.7% were individuals who had completed post-secondary education, and 31.9% were respondents who completed tertiary education. It is important to note that the vast majority of those who stopped at Primary or Secondary level most commonly mentioned *‘embryo freezing and/or gamete freezing’* and *‘blood banks with the purpose of blood transfusion/ donation’*. Whereas those with a post-secondary or tertiary level of education were more likely to mention the other types of biobanks.

Table 2 shows the demographics of gender of the 91 respondents who initially claimed they did not know what a biobank is, and later gave a valid answer to the question, *‘What do you think a biobank is?’*. Male gender is seen to have a marginal association with valid answers.

Table 2: Valid answer to Q4 by gender.

Q4 ‘What do you think a Biobank is?’: Valid answers by gender.

		Frequency	Percentage out of 91	Percentage out of 387
Gender	Male	54	59.3	13.9
	Female	37	40.7	9.6
	Total	91	100.0	23.5

As mentioned previously, 27.6% (8 participants) of those who answered ‘yes’ when initially asked ‘Do you know what a biobank is?’, went on to give an invalid answer when asked the question ‘What is a biobank?’. Here again, the suffix ‘bank’ is dominant as most of these assumed, and seemed to be sure, that a biobank had to do with finance.

However, it can be observed the majority of those who answered the first question (**Q1**: Do you know what a biobank is?) with a yes, had a valid answer to the follow up question (**Q2**: What is a biobank?). Out of these valid responses 9 respondents mentioned biobanks which store tissue for the purpose of medical research. Another 7 participants mentioned biobanks which store gametes or frozen embryos, 4 participants mentioned biobanks which store organs for the purpose of organ donation. One participant mentioned private stem cell stores.

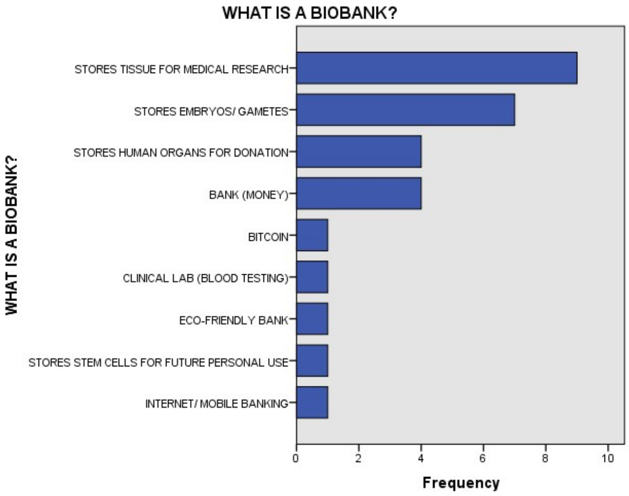

Figure 3: Responses to Q2 ‘What is a biobank?’ by individuals who claimed they knew ($n = 29$).

3.3 Mentioning Biomedical Research as the Function of a 'Biobank'

Other than evaluating the level of comprehension of the term 'biobank' in general, the specific link of biobanking activity with biomedical and genomic research was also examined. Data show that this level of understanding is minimal with only 2.3% (9 participants) of the whole sample initially stating that 'yes' they know what a biobank is, and then mentioning biobanks which 'store tissue for biomedical research'. A significant majority of 77.7% (of the 9 participants) consisted of individuals who have completed tertiary education, and 6 of these were male.

There were also 10 respondents (2.6% of the whole sample) who had initially stated that they did not know what a biobank is, who then also went on to suggest that it might be a place which stores tissue for the purpose of medical research. As expected, the level of educational attainment is also a significant variable here. None (0%) of those with a primary/secondary level of education, who stated that they did not know what a biobank is, mentioned the storing of tissue for biomedical research when asked the follow up question. 60% of those who did mention biobank which store tissue for medical research were tertiary education graduates, and 40% were respondents who have continued their studies to post-secondary education.

3.4 Sources of Information About Biobanks Used for Research Purposes

All those who mentioned biobanks which 'store tissue for medical research' ($n = 19$), after being asked the follow-up question, **Q2**: *"What is a biobank?"*, were asked a third question, **Q3**: *"How did you learn about biobanks?"*. This includes both those who answered the first question (Do you know what a biobank is?) with a 'yes' and those who answered with a 'no/ not sure' answer, but ultimately mentioned the *'storing of tissue for biomedical research'*.

The bar chart Fig. 4 illustrates how each of the 19 respondents who mentioned biobanks which 'store tissue for biomedical research' learnt about the function of a biobank.

Seven respondents were students who are currently enrolled in a course related to pharmacy, medicine or health science.

Six respondents were people who were not involved in pharmaceuticals or medicine but stay updated through independent reading.

Five respondents said that they learnt about biobanks for the purpose of medical research through their work in the pharmaceutical/ medical sector.

One respondent claimed that they learnt about biobanks after being asked to donate their own DNA sample for medical research.

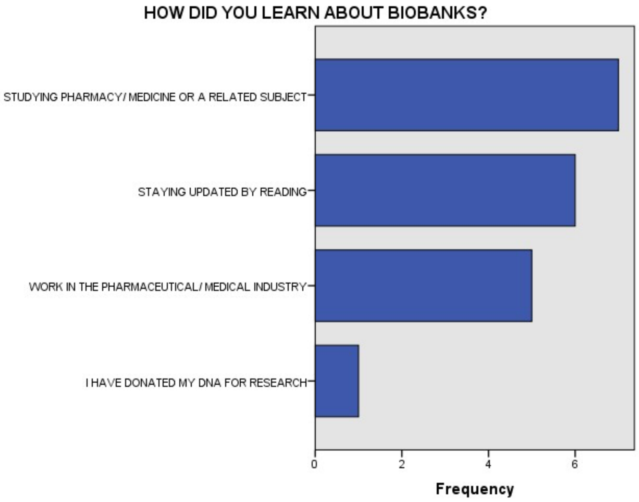

Figure 4: Responses to Q3: 'How did you learn about biobanks?' ($n = 19$)

Clearly, the majority of respondents attributed their knowledge about the use of biobanks for research purposes to their career, career prospects and/or interest in the process. There is no mention of knowledge drawn from local public information campaigns or recruitment drives.

3.5 A Breakdown of Numbers Indicating the Level of Biobank Awareness Amongst the Maltese Population

Table 3 is a simplified illustration of the numbers which unveil the level of awareness of biobanks amongst the general public. The first row indicates the distribution of responses to the first question of the survey questionnaire (Q1: "Do you know what a biobank is?"). The second row highlights the validity of the responses when the research participants were asked the follow-up question to elaborate about the purpose of a biobank (Q2: "What is a biobank?" & Q4: "What do you think a biobank is?").[1]

Table 3: Breakdown of responses to Q1, Q2 & Q4.

Yes, know what is a biobank is: **29**		No, don't know what a biobank is (includes those who claimed 'don't know'): **358**	
Legitimate answer to Q2: **21**	Non-legitimate answer to Q2: **8**	Legitimate answer to Q4: **91**	Non-legitimate answer to Q4: **267**

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[1] Q2 was asked to those who initially claimed that they know what a biobank is, whereas Q4 was asked to those who initially claimed that they don't know what a biobank is and to those who claimed that they are not sure whether or not they know what a biobank is.

4 Discussion

The dominant finding is that the overwhelming majority of the population does not know what a biobank is. Looking at the respondents who instantly claimed that they do not know what a biobank is ($n = 358$), when pressed to speculate about what it might be, a significant proportion of these (48.6%) gave an invalid answer and went on to mention something completely unrelated to biology and/or science. Another 12.6% linked the term to health and/or science but went completely off-track and did not mention anything related to biobanks when asked to explain further. 13.4% claimed that they had "no idea at all". The remaining 25.4% gave a valid answer when asked to think about what a biobank might be, however only a marginal 2.8% mentioned biobanks which store blood and/or tissue for the purpose of biomedical research.

To those who have no knowledge about its meaning, the word 'biobank' has strong associations with 'money', indeed the suffix 'bank' in 'biobank' is dominant in forming perceptions in the Maltese public, with 40% of those who were invited to speculate on the meaning of 'biobank' assuming it referred to a financial institution. Three respondents seem to have taken this to its most cutting-edge, by speculating that the word might mean a form of bitcoin currency. The prefix 'Bio' also proved to be influential, though much less so, with respondents who were asked to speculate about the meaning of the word 'biobank' suggesting it was some eco-friendly or 'organic' institution/product.

The dominant misconception, by far, is that a 'biobank' is some form of financial institution. This said, a noteworthy number of respondents did make the link between the term 'biobank' and medicine, biology, science and/or nature, with 28.9% (112 respondents) of the whole sample mentioning a valid type of biobank. The most common response in this respect was a biobank which stores gametes and/or embryos for the process of IVF. It is highly likely that the frequency of this response is due to the fact that during the time when the field research was conducted, the Maltese parliament and media were actively debating the matter of IVF, gamete donation and embryo freezing, and the debate was predominant in public discourse in the everyday social environment. When analysing the gender of the respondents who initially claimed they did not know what a biobank was, and then went on to suggest an appropriate response when asked to speculate, male gender was seen to have a marginal association with valid answers.

Results highlight the fact that the Maltese general public's awareness of biobanks used for the purpose of biomedical research is minimal, and that when the term 'biobank' is associated with science, then there is a stronger association with gametes and sperm collection/storage for IVF than with molecular medicine/genomic research. Indeed, only a total of 19 respondents (4.9% of the whole sample) mentioned biobanks which store tissue for the purpose of biomedical research. Ten of these participants had initially stated that they did not know what a biobank was, and only mentioned this type of biobank after thinking further about what the term 'biobank' could mean. It is noteworthy that only 2.3% (9 participants) of the whole sample were prompt to claim that they know what a biobank is and mentioned biomedical research when asked to explain further. This indicates that the awareness of the research process in molecular medicine is higher than the awareness of the term 'biobank', despite noting that awareness about both is significantly low.

Clearly the level of education of the research participants is significant when analysing all those who mentioned biobanks which store tissue for the purpose of medical research, with a significant positive correlation between tertiary educational achievement and mentioning such type of biobanks. Within this caveat, and flagging this with caution as the numbers involved here are small, gender becomes a significant variable when associated with education and career choice as 63.2% (12 respondents) of those who mentioned biobanks for biomedical research are either studying or working in the pharmaceutical or medical sectors, and the significant majority of those were male.

5 Conclusion

It is clear from the data presented herein, that the vast majority of the Maltese general public does not know what the term 'biobank' means, and does not associate the term with biomedical or genomic research. The suffix 'bank' is the most influential in forming the general public's assessment of what it might be, with the vast majority mentioning a financial institution as a possibility. The prefix 'bio' also has an interesting impact on public perception of what a biobank might be, with a small proportion suggesting it may be linked to some eco-friendly or 'organic' institution/product. Results indicate that there is great scope for increasing science communication in relation to research linked biobanking in order to enhance the process of biomedical and genomic research in Malta.

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Research Article

Maltese Microalgae and Global Climate Variability

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Abstract. The biodiversity of cyanobacteria and microalgae growing in terrestrial and marine habitats around the Maltese islands is currently being investigated, as limited knowledge exists about the phototrophic microorganisms inhabiting this geographical area. New strains of the genera *Oculatella*, *Albertania*, *Nodosilinea*, *Nostoc*, *Lyngbya*, *Oscillatoria*, *Calothrix* and *Jenufa* have been recorded recently. Since the phototrophic microbial biodiversity is currently largely unknown, there is an imminent risk of undescribed microorganisms being lost as a result of changes in microbial community structures due to global climate variability (GCV). We describe a six-month experiment to assess the effects of GCV on two Maltese microorganisms, the filamentous heterocytous cyanobacterial *Nostoc* strain AD0303 and a coccal microalgal *Jenufa* strain AD0402. Each strain was cultured under environmental conditions associated with GCV; a temperature (T) of 26 °C, enhanced ultraviolet radiation (UVR) and an increased $\mathrm{CO_2}$ concentration. Elevated T stimulated growth and biomass accumulation of *Nostoc* AD0303, whereas growth of *Jenufa* AD0402 was partially inhibited. Increased UVR had the most prominent effect on cellular morphology. *Nostoc* AD0303 presented as aggregated filaments, whereas *Jenufa* AD0402 exhibited thicker cell walls. These UV-protecting mechanisms allowed both strains to accumulate biomass at a significantly higher rate than the control. An increase in $\mathrm{CO_2}$ concentration resulted in inhibition of growth in *Jenufa* AD0402 and bleaching of filaments in *Nostoc* AD0303, both leading to culture death. A lower $\mathrm{CO_2}$ concentration and re-introduction of air subsequently allowed *Jenufa* AD0402 to grow. So far, this demonstrates that the effects imposed by climate variability are strain-specific, making changes at an ecosystem level difficult to predict.

Keywords: cyanobacteria, microalgae, climate change, *Nostoc*, *Jenufa*

1 Introduction

Global climate variability (GCV) has affected organisms throughout evolutionary history. Currently, however, advocates in the scientific community argue that there is a rapid pace of change (Bradshaw, 2006). This rapid change in climatic parameters on a global scale could be accounted for by anthropogenic greenhouse gas emissions. An increase in temperature (T) has resulted, particularly, but not exclusively, due to $\mathrm{CO_2}$ emitted from fossil fuel combustion (Montzka, Dlugokencky & Butler, 2011). Global levels of $\mathrm{CO_2}$ reached an average concentration of 406.00 ppm in October 2018 (NOAA, 2018). Foster and colleagues suggest that the $\mathrm{CO_2}$ level will soon reach its highest in at least 50 million years; at around 600 ppm by 2050 (Foster, Royer & Lunt, 2017). Different climatic models have predicted that this value will continue to rise and may even double by the year 2100. Ozone layer depletion, through the past use of chlorofluorocarbons, is another significant consequence, and this is expected to expose the Earth's surface to higher levels of ultraviolet radiation (UVR) (Sivasakthivel & Reddy, 2011).

The latest report from the Intergovernmental Panel on Climate Change discusses the consequences of climate change that could be avoided by limiting global warming to 1.5 °C, rather than 2 °C or more (IPCC, 2018). The aim is to reduce challenging impacts on ecosystems, human health and global well-being.

To date, most research studies focusing on the effects of GCV on ecosystems and biodiversity have been concerned with higher animals and plants (Parmesan,

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2006). Research studies conducted on the effects of GCV on microorganisms have been few in comparison, and most have focused on bloom-forming cyanobacteria and their toxicity to humans and other animals (Paerl & Paul, 2012; Hense, Meier & Sonntag, 2013; Moe, Haande & Couture, 2016). On the other hand, research into the effects of GCV on non-pathogenic strains of eukaryotic microalgae and cyanobacteria is generally lacking. Since autotrophic microorganisms are important primary producers in both marine and freshwater ecosystems, a change in their populations may cause a cascading effect throughout the biocoenosis (Gragnani, Scheffer & Rinaldi, 1999).

Most of the research into the effects of elevated temperature, CO_2 and UVR has been concerned with marine and freshwater cyanobacteria and microalgae (Karlberg & Wulff, 2012; Lürling, Mello, van Oosterhout, de Senerpont Domis & Marinho, 2018; Visser et al., 2016). Studies about the ecophysiology of terrestrial cyanobacteria and microalgae such as the *Nostoc* and *Jenufa* strains applied in this research study have been mostly overlooked. The effect of changes in environmental parameters on terrestrial organisms may be different to that observed in aquatic organisms. Exposure may be higher in terrestrial microalgae and these may be susceptible to desiccation, which alters their growth and photosynthetic rates (Häubner, Schumann & Karsten, 2006).

The main objectives of this research were to investigate the biodiversity of cyanobacteria and microalgae in the Maltese islands, which is severely understudied at present, and to monitor the effects of GCV on two strains isolated from microalgal biofilms. These included a filamentous heterocytous cyanobacterial *Nostoc* strain AD0303 and a coccal microalgal *Jenufa* strain AD0402. These were subjected to future environmental conditions related to climate variability within a laboratory setting, i.e. an increase in T, UVR and CO_2 concentration.

The cyanobacterial strain selected for this study is morphologically classified within the genus *Nostoc* Vaucher ex Bornet & Flahault, 1886 (Nostocaceae, Nostocales). This strain of *Nostoc* forms a very thick subaerial biofilm in the entrance chambers of hypogea (Zammit, Billi, Shubert, Kaštovský & Albertano, 2011; Zammit, Sánchez-Moral & Albertano, 2011). Morphologically, colonies of *Nostoc* consist of trichomes surrounded by an extracellular sheath or a gelatinous matrix. Heterocytes may be present within the trichome, however this is dependent on the amount of nitrogen available (M. D. Guiry & Guiry, 2018).

The microalga chosen for this study belongs to the genus *Jenufa* Němcová, M. Eliáš, Škaloud et Neustupa and was described in 2011 as part of the Chlorophyceae (incertae sedis). *Jenufa* strains form subaerial biofilms on stone surfaces in humid environments (Zammit, Billi, Shubert et al., 2011; Zammit, Sánchez-Moral & Albertano, 2011). Morphologically, this is a coccal microalga with roughly spherical or ovoid cells and a lobed chloroplast.

Morphological features were noted at the beginning of the study since changes in morphology brought about by T, CO_2 and UVR have been observed in other species of cyanobacteria and microalgae (Giordanino, Strauch, Villafañe & Helblung, 2011).

2 Materials and Methods

2.1 Sampling of Microbial Communities

Phototrophic biofilms and microalgal biomats were sampled from different calcareous substrates by non-invasive sampling methods which employed different techniques to remove the biofilm but not the underlying substrate (Zammit, De Leo, Albertano & Urzì, 2008). Biofilm communities were sampled from various microhabitats in hypogea and along rocky shores, such as smooth or rough rock surfaces and crevices. In littoral zones, biofilms in rock pools were either submerged in seawater or located in the splash zone. The diversity of microorganisms within these biofilms and biomats was observed by light microscopy as described below.

2.2 Microscopy

The morphological features of the microorganisms present in the microbial communities and those of isolated strains were examined by light microscopy. Temporary slides were prepared for biofilm and biomat samples and these were observed via a Nikon Eclipse Ti-S inverted microscope at a 200x and 400x magnification. Light micrographs were taken using a Nikon camera via NIS-Elements microscope imaging software. Cultured strains were observed using an Olympus BX-51 microscope equipped with DIC and a DP73 camera at a 1000x magnification. Identification was based on reference books for microalgae, cyanobacteria and diatoms (John, Whitton & Brook, 2002; Komárek & Anagnostidis, 1998, 2005; Komárek, 2013; Ettl & Gärtner, 2014; Škaloud, Rindi, Boedeker & Leliaert, 2018).

2.3 Culture Conditions

Cyanobacterial biofilms were grown on BG-11 medium (Rippka, Deruelles, Waterbury, Herdman & Stanier, 1979) and microalgal mats on Bold's Basal Medium (BBM) (Nichols & Bold, 1965), f/2 (Guillard, 1975) and SN (Waterbury, Watson, Valois & Franks, 1986) media. Individual strains were isolated by subsequent transfer onto fresh media. All isolated microorganisms were added to the Maltese Microalgal Culture Collection (MMCC) (Zammit, 2016).

The two strains chosen for the initial experiment related to climate variability were the cyanobacterium *Nostoc* AD0303 and the microalga *Jenufa* AD0402, strains of which have been found to be ubiquitous in terrestrial habitats. An isolated colony of *Jenufa* AD0402 from solid nutrient medium was inoculated into 100 mL of liquid BBM and an isolated colony of *Nostoc* AD0303 was inoculated into liquid BG-11 medium. These strains were cultured under environmental conditions that were similar to those of the natural environment from where they were sampled; 18 °C T, 75% RH and at a light intensity of 40 µE for a 10-hour photoperiod. Strains were allowed to accumulate biomass until they reached an optical density of 0.6 during exponential growth phase, measured as described in Section 2.5.

2.4 Experimental Design

Three experimental set-ups and two controls were included in the six-month study. These were prepared as follows. For each experiment, 25 mL of the liquid culture was made up to 1 L with autoclaved medium in a glass culture flask. Experiments were carried out in duplicate.

In the first experiment, cultures were grown at an elevated temperature of 26 °C, 75% RH and at a light intensity of 40 µE for a 10-hour photoperiod. In the second experiment, a UV-chamber was designed in which light was provided by a UV-lamp (PHILIPS Blacklight Blue F8 T5 TL 8W/ 08) to emit UV-A (340 nm–400 nm) over a 10-hour photoperiod. The cultures were maintained at 18 °C, 75% RH and at a light intensity of 40 µE, also for a 10-hour photoperiod.

For the CO_2 experiment, the culture flasks were fitted with a Duran GL-45 dual port cap to allow an inflow of filtered CO_2 from one port, and a filtration system using a Duran pressure compensation attachment, fitted with a 0.2 µm filter, on the second port. The flow rate of CO_2 was set at 0.2 L/ min. Five different CO_2 experiments were carried out varying the amount of CO_2, as follows: 10 hours of CO_2 daily for 2 weeks, 2 hours of CO_2 daily for 2 weeks, 30 minutes of CO_2 daily for 1 week, 15 minutes of CO_2 daily for 2 weeks and finally, 15 minutes of CO_2 and 15 minutes of air weekly for 8 weeks.

Two controls were set up. The first was used as a control for the increased T and increased UVR experiments. Here the strains were cultured at 18 °C, 75% RH and at a light intensity of 40 µE for a 10-hour photoperiod. In the second control, cultures were grown under the same conditions described above, but in addition, air was provided via a pump, in order to replicate the agitation created by the inflow of CO_2.

2.5 Analysis

2.5.1 Growth Curves and pH

2 mL aliquots of each culture were used to measure *in vivo* absorbance for chlorophyll *a* at a wavelength of 680 nm and the optical density at 790 nm respectively using a Shimadzu UV-2501PC spectrophotometer. The pH of each aliquot was also measured. Both tests were carried out bi-weekly.

2.5.2 Morphology

Wet-mount slides of each culture were prepared bimonthly and viewed microscopically as described in Section 2.2. For each of the strains, different morphological characters were noted and the dimensions of individual cells were taken.

2.5.3 Pigment Assays

Estimation of chlorophyll *a* and *b*, carotenoids and phycobiliproteins were conducted at the end of the study. Cell lysis was carried out as follows. 10 mL of culture were aliquoted into 15 mL centrifuge tubes and centrifuged at 6000 rpm until a cell pellet formed. The supernatant was removed, the pellet transferred to a 2 mL microcentrifuge tube and washed with 2 mL of distilled water. The samples were frozen at −20 °C overnight, thawed and placed in a sonicator for 15 minutes at 250 Hz. Additionally, for the strain *Jenufa* AD0402, a 4-hour drying period was carried out at 60 °C.

Analyses for chlorophyll *a*, *b* and carotenoids were conducted on both strains. 0.25 mL of glass beads and 1.5 mL of pure methanol were added to the cell pellet, the tubes were vortex shaken for 15 minutes and incubated in darkness overnight at 4 °C. The concentration (µg/ml) of chlorophyll *a* (C_a), chlorophyll *b* (C_b), and total carotenoids (C_{x+c}) were calculated using the equations proposed by Lichtenthaler (1987).

A phycobiliprotein assay was also carried out for cultures of *Nostoc* AD0303. Following cell lysis, 0.25 mL of glass beads and 1.5 mL of phosphate buffer (0.01 M NaH_2PO_4, 0.15 M NaCl, pH 7) were added to the pellets and the tubes were vortex shaken for 15 minutes. The samples were then incubated overnight in darkness at 4 °C. The concentration of phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) were calculated using the equations proposed by Bennett and Bogorad (1973).

2.5.4 UV-Absorbing Compounds

Cultures were tested for the presence of scytonemin and mycosporine-like amino acids (MAAs) at the end of the six-month study, using a method adapted from Rastogi and Incharoensakdi (2013). The solvent was evaporated using a Savant SpeedVac SVC 100H Centrifugal Evaporator. The extracts were scanned for typical peaks of both MAAs and scytonemin between 200 nm and 800 nm using a Shimadzu UV-2501PC spectrophotometer.

2.5.5 Biomass Yield

The biomass yield for each culture was calculated by freeze-drying at the end of each experiment. 50 mL of culture was centrifuged at 4000 rpm until a cell pellet was formed. The pellet was frozen at −20 °C and lyophilised to a powder. The tube was weighed beforehand, and the amount of biomass was calculated by subtracting the mass of the empty tube from the mass of the tube containing the powder.

2.5.6 Data Analysis

Means were calculated from measurements taken in duplicate. Differences between the means ($P < 0.05$) for treatment and experimental controls were determined using the independent *t*-test on IBM SPSS v. 25.

3 Results

3.1 Microbial Communities

In both terrestrial hypogean environments and the marine littoral zone, the biofilms and biomats consisted of highly diverse microbial communities made up of both phototrophic and heterotrophic microorganisms. In each habitat investigated so far, filamentous cyanobacteria were found to be the dominant microorganisms making up the microbial biofilm structure. Upon initial transfer to culture conditions, the biofilms and their constituent microorganisms grew at a very slow rate. Many of the microorganisms exhibited intricate relationships and proved to be difficult to isolate in culture.

Cyanobacteria possessing fine filaments and having a simple morphology, were found in all biofilm and biomat samples. They belonged to the genus *Leptolyngbya* (Fig. 1a) and the recently described genera *Nodosilinea* (Perkerson et al., 2011), *Oculatella* (Zammit, Billi & Albertano, 2012) and *Albertania* (Zammit, 2018).

For biofilms isolated from terrestrial environments, filaments of the heterocytous genera *Nostoc* and *Fischerella*, as well as coccal cyanobacteria belonging to *Chroococcidiopsis*, *Gloeocapsa* and *Asterocapsa spp.* were isolated in culture. Microalgal strains belonged to the genera *Jenufa*, *Pseudopleurococcus* and *Trentepohlia*. All these strains are presently being characterised by a more comprehensive multiphasic approach. The characteristics of the Maltese *Jenufa* strains are described in a separate study (Zammit & Agius, 2019).

Larger cyanobacterial filaments (Fig. 1b–d) were also observed in communities colonising the calcareous rock of the marine littoral, including Oscillatorialean strains belonging to the genera *Lyngbya* (Fig. 1b), *Oscillatoria*, as well as the heterocytous *Calothrix* (Fig. 1d) and *Scytonematopsis*. Large spherical cyanobacterial cells of *Stanieria cf. sublitoralis* produced baeocytes by spontaneous multiple fission to form red colonies on the surface of biofilms from the littoral zone. Other coccal cyanobacteria could be morphologically classified into *Aphanocapsa* and *Gloeocapsa spp.* Diatoms of *Navicula* spp. were ubiquitous in the same biofilms and biomats. Developing macroalgal filaments belonging to species of the green macroalgae *Cladophora* and *Ulva* also frequently contributed to the structure of marine biofilms and biomats. These microbial communities also included cryptic coccal microalgae, bacteria, protozoans and fungi.

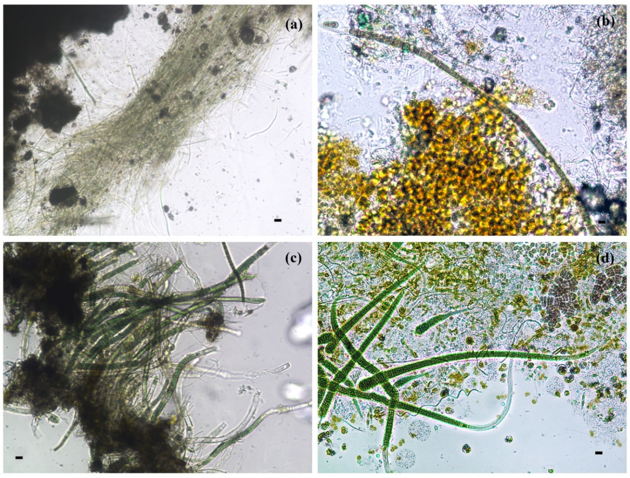

Figure 1: Light micrographs showing biofilm and biomat microorganisms from a rocky shore in Sliema, Malta, (a) simple filaments of the Leptolyngbyaceae, (b) *Lyngbya* filament and coccal microalgae, (c) tufts of cyanobacterial filaments, (d) *Calothrix* filaments with basal heterocyte, surrounded by different types of coccal cyanobacteria and microalgae. Scale bar = 10 µm in (a), (b), (d). Scale bar = 20 µm in (c).

For the climate change experiment, the filamentous heterocytous cyanobacterial *Nostoc* strain AD0303 and a coccal microalgal *Jenufa* strain AD0402 were used. Both of these were of common occurrence in biofilms colonising hypogea (Zammit, Billi, Shubert et al., 2011; Zammit, Sánchez-Moral & Albertano, 2011).

3.2 Growth Experiment

Under all treatments, both *Nostoc* and *Jenufa* strains displayed a typical sigmoidal growth curve based on the absorbance of chlorophyll *a* (Fig. 2) and cell density. In this study, two subsequent growth curves were observed, since fresh medium was supplied again midway through the experiment. Under elevated UVR, both strains exhibited growth in culture for the duration of the experiment (Fig. 2).

At elevated temperature, *Nostoc* AD0303 accumulated a higher mean cell density than the control. On the other hand, *Jenufa* AD0402 grown under elevated

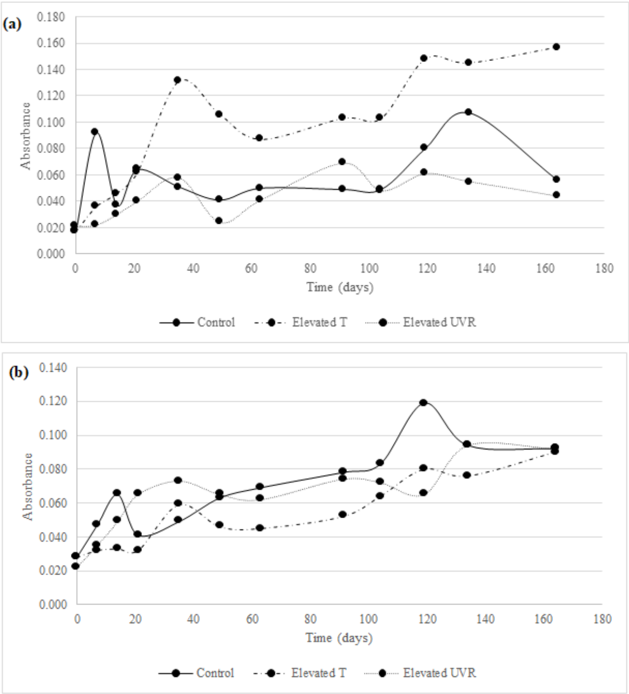

Figure 2: Representative growth curves based on the absorbance of chlorophyll *a* at 680 nm for (a) *Nostoc* AD0303 and (b) *Jenufa* AD0402.

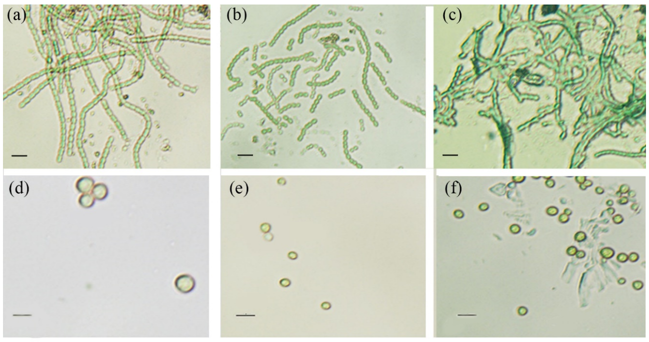

Figure 3: Light micrographs of *Nostoc* and *Jenufa* cultures taken at the end of the six-month study. *Nostoc* AD0303 cultivated (a) under control conditions (b) at elevated T (c) at elevated UVR. *Jenufa* AD0402 grown (d) under control conditions (e) at elevated T (f) under elevated UVR. Scale bars = 10 µm.

temperature displayed an extended lag phase and a decrease in growth rate but continued to grow steadily over the six- month period.

Nostoc AD0303 exhibited filament bleaching leading to culture death in all CO_2 treatments. Culture death was also observed in *Jenufa* AD0402 growing under increased CO_2 concentration. However, the subsequent introduction of air in the final experiment, allowed cultures of *Jenufa* AD0402 to recover. In the same experiment, filaments of *Nostoc* AD0303 were bleached throughout, and only started to recover 8 weeks after the experiment was terminated.

3.3 Morphology

Cultures of *Nostoc* AD0303 grown at elevated T were characterized by short, rapidly dividing filaments (Fig. 3). A significant reduction ($p < 0.05$) of 0.66 µm was observed in the widths of cells grown at elevated T when compared to the control. Cells of *Jenufa* AD0402 also showed a significant reduction in diameter ($p < 0.05$) of 0.45 µm when compared to that of the control (Fig. 3e).

Cultures of *Nostoc* AD0303 grown under elevated UVR showed the greatest variation in morphology. Filaments became densely aggregated and individual filaments could not be easily discerned under the microscope (Fig. 3c). Cultures of *Jenufa* AD0402 grown under elevated UVR exhibited thicker cell walls (Fig. 3f). Heterocytes were observed in cultures of *Nostoc* AD0303 grown under increased CO_2 concentration, prior to culture death. Heterocytes were not observed in any of the other experimental cultures. Cultures of *Jenufa* AD0402 grown under elevated CO_2 exhibited a significant reduction in cell diameter ($p > 0.05$) of 1.38 µm.

3.4 Pigment Analysis

Cultures of *Nostoc* AD0303 grown under elevated T and UVR had significantly lower concentrations ($p > 0.05$) of allophycocyanin (93.5%) (Fig. 4a). When grown under elevated UVR, *Nostoc* AD0303 cells contained a significantly lower ($p > 0.05$) concentration of phycocyanin (reduced by 89.7%) and carotenoids (90% lower) (Fig. 4b). Cultures of *Jenufa* AD0402 showed no significant changes in pigment concentration in all experimental treatments (Fig. 5a, b).

3.5 Photoprotective Pigments

No typical absorption maxima for scytonemin and mycosporine-like amino acids were detected.

3.6 Biomass

Nostoc AD0303 produced a significantly greater biomass when cultured at an elevated T, whereas *Jenufa* AD0402 produced a significantly greater biomass when cultured at elevated UVR (Fig. 6). For the CO_2 parameter, the data shown in Fig. 6 is for the final experiment, in which cultures were incubated for 8 weeks.

4 Discussion

The biofilms and biomats studied so far are diverse in morphology and are composed of heterogenous communities of microorganisms. A high diversity of morphologically distinct cyanobacteria and microalgae were found to make up these microbial communities. The species composition varied between different types of biomat communities, while the biofilm samples had

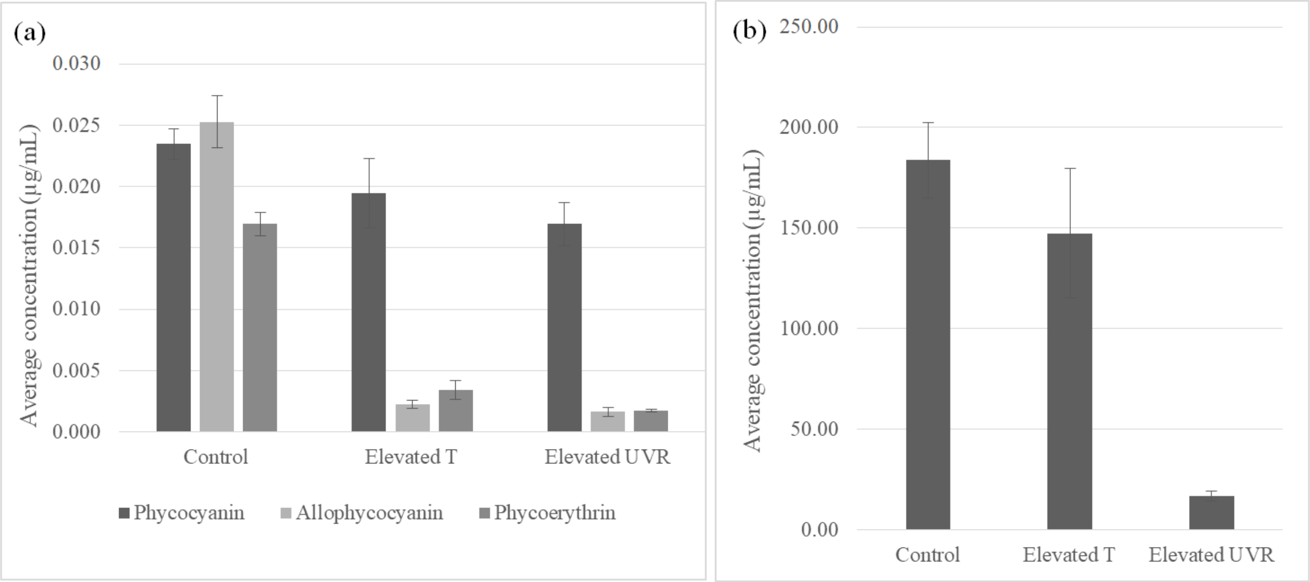

Figure 4: Pigment concentrations of *Nostoc* AD0303, (a) phycobiliproteins, (b) carotenoids. Error bars denote the standard deviation of duplicate samples.

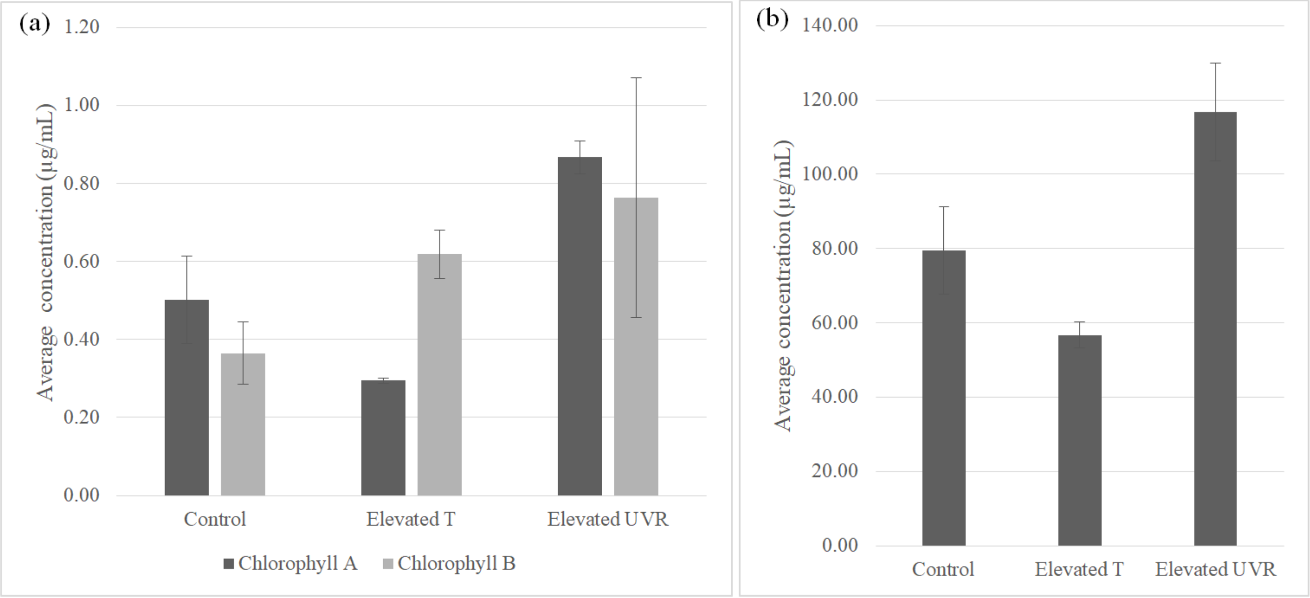

Figure 5: Pigment concentrations of *Jenufa* AD0402 (a) chlorophylls *a* and *b* (b) carotenoids. Error bars denote the standard deviation of duplicate samples.

similar compositions. Thus, the identity and relationships between these microorganisms may be important in shaping the community together with the prevalent biotic and abiotic components. Distinct layers of terrestrial biomats were composed of different types of microalgae and cyanobacteria. On the other hand, aerophytic biofilms in the marine littoral were commonly dominated by cyanobacterial filaments that were arranged in tufts attached to the rock surface (Fig. 1c).

Both *Jenufa* and *Nostoc* strains responded differently to changes in environmental parameters (Fig. 2). The cultures follow a typical sigmoidal growth curve, with a second spurt of log phase growth after three months, when cultures were supplemented with liquid medium. Temperature yielded a positive effect on the growth rate of *Nostoc* AD0303. The final mean cell density recorded for cultures grown at elevated temperature was twice that recorded in the control. The final

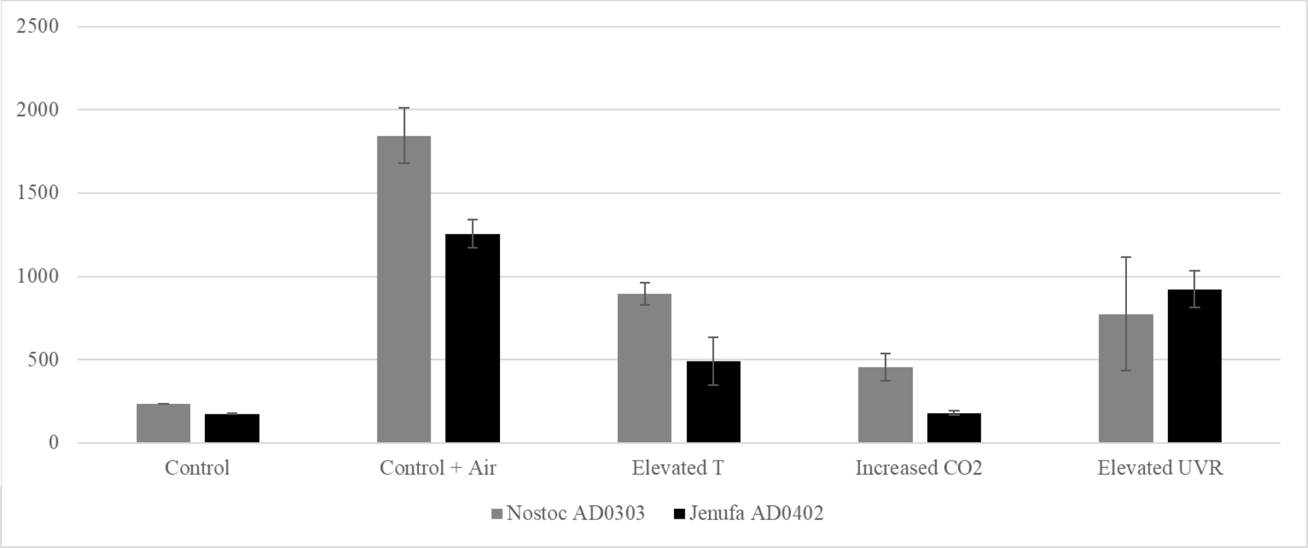

Figure 6: Final biomass yield (µg/L) for both strains. Error bars denote the standard deviation of duplicate samples.

biomass yield at the end of the experiment was four times higher than that of the control. A similar positive effect of temperature on growth has been documented extensively in cyanobacteria, as in the marine cyanobacterium *Synechococcus* and the terrestrial cyanobacterium *Nostoc flagelliforme* (Fu, Warner, Zhang, Feng & Hutchins, 2007; Li et al., 2016). The effects on growth rate are influenced by the photosynthetic rate. A higher photosynthetic rate is determined by carbon fixation rate of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, (RuBisCO), which increases at higher temperatures (Beardall & Raven, 2004). Microscopic observation of *Nostoc* AD0303 showed that cultures consisted of short rapidly dividing filaments throughout the entire experiment (Fig. 3b). Temperature was found to have no effect on the concentrations of chlorophyll *a*, *b* and carotenoids of *Nostoc* AD0303, however significant reductions in allophycocyanin and phycoerythrin were recorded (Fig. 4a, b). This suggests that other pigments may be contributing to the increase in growth rate. This significant reduction in phycobiliproteins contradicts findings published for other cyanobacteria. For instance, the production of phycobiliproteins in *Spirulina platensis* showed the highest concentrations of phycoerythrin and allophycocyanin at temperatures between 30–35 °C (Kumar, Kulshreshtha & Singh, 2011).

Temperature had a different effect on the growth of *Jenufa* AD0402. Unlike cultures of the same strain grown under the different experimental conditions, these cultures showed a lag phase at the start of the study. Moreover, one of the replicates grown under elevated temperature failed to grow and the culture died at day 50. This implies that this specific subaerophytic strain is acclimatised to grow at lower temperatures in its natural habitat and the cells do not easily adapt to changes in temperature. This is corroborated by the significant reduction in cell diameters observed in *Jenufa* AD0402 within the first month. No significant differences in growth rate, cell size or pigment content was noted in experimental cultures compared to the control after six months. This suggests that growth in *Jenufa* cells was not completely inhibited by elevated temperature, but that the cells took longer to acclimatize.

Increasing the concentration of CO_2 had an inhibitory effect on the growth rate of both strains. Elevated CO_2 resulted in culture decline and death of one or both strains. In the first experiment, *Jenufa* and *Nostoc* culture death was attributed to the 10-hour duration of CO_2 flow which caused the liquid medium to become acidic ($\mathrm{pH} = 4.7$). The bleaching of cells due to an acidic pH was also documented in *Synechococcus* at a pH lower than 5.0 (Sakomoto, Delgaizo & Bryant, 1998). In our experiment, the flow of CO_2 was subsequently decreased in order to maintain the pH of the media; however, culture growth of both strains was still inhibited. This suggests that the CO_2 was the direct cause of growth inhibition.

In the final CO_2 experiment, a limited weekly supply of CO_2 for 15 minutes was followed by an additional 15 minutes of air. In this experiment cultures of *Jenufa* AD0402 grew steadily till the end of the experiment. During a 20-day period, the mean cell density was the same in the experimental culture and in the aerated control, suggesting that oxygen deprivation had been the cause of culture death in previous experiments. However, filaments of *Nostoc* AD0303 remained bleached and took over two months to recover after the experiment was terminated.

UVR, specifically UV-A (emission maximum at 320–400 nm) influenced the morphology of both *Jenufa* AD0402 and *Nostoc* AD0303. In general, cyanobacteria may employ a range of mechanisms which allow them to tolerate or avoid exposure to UVR. For instance, motile species may glide away from UVR exposure, however both strains used in this study are non-motile. The formation of a microbial mat consisting of various layers of cells or filaments for protection, is also a possible response (Singh, Häder & Sinha, 2010). In fact, both strains were present as biofilms or biomats in their natural habitat. However, this six-month study was probably too short to result in biomat or biofilm formation. At the end of the UV experiment, cultures were screened for UV-absorbing compounds scytonemin and MAAs. MAAs may occur in both strains but the production of scytonemin is limited to cyanobacteria. *Nostoc flagelliforme*, for instance has been documented to produce both scytonemin and MAAs (Ferroni, Klisch, Pancaldi & Häder, 2010). Scytonemin was also isolated from *Nostoc commune* (Matsui et al., 2012). However, even though the production of UV-absorbing compounds has been recorded in several strains, the *Nostoc* strain AD0303 used in this experiment produced neither of these compounds under the experimental conditions provided here. *Jenufa* AD0402 did not produce any UV-absorbing compounds either. On the other hand, the mechanism that allowed the cultures to thrive in elevated UV-A was a change in cell and filament morphology. In fact, filaments of *Nostoc* AD0303 became aggregated to a point where it was hard to distinguish between individual filaments. This aggregation allowed for the formation of clusters of filaments in which the outer filaments sheltered the inner filaments. The compact structure was the result of thicker sheaths and an extracellular polysaccharide (EPS) matrix around the cells. On the other hand, *Jenufa* AD0402 grown under elevated UVR exhibited thicker cell walls and a significant reduction in cell diameter (Fig. 4f). Similar reactions to UVR exposure have also been documented in the macroalgae *Gelidium floridanum* (Schmidt et al., 2012) and the microalga *Chlamydomonas reinhardtii* (De Lange & Van Donk, 1997). The change in morphology in both *Nostoc* and *Jenufa* strains must have protected the cells adequately to allow for biomass accumulation and growth under elevated UVR.

5 Conclusion

Further knowledge is constantly being gained from the continued study of the biodiversity and ecology of biofilm and biomat forming microorganisms growing in different microhabitats around the Maltese islands. The number of new taxa being discovered routinely suggests that more intensive research in this field is required. The effects of changes in environmental parameters on two of these phototrophic microorganisms, a cyanobacterium and a microalga, were found to be strain specific.

Under increased CO_2, cyanobacterial filaments of *Nostoc* AD0303 became bleached and proved to be less resistant than the microalgal *Jenufa* AD0402 cells. Under elevated UV-A levels, both strains underwent changes in morphology which allowed them to proceed with photosynthesis and growth. This implies that the photosynthetic apparatus was not irreparably damaged. *Nostoc* AD0303 benefitted positively from an elevated temperature, whereas *Jenufa* AD0402 took a longer time to acclimatize, however, at the end of the study there was no significant difference between the experimental culture and the control.

Further studies on different strains of Maltese cyanobacteria and microalgae and their response to GCV should be undertaken, since some of these strains might be used as early indicators of climate change in particular habitats. In view of this, our current studies are investigating the effects of GCV on phototrophs isolated from Maltese rocky shore environments, which are seasonally exposed to stresses such as high UV-radiation, temperature and desiccation.

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Research Article

Time Variation, Asymmetry and Threshold Effects in Malta's Phillips Curve

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Abstract. This paper estimates a Phillips curve for Malta using data since the 1960s, using Bayesian methods to estimate a time-varying parameter model with stochastic volatility. It presents evidence that the curve has flattened over time. This implies that the link between inflation and economic activity has weakened, consistent with findings for other countries. This phenomenon is driven by downward price stickiness and threshold effects, where inflation is generally unresponsive to domestic economic conditions unless the economy is going through a strong boom. Meanwhile, this study finds an increasingly important role for import price shocks in driving inflation in the Maltese economy, owing to its increased openness and trade integration. The estimated variance of shocks to inflation was high in the 1980s, but has fallen greatly since then, rising somewhat in the run-up to the Great Recession.

Keywords: Inflation, NAIRU, time-varying parameters, Bayesian methods, Metropolis-Hastings, Gibbs sampling

1 Introduction

> *Understanding inflation dynamics has become particularly important in view of the low inflation regime now prevailing and because the traditional relationship between slack in the economy and inflation seems to have weakened significantly in some countries.*
>
> *If confirmed, the flattening of the Phillips curve would be relevant for monetary policy because that relationship was the traditional linchpin of the transmission mechanism that gave central banks control of inflation. The subsequent focus on the role of expectations and their management in the toolkit of monetary policy reduced but did not eliminate the relevance of the traditional mechanism.*
>
> —Vítor Constâncio[1]
> Former ECB Vice-President

Central banks have striven to earn credibility in their quest to control consumer price inflation by, *inter alia*, improving their communication through the announcement of a preferred inflation rate. In the euro area, monetary policy is conducted with the primary objective of keeping inflation "below, but close to, 2% in the medium term" (ECB, 2001). When analysing economic developments, econometric models help shape views about the current and medium-term outlook for economic activity and inflationary pressures.

Since the financial crises of 2008 and the ensuing Great Recession, research is being directed at studying additional important channels through which shocks propagate. At the same time, economists observe that models which enjoyed a good track record at forecasting inflation tended to perform badly during the past ten years, predicting a more significant drop in inflation than what materialised. This was termed the period of the 'missing deflation' (Ball & Mazumder, 2011; Stock, 2011; Ball & Mazumder, 2015).

Economists believe that in the short run inflation moves in line with economic conditions. This relationship, known as the Phillips curve, traces its origins to an empirical exercise conducted in the late 1950s, showing a negative relationship between nominal wage growth and unemployment in the United Kingdom (Phillips, 1958). During times of strong demand, firms employ more workers, leading to a tighter labour market. This puts upward pressure on wage claims, and therefore also on

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[1] Costâncio (2015)

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firm operating costs, which are reflected in higher prices for goods and services, and so on. Low demand should generate the opposite effect. Thus, demand shocks boost economic activity, lowering unemployment and increasing inflation.

In the past, policymakers believed they could exploit this trade-off, reducing unemployment at the cost of higher inflation. However, advances in the theory behind the Phillips curve, in particular the incorporation of people's expectations in the late 1960s, as well as a better framework for firms' pricing behaviour in the 1970s and 1980s, showed that exploiting this trade-off did not really pay off in the medium to long run. Economists realised that as people come to expect higher inflation, unions would call for higher wage growth, which would increase unemployment back to an equilibrium level. The economy would return to the previous unemployment rate, living with a higher rate of price inflation. Thus, the Phillips curve is vertical in the long run, unrelated to economic activity.

Today the Phillips curve is a complex but important component of the New Keynesian DSGE model, which is the workhorse model in academia, central banks and other policy-making institutions. Despite the rich theory behind it, recent studies have shown that simple versions of the Phillips curve can capture inflation dynamics reasonably well. A more detailed review of the literature is given in Section 2.

The Phillips curve in Malta is relatively unexplored. It is embedded within the Central Bank of Malta's macro-econometric model (O. Grech & Micallef, 2014), where inflation responds to economic activity in the short run. A. Grech (2015a) plots annual data for the unemployment rate and inflation over the period 1960-2014 and finds a negative relationship, in line with theory. He argues however that the link is weak, implying that a tight labour market does not lead to strong upward pressure on prices, and vice versa.

This paper contributes to the literature by estimating the Phillips curve over a long time frame, using state-of-the-art techniques.[2] It also explores whether the relationship changed over time. The link between economic activity and inflation was high in the mid-1980s but fell progressively over the 1990s and was very weak thereafter. Further analysis shows that size and nature of economic disequilibrium also matters. Inflation tends to rise during strong economic booms, but does not fall during recessions. In recent years however, this relationship seems to have disappeared. Foreign price pressures play a strong and increasingly important role in driving inflation in Malta, since it is a small and very open economy.

Increased globalization and lower barriers to trade, mainly through EU accession, have led to an increase in competition, putting a lid on price pressures. In addition, increased participation in the labour market, especially female participation, together with a strong inflow of foreign workers boosted the labour supply, reducing labour market tightness. These factors can explain the observed 'flattening' of the Phillips curve.

2 The Phillips Curve

In this section I discuss the canonical specification of the Phillips curve, which has been rigorously developed over time, particularly with the incorporation of inflation expectations in the late 1960s, as well as micro-founded derivations of profit maximisation subject to nominal rigidities in the 1970s and 1980s.[3] The hybrid version of the New Keynesian (NK) Phillips curve (Gali & Gertler, 1999; Gali, Gertler & Lopez-Salido, 2001; Gali, 2008) is given by

$$\pi_t = \gamma_f \mathbb{E}_t \pi_{t+1} + \gamma_b \pi_{t-1} + \lambda \widehat{mc}_t, \tag{1}$$

where the parameters γ_f ,γ_b and λ are functions of structural parameters, $\mathbb{E}_t\pi_{t+1}$ is expected future inflation, reflecting forward-looking behaviour, π_{t-1} is lagged inflation, capturing inflation inertia, and $\widehat{mc}_t$ is real marginal cost of production, which is the activity variable through which prices are affected. The latter term has been shown to be proportional to the output gap, under a number of assumptions (Gali & Gertler, 1999; Gertler & Leahy, 2008). For this reason, empirical studies proxy real marginal costs by a measure of the output gap (see Bermingham, Coates, Larkin, O'Brien & O'Reilly, 2012; Jordan & Vilmi, 2014).[4]

Other studies use the deviation of the unemployment rate from the Non-Accelerating Inflation Rate of Unemployment (NAIRU) as the activity variable, referred to as cyclical unemployment or the unemployment gap (see Ball & Mazumder, 2011; Peach, Rich & Cororaton, 2011; Bermingham et al., 2012; Kajuth, 2012; Simon, Matheson & Sandri, 2013; ECB, 2014; Speigner, 2014). Using cyclical unemployment as the activity variable is more reminiscent of the traditional Phillips curve.[5]

The definition of expected inflation varies across empirical studies. Expectations are proxied either by survey-based measures of expected inflation (Jordan & Vilmi, 2014), announced central bank targets (Simon

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[2] Studies using data with long time spans for the Maltese economy are relatively scarce.

[3] See Kajuth (2012) for a list of the important contributions to this area.

[4] However it has been argued that for such specifications the proper proxy for marginal costs is the labour share of income; see Gali and Gertler (1999).

[5] Other studies use more complex specifications which take into account asymmetric/threshold effects and differences between the short term and long term unemployment; see *inter alia* Laxton, Rose and Tambakis (1999), Bermingham et al. (2012), Speigner (2014) and Ball and Mazumder (2015).

et al., 2013), or a long run average of realised inflation (Ball & Mazumder, 2011).[6]

In empirical studies aimed at determining the size and significance of the coefficients relating to the determinants of inflation, inference is based on a reduced-form Phillips curve. A number of authors have recently introduced time variation in the parameters, allowing the relationship between inflation and its determinants to change over time (Simon et al., 2013; Stevens, 2013; Álvarez & Urtasun, 2013; Oinonen, Paloviita & Vilmi, 2014; Riggi & Venditti, 2015). This was partly motivated by the poor forecasting performance for inflation during and after the financial crisis (ECB, 2015). While one reason behind the large forecast errors were incorrect real-time estimates of activity gaps, it has also been shown that the sensitivity of inflation to activity has changed recently. This topic has also been re-visited in a recent ECB conference (see Hartmann & McAdam, 2018). This highlights the importance of allowing for structural change in empirical models.

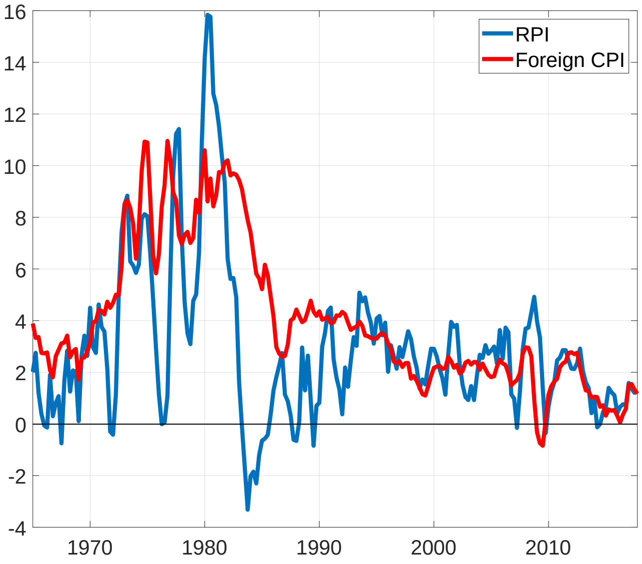
There are other factors which can explain apparent 'shifts' in the Phillips curve, for example, it may be subject to threshold and asymmetry effects (Laxton et al., 1999; Musso, Stracca & van Dijk, 2009; Bermingham et al., 2012; Speigner, 2014). Very strong booms or deep recessions may affect inflation differently than smaller, more typical booms and recessions. Owing to downward price rigidity, inflation may also not turn negative during recessions. These issues are explored in Section 4.3.

3 Data

This section describes the data that were used in this study. The main variables of interest are the Retail Price Index (RPI), registered unemployment, real GDP, and consumer price indices (CPI) of Malta's key trading partners. Further information on the data sources and workings can be found in Appendix Appendix A.

Fig. 1 shows yearly growth in the RPI and an index of foreign consumer prices since the mid-1960s.[7] The long-run co-movement between these two price series has been high, which implies that both series were driven by common factors, such as the oil price shocks in the 1970s and 1980s. The two series deviate somewhat in the mid-1980s, partly on account of the price controls that were enacted on some consumer goods at the time in Malta. Subsequently, both foreign and domestic inflation comove and stabilise around lower levels.

Figure 1: Inflation indicators (yearly growth (%)).



Figures 2 and 3 show GDP growth and the unemployment rate and their underlying (and unobserved) trends, potential output and the Non-Accelerating Inflation Rate of Unemployment (NAIRU). The trends were estimated using an Unobserved Components Model (UCM) based on the Kalman filter; see Appendix Appendix B for details. The data match the stylised facts for a number of advanced economies, in which GDP growth becomes more stable after the 1980s (Summers, 2005) and the Maltese economy, being small and very open, enjoyed the spillovers of the so-called Great Moderation. The output gap and cyclical unemployment, defined as the difference between the data and estimated trends, are treated as indicators of excess demand. A positive output gap implies output growing at a faster rate than potential growth, and vice-versa. Similarly, negative cyclical unemployment indicates an excess demand for labour, and vice-versa.[8]

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[6] Some studies adopt the hybrid approach, including both leads and lags of inflation. For instance, Simon et al. (2013), Blanchard, Cerutti and Summers (2015) and Blanchard (2016) list the following specification:

$$\pi_t = (1-\vartheta)\pi_{t-1} + \vartheta\pi_t^e - \kappa\bar{u}_t + \gamma\pi_t^m + \varepsilon_t, \qquad (2)$$

where ϑ measures the relative importance given to expectations of future inflation during wage and price setting, relative to information from past inflation, κ measures the slope of the Phillips curve on the activity variable (in this case cyclical unemployment $\bar{u}_t$) and γ measures the impact of imported inflation.

[7] The foreign CPI is an index based on CPI developments in France, Germany, Italy and the United Kingdom, which were historically the most important trading partners.

[8] The output gap and unemployment gap, as expected, are negatively correlated. Developments in GDP growth typically precede developments in the labour market, with a lag of about 2 to 3 quarters.

Figure 2: Real GDP and potential output growth (annual, %).

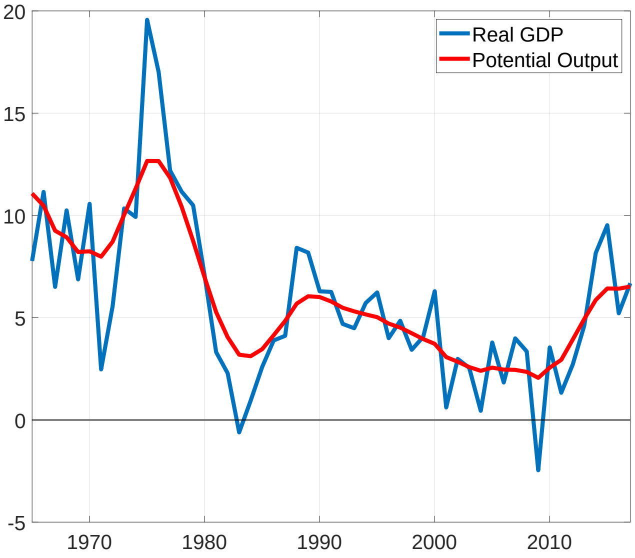
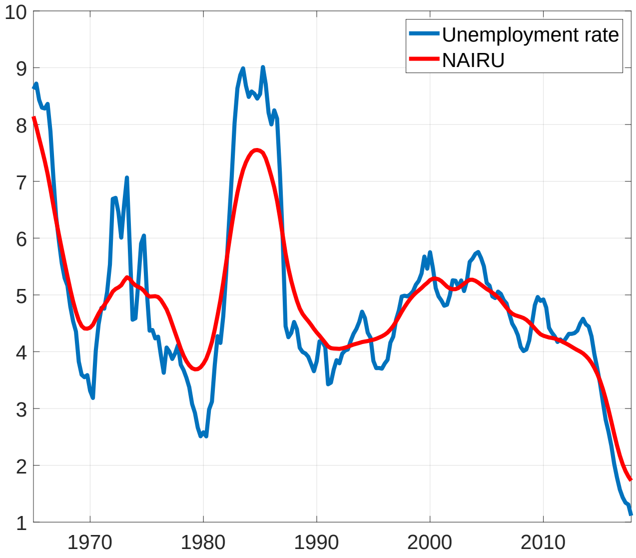


Figure 3: Registered unemployment rate and the NAIRU (%).



4 Empirical Results

In this section, I first present estimates of the Phillips curve under the assumption of constant parameters over the entire sample. I then relax this assumption and use a more sophisticated technique to uncover possible changes in the coefficients over time, and allow different inflation responses during booms and recessions.

4.1 A Standard Phillips Curve

The Phillips curve specification that is used in this paper is

$$\pi_t = c + \alpha X_{t-i} + \gamma \pi^f_{t-i} + \sum_i \rho_i \pi_{t-i} + \varepsilon_t, \qquad (3)$$

where π is annual RPI inflation, X is the activity variable and π^f is relative import prices proxied by annual growth in foreign CPI less RPI growth. The appropriate lag length i for each variable is determined empirically. Note that this specification assumes that inflation expectations are purely backward-looking, or adaptive. Eq. (3) is estimated using both cyclical unemployment $\tilde{u}$ and the output gap $\tilde{y}$ as the activity variables ($X \in \{\tilde{u}, \tilde{y}\}$).[9]

Since $\tilde{u}$, $\tilde{y}$ and π^f are all autocorrelated over time, including several lags of these variables introduces high collinearity between regressors. Hence, after some testing, cyclical unemployment was only included in its third lag and imported inflation included only in its first lag. This choice was guided by the cross-correlogram for the dependant variable and the regressors, and the lag at which there was the highest correlation was chosen. Given the lagged co-movement between the output gap and cyclical unemployment, the output gap was lagged by 1 quarter. Since inflation is measured in annual percentage changes, the model includes the first four lags of inflation to control for residual serial correlation. Eq. (3) is estimated using Ordinary Least Squares (OLS), using quarterly data for the period 1965Q4–2017Q4.[10] Inference is based on Newey-West standard errors (Newey & West, 1987).

The OLS estimates are shown in Table 1 below in column (1). The 'slope' of the Phillips curve, α, is statistically significant at conventional levels only when cyclical unemployment is used as the activity variable. This shows some link between economic activity and prices. Cyclical unemployment is arguably a more indicative measure of economic activity than the output gap, as transitory shocks to GDP, which affect the output gap, may be absorbed by firms and thus not reflected in employment through the extensive margin. Column (1) also confirms the important role of import price shocks on domestic inflation. The measure of fit of both models, assessed using the adjusted R-squared ($\overline{R}^2$) is very

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[9] This specification is typically used in empirical studies; see *inter alia* Simon et al. (2013). It nests the hybrid NK Phillips curve since it is assumed that expected inflation is equal to inflation in the previous period and marginal costs are approximated by the slack variables $X \in \{\tilde{u}, \tilde{y}\}$. In open economy versions of the NK Phillips curve an additional variable is the (change in the) terms of trade Gali and Monacelli (2005). Other foreign variables appear in the marginal cost variable, but given that estimation is based on a reduced-form representation, foreign prices are explicitly included as a separate variable.

[10] Although inflation and unemployment are available in quarterly frequency, GDP, from which the output gap is derived, was only measured in annual frequency during roughly the first half of the sample. Appendix Appendix A explains how this data was interpolated to quarterly frequency.

high, although most of the fit can be attributed to inflation being explained by its history.

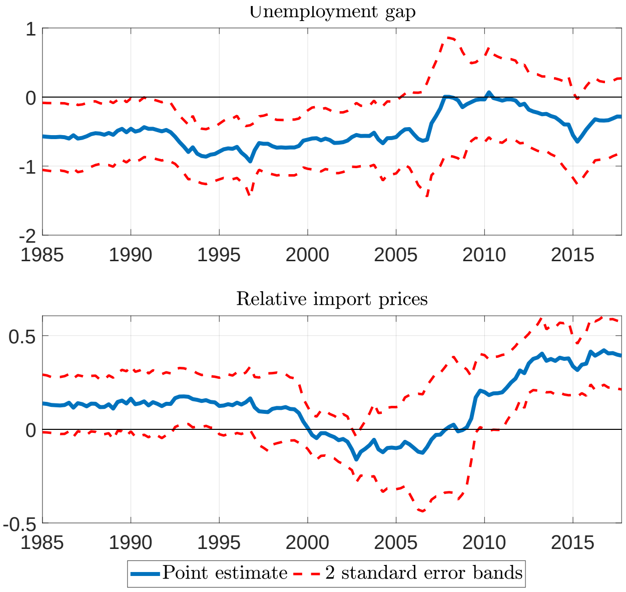
These estimates are based on a relatively long time series, during which the Maltese economy witnessed significant structural and socio-economic changes.[11] It is therefore likely that the relationship presented above might have changed over time. Two approaches are used to test the stability of the parameters. The first is to split the sample into two, an 'early' period spanning 1966–1995, and a more recent period over 1996–2017, and estimate the Phillips curve for each sub-sample.[12] The results are in columns (3) to (6) respectively in Table 1. By comparing the estimated coefficients over the two periods, we can assess any material shifts in the coefficients for economic slack and imported inflation over the two periods.

A second approach is to estimate rolling regressions for Eq. (3) to track the evolution, if any, of the parameters over time.[13] Starting from 1965Q4, the first 80 observations, or 20 years worth of data (the 'window') are used to estimate the Phillips curve. The estimated coefficients are saved, and the sample is moved by one period forward in time, while keeping the same window length. I repeat the process until the end of the entire sample (2017Q4). The estimates from each recursion track 'smooth' changes in the parameters and report them as time series. I use the unemployment gap as the relevant activity variable, and show the results in Fig. 4.[14]

Both the sub-sample and rolling regression approaches provide evidence of a change in the slope of the unemployment gap version of the Phillips curve, α, over time. The parameter in the first sub-sample is -0.535 and statistically significant (column (3)), while in the second it is lower in absolute terms (-0.209) and not statistically different from zero (column (4)), indicating a significant change. This development is not easily seen in the output gap version, as in both sample periods the coefficient is negative and insignificant. As discussed above, this latter result could be due to the fact that the output gap is somewhat volatile, carrying less information about the cyclical position of the economy compared to the unemployment gap, which is more persistent.

These results are supported from the rolling regression estimates. The top panel of Fig. 4 shows that the slope of the Phillips curve was relatively stable for a long time up to the late 2000s, after which it tended to zero. The same dynamics occur at a slightly earlier stage for the economy's sensitivity to import price shocks. It is interesting to note that the drop in the inflation sensitivity to economic slack and import price shocks, and wider error bands, coincide with the Great Recession.[15] From 2010 onwards we observe a reversal, especially in the coefficient for imported inflation.

Figure 4: Rolling regression parameter estimates.



These results are interesting as they imply that significant changes, potentially structural, took place in the Maltese economy. Furthermore, and as noted above, volatility in inflation was high in the 1970s and 1980s, but then fell markedly since the 1990s. This phenomenon is also observed in many major economies as one of the characteristics of the Great Moderation. The fall in inflation and its volatility across advanced economies is argued to be primarily a consequence of credible inflation targeting central banks, which stabilized long-term inflation expectations (Simon et al., 2013; Carney, 2015).

4.2 Allowing for Stochastic Volatility: A TVP-SV Model

A model which allows the parameters to change over time but ignores the changing volatility in the dependant variable is likely to overestimate or lead to spurious variation in the coefficients, as these 'soak up' some of the variance of the residuals (Cogley & Sargent, 2005;

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[11] See A. Grech (2015a).

[12] The choice for splitting the sample around 1995 was made mainly to have roughly balanced sub-samples.

[13] This approach is frequently used in the literature, see Oinonen et al. (2014).

[14] The first parameter estimates are as at 1984Q4. The results using the output gap version of the model are not shown, but are qualitatively the same.

[15] Admittedly, the timing of these dynamics is somewhat sensitive to the window length used in estimation. A shorter window of 60 observations shows the same dynamics occurring earlier in time. However, the key point is the indication of instability in the relationship over time.

Table 1: OLS regression results.

	(1) 1965Q4–2017Q4	(2) 1965Q4–2017Q4	(3) 1965Q4–1995Q4	(4) 1996Q1–2017Q4	(5) 1965Q4–1995Q4	(6) 1996Q1–2017Q4
$\tilde{u}_{t-3}$	-0.450^{**}		-0.535^{***}	-0.209		
$\tilde{y}_{t-1}$		0.016			0.010	-0.046
π^f_{t-1}	0.130^{***}	0.118^{**}	0.144^{**}	0.381^{***}	0.137	0.392^{***}
$\overline{R}^2$	0.863	0.862	0.871	0.668	0.865	0.668
Obs.	209	209	121	88	121	88

Notes: *** and ** imply statistically significant coefficients at the 1% and 5% level of significance respectively and are based on Newey-West standard errors. Obs. is the number of observations used in the estimation. The coefficients on the autoregressive terms are not shown, but throughout the regressions are jointly statistically significant and sum up to less than one, implying that RPI inflation is a stationary process.

Primiceri, 2005; Nakajima, 2011). For this reason, the Time-Varying Parameter model with Stochastic Volatility (TVP-SV) is explored next. This model allows both the parameters and the volatility of shocks to inflation to change over time.[16] I use this technique on the unemployment gap version of the model.

The TVP-SV specification of the baseline Phillips curve is given by

$$\pi_t = c_t + \alpha_t \tilde{u}_{t-3} + \gamma_t \pi^f_{t-1} + \sum_i^4 \rho_{i,t}\pi_{t-i} + \varepsilon_t\sqrt{h_t}, \quad (4)$$

where coefficients now have a time subscript and shocks to inflation ε_t are augmented with a time-varying variance term h_t. The parameters of the model c, α, γ and ρ_i ($i \in [1,4]$), and the logarithm of h, are assumed to follow random walks. When stacked into the vector $B_t = [c_t\ \alpha_t\ \gamma_t\ \rho_{1,t}\ \rho_{2,t}\ \rho_{3,t}\ \rho_{4,t}]'$, the evolution of the parameters can be represented as

$$B_t = B_{t-1} + \nu_t, \quad (5)$$

where ν_t is a vector of shocks. The evolution of the (log) variance of shocks is given by

$$\log h_t = \log h_{t-1} + \eta_t, \quad (6)$$

where η_t is a disturbance term. This setup constitutes a non-linear state-space model, as the state variable h_t is not linear in the observation equation (Eq. (4)).

The model is estimated using Bayesian methods, specifically a Metropolis-within-Gibbs sampler, using the algorithm of Carter and Kohn (1994) to extract the path for all the elements in B_t in every iteration.[17] Following Primiceri (2005), a fraction of the data were used as a training sample to initialize the priors (1966Q1–1979Q4). Details on the estimation setup are available in Appendix Appendix C. The sample on which inference is based spans 38 years (1980Q1–2017Q4) and the estimation procedure is based on 20,000 iterations. The first 5,000 burn-in draws are discarded and the remaining draws are used to construct the posterior distributions of the parameters, shown in Fig. 5.

The time-varying Phillips curve slope, α_t, is estimated to have declined in absolute terms since the 1980s, implying a weakening in the relationship between economic activity and inflation. This confirms the findings of the previous section, and is a pattern that is observed in many advanced economies, in line with the findings in Simon et al. (2013) and Blanchard et al. (2015). Although there are no major changes during the period associated with the financial crisis, a slight but sudden change in the trend of the slope can be seen starting in 2010.

The estimates for γ_t show that the role of import prices was weakest during the period of the price controls in the early 1980s. Thereafter, imported price shocks played a progressively stronger role in explaining inflation in Malta. This trend is also in line with other studies; Stevens (2013) and Simon et al. (2013) find the same behaviour in the economies of the EU and of a number of OECD countries, respectively. The variance of shocks to inflation, h_t (bottom right panel), exhibited significant time variation, being high in the early 1980s but then falling significantly. Inflation volatility rose temporarily just before the 1990s and more recently in 2007, on account of the food price shocks that preceded the financial crises.

Inflation persistence fell significantly since the 1980s, implying that when everything else is kept constant, shocks to inflation used to die off much slower in the past. The anchoring of expectations in many major eco-

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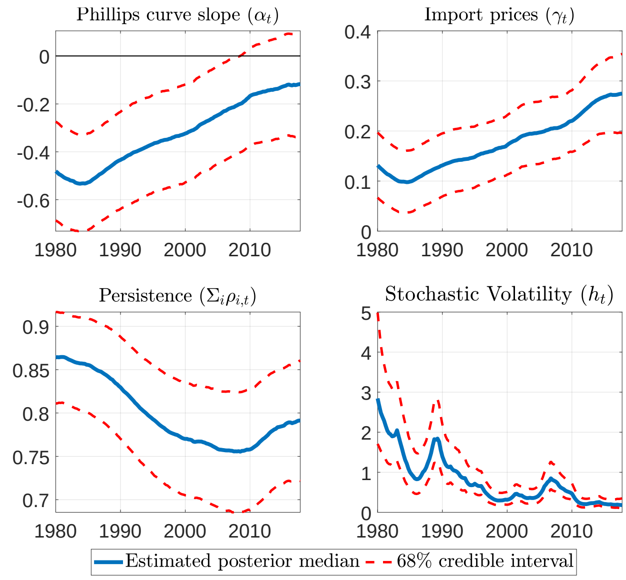
[16] See Cogley and Sargent (2005) and Primiceri (2005) and the literature cited therein for a discussion of this model. An explanation of this setup for a univariate framework can be found in Nakajima (2011, p.109). More details are provided in Appendix Appendix C.

[17] See Jacquier, Polson and Rossi (1994) and Blake and Mumtaz (2012) for a discussion of Monte Carlo Markov Chain (MCMC) Bayesian inference in such models.

Figure 5: TVP-SV estimation results.



nomies and the increased synchronisation of the Maltese economy with such developments (through a rise in γ), are perhaps the key reasons for the decline in local inflation persistence. The uncertainty surrounding the time-varying parameter estimates is quite high, as the 68% credible intervals are relatively wide for α, γ and ρ_i. Nevertheless, these results highlight the possible changes that have occurred in the macroeconomy and are discussed further in Section 5. A model of the Maltese Phillips curve which assumes constant parameters and constant residual volatility, is therefore unable to capture all these interesting developments.

All the estimates presented above and in previous sections could be sensitive to how economic slack is measured. To this end the model is re-estimated using a measure of cyclical unemployment derived from the Hodrick-Prescott filter, which is another frequently used trend-cycle decomposition technique. The flattening of the Phillips curve is also observed on the basis of this activity measure. The sudden reversal in the slope starting in around 2010 is also confirmed in this set of estimates. Other robustness checks are discussed in a working paper version of this article (Gatt, 2016).

4.3 Asymmetry and Threshold Effects

The model specified above postulates that the economy behaves the same way irrespective of the state it is in, that is, irrespective of whether it is going through a boom or recession. This goes against the literature on downward nominal rigidities and Phillips curve convexity, which argues that one should not expect the same relationship at different points along the Phillips curve.[18] Empirical evidence shows that the response of inflation to slack may be state-dependent. For example, Demers (2003) and Barnes and Olivei (2003) find that the slope of the Phillips curve can change according to the state of the economy within a business cycle. Bermingham et al. (2012) also find the existence of threshold effects, whereby the link between economy and inflation is stronger during recessions compared to booms. Further discussion can be found in Musso et al. (2009).

To allow for the possibility of asymmetries in the Phillips curve, I modify the model in the previous section to allow different Phillips curve slopes conditional on booms ($\tilde{u} < 0$) and recessions ($\tilde{u} > 0$):

$$\begin{aligned}\pi_t = c_t + \alpha_t^b \tilde{u}_{t-3}\mathbb{1}_{(\tilde{u}<0)} \; + \; \alpha_t^r \tilde{u}_{t-3}\mathbb{1}_{(\tilde{u}>0)} \\ + \gamma_t \pi_t^f + \sum_i^4 \rho_{i,t}\pi_{i,t-1} + \varepsilon_t\sqrt{h_t},\end{aligned} \tag{7}$$

where $\mathbb{1}_{(.)}$ is the indicator function which takes a value of 1 when the condition within the brackets holds. As above, the parameter vector B_t and the log variance $\log(h_t)$ evolve as random walks. Estimation follows the same procedure as above and the results are summarized in Fig. 6. The drifts of the parameters γ and $\sum_i^4 \rho_i$ are similar to those in Fig. 5, so are not shown again.

Besides time variation, there is also evidence of asymmetry in the Maltese Phillips curve. The slopes associated with booms α_t^b and recessions α_t^r have both fallen in absolute terms since the 1980s. However, the relationship during a boom was much stronger throughout the sample period. Thus, the flattening phenomenon appears to be driven mainly by behaviour during economic booms, since the relationship during recessions was always weak.

The estimated unemployment gaps have varied in magnitude over time. Large deviations from the NAIRU occurred mostly during the early part of the sample. It may be the case that the Phillips curve relationship may also be sensitive to the *size* of labour market slack, as discussed in Barnes and Olivei (2003). The implication of this argument would be that the observed flattening may not reflect a change in the relationship, but merely the fact that prices are today reacting to much smaller shocks than in the past. Below a given threshold, inflation may respond very weakly (potentially in both directions), say due to menu costs.

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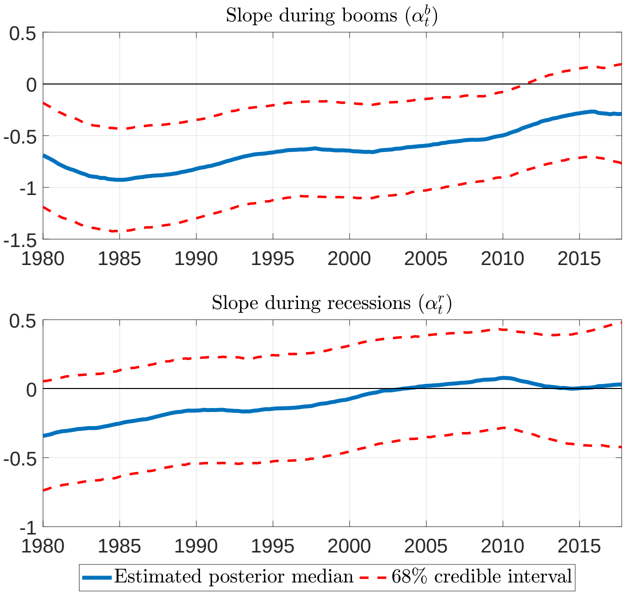
[18] See Laxton et al. (1999) and Speigner (2014). A theoretical account of how rigidities affect the convexity of the Phillips curve is given by Daly and Hobijn (2014).

Figure 6: Asymmetric Phillips curve slopes.



To test this formally, while maintaining the separation between booms and recessions, I modify the Phillips curve to the following form

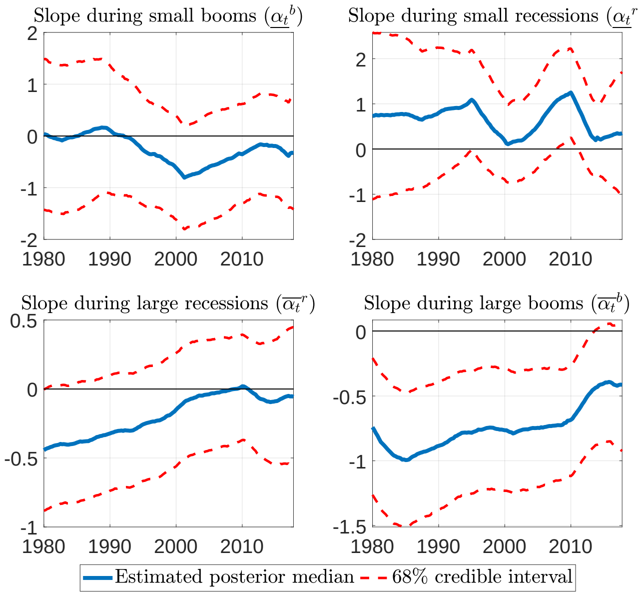
$$\begin{aligned}\pi_t = &\Big[\underline{\alpha}_t^b \tilde{u}_{t-3}\mathbb{1}_{(\tilde{u}<0)} + \underline{\alpha}_t^r \tilde{u}_{t-3}\mathbb{1}_{(\tilde{u}>0)}\Big]\mathbb{1}_{(|\tilde{u}|<\kappa)} \\ &+ \Big[\overline{\alpha}_t^b \tilde{u}_{t-3}\mathbb{1}_{(\tilde{u}<0)} + \overline{\alpha}_t^r \tilde{u}_{t-3}\mathbb{1}_{(\tilde{u}>0)}\Big]\mathbb{1}_{(|\tilde{u}|>\kappa)} \\ &+ \gamma_t \pi_t^f + \sum_i^4 \rho_{i,t}\pi_{i,t-1} + c_t + \varepsilon_t\sqrt{h_t}, \qquad (8)\end{aligned}$$

where the indicator function outside square brackets switches on during periods of low ($|\tilde{u}| < \kappa$) or high ($|\tilde{u}| > \kappa$) labour market slack respectively. This specification nests that in equation (7), so that the threshold effect is tested along with the asymmetry effect discussed above.[19] The threshold value κ was set at 1 standard deviation of the unemployment gap.[20] This specification effectively allows the economy to be in four distinct states and returns four slope parameters, which describe the relationship between inflation and slack during shallow and deep recessions ($\underline{\alpha}^r$ and $\overline{\alpha}^r$ respectively) and small and large booms ($\underline{\alpha}^b$ and $\overline{\alpha}^b$ respectively).

The results, shown in Fig. 7, shed further light into the degree of asymmetry and sensitivity of inflation to small and large shocks. The estimates are subject to a higher degree of uncertainty, given that few observations are available in each state, so these results should be interpreted with some caution.[21]

Nevertheless, the results related to the asymmetry of the Phillips curve discussed above remain valid. Shallow recessions have not been associated with a drop in inflation, and although there is evidence that deep recessions may have put downward pressure on inflation in the 1980s, the link has since then disappeared. Similarly, small expansions have not been associated with a rise in inflation. The asymmetry takes effect only during large expansions, and these estimates suggest that this relationship has also weakened somewhat since the 1980s.

Figure 7: Asymmetric-threshold Phillips curve slopes.



5 What Caused a Flat Phillips Curve?

I now discuss possible drivers of a falling Phillips curve slope. A number of theories have been fielded to explain this phenomenon, which as Simon et al. (2013) show, seems to be widespread across several advanced economies, with varying degrees of openness. The central argument raised in studies which report a flattening of the Phillips curve, is a general move towards 'anchored inflation expectations' (Simon et al., 2013; Ball & Mazumder, 2015; Blanchard et al., 2015). People's belief of moderate and stable future inflation, brought about by successful central bank monetary policy, reduced pressure on wages by workers and unions seeking to maintain the purchasing power of income. Anchored inflation expectations were attributed to the so-called 'missing deflation' in OECD countries, in which economic activity

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[19] Indeed, it also nests the fixed parameter model estimated using OLS.

[20] Similar results were obtained at a lower (0.5 standard deviation) and higher (2 standard deviations) threshold.

[21] There were 50 quarters of shallow recessions, 55 quarters of moderate booms, 25 quarters of deep recessions and 22 quarters of strong booms in the period 1980–2017.

dropped significantly but inflation did not turn negative.

Another key argument is the role of globalisation. Lower global inflation, in part due to increased openness to trade and cheaper imported goods – the so-called "China effect" (Lewis & Saleheen, 2014) – lowered domestic inflation. To this end, changes over both the general level of mark-ups and their relation to the economic cycle might have also changed pricing behaviour, and hence affected the Phillips curve slope (Carney, 2015). A theoretical account of how globalization drives a flat aggregate supply curve is given in Razin and Binyamini (2007). In fact, the empirical work of Borio and Filardo (2007) presents cross-country evidence of an increased role for global factors in explaining domestic price developments, especially since the 1990s. Furthermore Sbordone (2007) argues that globalisation may have led to a low inflation environment by moderating growth in marginal costs through increased competition.

Using a NK Phillips curve, as specified in Eq. (1) above, Kuttner and Robinson (2010) argue that changes in the persistence of marginal cost fluctuations can lead to a flattening of the slope, which is typically observed in reduced form estimates. However, they show that their estimate of the structural parameter, λ, linking developments in marginal costs and inflation in the United States, fell over time through an increase in the so-called Calvo parameter – the probability that firms in any point in time cannot revise prices.[22]

Since the Maltese economy is very small and open, the globalization argument is considered the prime mechanism driving a flatter Phillips curve. Lower barriers to trade over time, brought about by EU accession and later the adoption of the euro, led to increased competition, which controlled price pressures. This was coupled with low and stable inflation in trading partner countries. A more recent phenomenon, the rise of online purchases from abroad, marks an additional development in product market competition. In fact, whereas only 34% of Maltese households with internet access had purchased goods online in 2005, this percentage rose to 66% by 2015.[23] All of these developments have led to a decline in trend inflation in Malta (Gatt, 2014).

While estimates of the asymmetric Phillips curve show that the flat slope relates mainly to periods of subdued economic activity, the expected impact of a strong boom on prices is uncertain. Based on the 68% credible interval for estimated slope at the end of the sample, a 1 standard deviation downward shock to the unemployment gap is associated with an increase in inflation of between $[-0.09 - 0.93]$ percentage points on impact.

Developments in the labour market may have contributed to lower pressure on wage growth, through which we get a wage-price spiral. Trade unionisation rates have declined significantly from 33% in 1995 to 23% in 2013 (Micallef & Caruana, 2014). Labour participation rates, which were stable for decades, rose sharply after 1995, led by a near doubling of the female participation rate. This was also complemented by a significant inflow of foreign workers following EU accession (A. Grech, 2015b), and hence an overall increase in the labour supply may have dampened wage claims. These developments may be behind the stabilization in trend wage inflation, and thus explain the fall in the volatility of wage growth, as discussed in Gatt (2016). Therefore, while in Malta inflation tends to rise during an expansion, it does not fall during an economic slowdown.

6 Conclusion

This paper discusses and presents estimates of the Phillips curve in the Maltese economy using data starting from the mid-1960s. While OLS regression results show that the data fit the relationship over the full sample, sub-sample estimates point to a weakening of the relationship over time. Meanwhile, the same analysis shows an increase in the sensitivity of domestic inflation to import price shocks.

To analyse this further, I use a more flexible model which allows the Phillips curve parameters to change over time. Estimation of this model is based on a mix of Bayesian methods, and the results show significant changes in the parameters over time. The model is also able to track changes to the variance of shocks affecting inflation. The results show that, recently, shocks became smaller in magnitude on average compared to the 1980s, peaking only during the energy and food price shocks of 2007.

The decline in the slope of the Phillips curve is shown primarily to be due to an asymmetry in the relationship; the link between economic activity and inflation exists only during times of (strong) growth, implying downward price rigidity. The link between activity and nominal variables exists only when the shock to the economy is sizeable. However, increased openness and more stable economic growth are the key drivers for the observed flattening of the Phillips curve since the 1980s.

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[22] This will have an effect on the transmission of monetary policy in a simple New Keynesian DSGE model. A flat Phillips curve reduces the effectiveness of monetary policy which follows a Taylor rule for the nominal interest rate. An exogenous positive shock to inflation causes the same central bank to lift interest rates more aggressively when the Phillips curve is relatively flat. See Appendix Appendix D for simulations.

[23] See annual National Statistics Office reports titled 'ICT usage by households'.

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Appendix A Data

Historic data is sourced primarily from A. Grech (2015a). All recent data are subject to revisions.

A.1 Inflation

Inflation is measured using the Retail Price Index, an index which was first estimated in 1936 (Micallef & Caruana, 1996) and has been the key indicator used to track inflation in Malta for many decades. This data was obtained in monthly frequency for the period 1950-2014 from the National Statistics Office. Since the index was re-based several times since 1950, the index was spliced into a consistent series and re-based.[24] Quarterly averages of the price index P_t were obtained and inflation is defined as $\pi_t = [\log(P_t) - \log(P_{t-4})] \times 100$.[25]

A.2 Unemployment Rate

Data for registered unemployed and the labour supply were obtained from the Employment and Training Corporation (ETC; nowadays JobsPlus) from 1965 to 2014 in monthly frequency and spliced into a consistent series. The series was then seasonally adjusted using Census X12.

A.3 GDP

Annual data for GDP was obtained as a spliced series from different statistical methodologies over time.[26] The whole dataset was re-based in terms of millions of euros based on 2010 prices and was interpolated from annual to quarterly frequency using the Litterman interpolation technique (Litterman, 1983).[27,28]

A.4 Foreign CPI

This index was constructed as a weighted average using the consumer price indices of four of Malta's major trading partners, namely the United Kingdom (UK), France, Germany and Italy. These countries accounted on average for more than 80% of all trade within the EU and just under 60% of all of Malta's trade between the period 1980-2014. CPI indices are those found in OECD (2010), and were extracted from the FRED© database. Relative weights were obtained from the historical Direction of Trade statistics that can be found on the Central Bank of Malta's website. Data prior to 1980 was not available, and thus the earliest datapoint available was held constant to the past.

Appendix B Extracting Cyclical Indicators

This section describes the Unobserved Components Model (UCM) that is used to extract potential output growth, the output gap, the NAIRU and cyclical unemployment (as shown in Figs. 2 and 3) from the GDP and unemployment data. The trend-cycle decomposition is based on a state space representation of the system

$$\Delta GDP_t = \tau_t + \mu_t + \epsilon_t^{\Delta GDP}, \tag{B.1}$$

$$U_t = N_t + \lambda_t + \epsilon_t^U, \tag{B.2}$$

$$\tau_t = \tau_{t-1} + \epsilon_t^\tau, \tag{B.3}$$

$$N_t = N_{t-1} + \epsilon_t^N, \tag{B.4}$$

$$\mu_t = \varrho_1 \mu_{t-1} + \varrho_2 \mu_{t-2} + \epsilon_t^\mu, \tag{B.5}$$

$$\lambda_t = \theta_1 \lambda_{t-1} + \theta_2 \lambda_{t-2} + \theta_3 \mu_{t-4} + \epsilon_t^\lambda, \tag{B.6}$$

where ΔGDP_t is yearly GDP growth, U_t is the unemployment rate, τ is potential output, μ is the output gap, N is the NAIRU, λ is cyclical unemployment, $\epsilon_t^{\Delta GDP}$ and ϵ_t^U are measurement errors, and ϵ^i random shocks to $i \in \{\tau, N, \mu, \lambda\}$.

Eqs. (B.1) and (B.2) are the observation equations, which state that the left-hand side variable in each is the sum of a trend, a cyclical component and an irregular component which accounts for measurement errors. These sub-components are the unobserved state variables which the framework tries to identify. Therefore, τ and N represent potential output growth and the NAIRU respectively, and these are modelled in Eqs. (B.3) and (B.4) as random walks which are subject to white noise shocks $\epsilon_t^\tau \sim N(0, \sigma_\tau^2)$ and $\epsilon_t^N \sim N(0, \sigma_N^2)$.

The output gap and cyclical unemployment are modelled as μ and λ respectively in Eqs. (B.5) and (B.6). Owing to their cyclical nature they are modelled as stationary AR(2) processes, however the process generating cyclical unemployment is also a function of the output gap lagged by four quarters, in the spirit of Okun's law. This latter detail adds some economic structure to the decomposition implied by the system. Both of these processes are also subject to random shocks $\epsilon_t^\mu \sim N(0, \sigma_\mu^2)$ and $\epsilon_t^\lambda \sim N(0, \sigma_\lambda^2)$. The measurement errors follow white noise processes $\epsilon_t^{\Delta GDP} \sim N(0, \sigma_{\Delta GDP}^2)$ and $\epsilon_t^U \sim N(0, \sigma_U^2)$. All disturbances are uncorrelated with each other.

The model was parameterised as shown in the Table B.1 and run through the Kalman Filter. These parameters were chosen such that the resulting trend variables τ and N are not excessively volatile but evolve progressively over time. In Gatt (2016) I show that the resulting

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[24] It should be noted that the coverage of the RPI basket was initially very limited; but was extended over time to include a wider range of goods and services.

[25] Unless otherwise stated, all growth rates are calculated using this formula.

[26] The data between 1954 and 2000 are those measured by the *System of National Accounts* (SNA) methodology, and two such series were available, one based on 1973 prices (which spans from 1954–1997) and another based on 1995 prices (data for which spans 1970–1995). GDP data from 2000 onwards is based on the *European System of Accounts 2010* (ESA2010) guidelines. All these series were spliced into a consistent and continuous series.

[27] *Litterman Match Sum* technique in Eviews 9.

[28] Although ESA2010 data is available in quarterly frequency, mixing this data with the interpolated quarterly frequency data would have introduced noise to a relatively smooth data series.

estimate for potential output growth is consistent with production function-based estimates of potential growth for the Maltese economy.

Table B.1: UCM model parameters

Coefficients		**Variances**	
ρ_1	1.6	σ^2_τ	0.9
ρ_2	−0.8	σ^2_N	1.0
ϱ_1	1.6	σ^2_μ	1.8
ϱ_2	−1.1	σ^2_λ	2.1
θ	−0.1	$\sigma^2_{\Delta GDP}$	5.0
		σ^2_U	12.5

Appendix C The Time-Varying Parameter Model with Stochastic Volatility

The TVP-SV model is given by

$$\begin{aligned}\pi_t = {} & c_t + \alpha_t \tilde{u}_{t-3} + \gamma_t \pi^f_{t-1} \\ & + \sum_i^4 \rho_{i,t}\pi_{t-i} + \varepsilon_t \sqrt{h_t},\end{aligned} \tag{C.1}$$

$$c_t = c_{t-1} + \epsilon^c_t, \tag{C.2}$$

$$\alpha_t = \alpha_{t-1} + \epsilon^\alpha_t, \tag{C.3}$$

$$\gamma_t = \gamma_{t-1} + \epsilon^\gamma_t, \tag{C.4}$$

$$\rho_{1,t} = \rho_{1,t-1} + \epsilon^{\rho_1}_{t,} \tag{C.5}$$

$$\rho_{2,t} = \rho_{2,t-1} + \epsilon^{\rho_2}_t, \tag{C.6}$$

$$\rho_{3,t} = \rho_{3,t-1} + \epsilon^{\rho_3}_t, \tag{C.7}$$

$$\rho_{4,t} = \rho_{4,t-1} + \epsilon^{\rho_4}_t, \tag{C.8}$$

$$\log h_t = \log h_{t-1} + \eta_t, \tag{C.9}$$

where shocks to inflation ε_t are augmented with a time-varying variance term h_t and the parameters of the model α, γ, ρ and c and the logarithm of h are assumed to follow random walks. Stacking these parameters in the vector $B_t = [c_t\ \alpha_t\ \gamma_t\ \rho_{1,t}\ \rho_{4,t}\ \rho_{3,t}\ \rho_{4,t}]'$, we can express the evolution of these parameters as

$$B_t = B_{t-1} + \nu_t, \tag{C.10}$$

where ν_t is a vector of shocks following normal distribution with mean 0 and variance Q

$$\nu_t \sim N(0, Q). \tag{C.11}$$

Similarly the stochastic component in the log-volatility transition equation follows a Normal distribution with mean 0 and variance g

$$\eta_t \sim N(0, g). \tag{C.12}$$

This setup constitutes a non-linear state-space model, as the state variable h_t is not linear in the observation equation. The model is estimated using Bayesian methods, specifically a Metropolis-within-Gibbs sampler, using the algorithm of Carter and Kohn (1994) to extract the path for all the elements in B_t in every iteration.[29]

Following Primiceri (2005), the prior for Q follows an inverse Wishart distribution ($Q \sim IW(Q_0, T_0)$) with scale matrix $Q_0 = (Q_{OLS} \times T_0 \times k)$, and T_0 degrees of freedom , where Q_{OLS} is the covariance matrix from an Ordinary Least Squares (OLS) regression of the Phillips curve on a training sample, T_0 is the number of observations in the training sample, and k is a scaling factor. The training sample spans 1966Q1–1979Q4 ($T_0 = 56$), and the value of k was set to 0.01, which is standard in the literature (see Primiceri, 2005; Cogley, 2005; Cogley & Sargent, 2005). A higher k reflects the prior belief of greater time-variation. Setting $k^* = 5k$ results in more changes in the parameters within B, while $k^* = \frac{k}{5}$ produces smoother dynamics, although in both cases the results remain qualitatively similar to those from the baseline settings.

Similarly, the prior for g, the variance of shocks to log volatility, follows the inverse Gamma distribution ($g \sim IG(\frac{V}{2}, \frac{S}{2})$), with prior degrees of freedom $V = 5$ and scale $S = 0.5$. This prior incorporates the belief that volatility shocks to inflation were historically large but places some uncertainty around this belief.

The estimation of this model proceeds in the following sequence:

1. **Sample the process h_t**
   This procedure is derived in Jacquier et al. (1994) and Jacquier, Polson and Rossi (2004), which involves specifying the distribution for h_t conditional on h_{t-1}, h_{t+1} and the data Y_t as the product of Normal and log-Normal densities:

$$\begin{aligned}& f(h_t|h_{t-1}, h_{t+1}, Y_t) = \\ & h_t^{-0.5} \exp\left(\frac{-\varepsilon_t^2}{2h_t}\right) \times h_t^{-1} \exp\left(\frac{-(\ln h_t - \mu)^2}{2\sigma_h}\right),\end{aligned} \tag{C.13}$$

   where $\mu = \frac{\ln h_{t+1} + \ln h_{t-1}}{2}$ and $\sigma_h = \frac{g}{2}$. An independence Metropolis-Hastings algorithm was used to draw from the candidate density, which is the second term in (C.13). To sample the initial value of h_t, i.e. h_0, the authors suggest assuming a prior for $\ln h_0$: $\ln h_0 \sim N(\bar{\mu}, \bar{\sigma})$ whose posterior density is given by:

$$f(h_0|h_1) = h_0^{-1} \exp\left(\frac{-(\ln h_0 - \mu_0)^2}{2\sigma_0}\right), \tag{C.14}$$

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[29] See Jacquier et al. (1994) and Blake and Mumtaz (2012) for a discussion of Bayesian inference in such models.

where $\sigma_0 = \frac{\bar{\sigma} g}{\bar{\sigma}+g}$ and $\mu_0 = \sigma_0 \left(\frac{\bar{\mu}}{\bar{\sigma}} + \frac{\ln h_1}{g} \right)$, which require a value for $\bar{\sigma}$, $\bar{\mu}$, h_1 and g. The hyperparameter $\bar{\mu}$ is estimated as the log of the variance of the residuals from an OLS regression, while $\bar{\sigma}$ is set to a high number to reflect the uncertainty around this estimate. In practice values for $\bar{\sigma}$ between 10 and 200 do not affect the results in a meaningful way. An estimate of the process h_t is obtained as the sequence of squared changes in the dependent variable in Eq. (C.2) (inflation), and the value for h_1 is simply the first number in this series. The value of g, the variance of the process $\ln h_t$, is initialised to 1. The process to sample the sequence $h_{t=1}$ to h_{T-1} (conditional on g and B_t) involves sampling from the density in (C.14) with $h_0 = h_t$, $\mu = \frac{\ln h_{t+1} + \ln h_{t-1}}{2}$ and $\sigma_h = \frac{g}{2}$. This draw is retained with probability

$$\chi = \min \left(\frac{h_{t,new}^{-0.5} \exp\left(\frac{-\varepsilon_t^2}{2h_{t,new}} \right)}{h_{t,old}^{-0.5} \exp\left(\frac{-\varepsilon_t^2}{2h_{t,old}} \right)}, 1 \right) > u \qquad \text{(C.15)}$$

for $u \sim U(0,1)$. That is, if χ is greater than a draw between 0 and 1 from the uniform distribution, the new draw $h_{t,new}$ is accepted, otherwise the previous draw is retained. Finally, the value for h_T is sampled from the same density in (C.14) with $\mu = \ln h_{t-1}$ and $\sigma_h = g$ and the same acceptance probability is calculated.

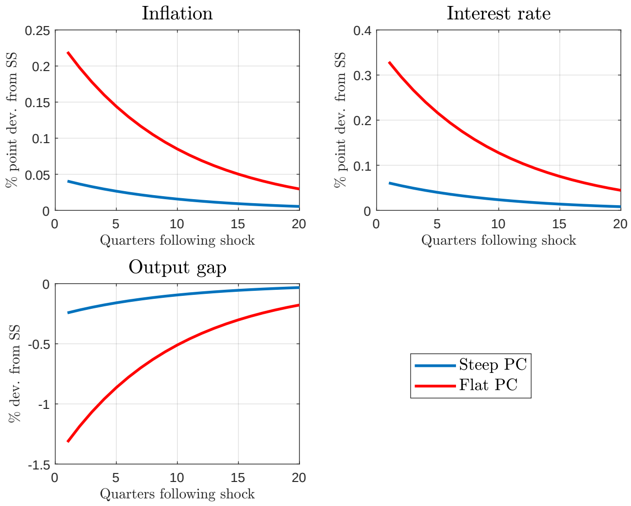
2. **Sampling** g
   For each full sequence h_t constructed above, the residuals η_t from the transition Eq. (C.9) are calculated and a value for g wiss drawn from the inverse Gamma distribution with degrees of freedom $T+V$ and scale $\Sigma {\eta_t}^2 + S$.
3. **Extracting** B_t
   Conditional on h_t and Q, the processes for the time varying parameters are drawn using the Carter-Kohn algorithm (Carter & Kohn, 1994).
4. **Sampling** Q
   Conditional on B_t, Q is sampled from the inverse Wishart distribution with scale matrix $(B_t - B_{t-1})'(B_t - B_{t-1}) + Q_0$ and degrees of freedom $T + T_0$.

Estimation is based on 20,000 repetitions of steps 1 to 4 above, from which the first 5,000 draws are discarded as burn-in draws. The posterior distributions of the parameters are based on the retained draws.

Appendix D Phillips Curve Slope and Monetary Policy Effectiveness

In this appendix, I show simulations from a benchmark closed economy New Keynesian DSGE model as in Walsh (2017, Ch.8), to which I add an exogenous and persistent shock process to the log-linearized New Keynesian Phillips curve.[30] The central bank follows a simple Taylor rule for the interest rate, and is only concerned with inflation stabilization. Fig. D.1 shows the dynamics of two economies, which are the same, except the Phillips curve in one is flatter than in the other, and are both hit by the same exogenous inflation shock. Variables are shown in deviation from their steady state (SS) values.

Figure D.1: Simulations: An exogenous inflation shock.



The Phillips curve slope is affected by changing the Calvo probability that a firm in any period cannot change prices ($\omega \in \{0.5, 0.75\}$). The central bank in the economy with a flatter Phillips curve ($\omega = 0.75$) will need to raise rates by more, causing the real interest rate to rise, and the economy to experience a larger (negative) output gap.[31] Inflation is nevertheless still higher, demonstrating that monetary policy in this economy is therefore, *ceteris paribus*, less effective.

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[30] The shock follows an AR(1) process with persistence $\rho = 0.9$. For more details on the model and calibration refer to the citation.

[31] The output gap is defined as the difference between supply and the level of output that is achieved under fully flexible prices.

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Review Article

The Development of the Sympathetic System of the Heart

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Abstract. Development of the sympathetic nervous system begins at about embryonic day 9 in mice with the migration of the neural crest cells to the dorsal aorta and the development of neurons and the sympathetic ganglia. This is followed by the axonal elongation towards the developing cardiac tissue. This process is directed by a series of signal ligands including ephrin-B1, semaphorin 3a (Sema3a), F-Spondin, bone morphogenetic proteins (BMPs), Wnt-1 protein, neurotrophin-3 (NT-3), nerve growth factor (NGF) and artemin (ARTN). Once at the developing heart, the nerve fibres follow the coronary veins in the subepicardium using NGF and the chemorepellent Sema3a as signals. Here they interact with the cardiac conduction system. Although these cardiac neural cells are part of the autonomic system, they are developed later, mainly on the epicardial surface. Bilateral innervation of the heart comes from the middle cervical stellate (MC-S) ganglion. Although the left ventricle and atrium receive noradrenaline from the MC-S on both sides, the right ventricle receives more from the MC-S from the left rather than from the right side. The development of the great vessels also contributes towards the pattern of development of cardiac innervation. The afferent fibres leaving the heart are also described. Their development relates to the sympathetic innervation of the heart and therefore to cardiac sensations. We hypothesise about how this reflects on the patterns of ischaemic cardiac pain.

Keywords: autonomic, sympathetic, visceral afferents, neural, embryology, cardiac development

1 Introduction

The heart is highly vascularised and extensively innervated by autonomic nerves. The autonomic nervous system comprises of both the sympathetic and parasympathetic systems, which work together to maintain homeostasis. Any impairment in the autonomic functions and innervation of the heart syncytium can lead to lethal arrhythmias (Dae et al., 1991; Hildreth, Anderson & Hednderson, 2009). The autonomic system is also vital for the heart during the development itself and the neonatal period, where it promotes the cardiac tissue to regenerate if it is damaged. Unfortunately, this is not the case in adult hearts (White, Gordon, Balkan & Hare, 2015). This highlights the importance of the neurovascular interactions during cardiac development.

The sympathetic nervous supply of the heart regulates chronotropy (contraction rate), inotropy (contraction force), dromotropy (atrioventricular node conduction speed) as well as lusitropy (myocardial relaxation) through the release of norepinephrine and the activation of cardiac β_1-adrenergic receptors (Kimura, Ieda & Fukuda, 2012; Fukuda, Kanazawa, Aizawa, Ardell & Shivkumar, 2015). The heart will suffer dysregulation and even myocardial injury without the sympathetic supply (Y. H. Jiang et al., 2015). The sympathetic innervation density is not uniform within the heart as it is higher in the subepicardium and within the special conduction system. This corresponds to the different areas of influence over cardiac performance (Kimura et al., 2012).

The sympathetic innervation is sculpted throughout development by chemoattractive and chemorepulsive factors such as nerve growth factor (NGF) and semaphorin 3a (Sema3a) respectively (Glebova & Ginty, 2004; Ieda et al., 2007). It is derived from neural crest cells and extends from neurons in the stellate ganglia,

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located on either side of the thoracic vertebra, to the heart. Arterial and venous vascular smooth muscle cells (VSMCs) mediate the proximal axon extension by the secretion of artemin (ARTN), neurotrophin-3 (NT-3) and endothelins. Although the proximal extension from the ganglia is well established, the mechanisms responsible for the distal extension to reach their target cells are not. During distal axon extension, nerves follow a typical pattern and path in target tissues, such as the subepicardium, prior to ultimately innervating the target cells (Nam et al., 2013). Besides the stellate ganglia, which is the main sympathetic supply to the heart, the superior cervical and the thoracic paravertebral ganglia also contribute to some cardiac sympathetic innervation in mice (Armstrong, Ryu, Chieco & Kuruvilla, 2011).

2.1 Formation of the Sympathetic Chain and Neuron Projection Along the Blood Vessels

The development of the autonomic innervation of the heart is composed of four phases: **(1)** the neural crest cells (NCCs) migrate to the dorsal aorta; **(2)** NCCs differentiate into neurons; **(3)** aggregation and migration of the neurons form the paravertebral sympathetic chains (the cardiac ganglia within the heart in the case of the parasympathetic); **(4)** the axon projections elongate towards and into the cardiac tissue until the terminal differentiation into their respective mature sympathetic or parasympathetic neuron (Hasan, 2013). The thoracic NCCs form paravertebral sympathetic ganglia at the dorsal aorta after migrating from the dorsal neural tube through somites during the fifth week of development of the embryo (Moore, Pernaud & Torchia, 2008). Table 1 shows the progress of the sympathetic development of the heart compiled from various animal studies.

The truncal NCCs are split into two groups, which migrate over different pathways at different stages of development. The first group to migrate are responsible for neurons and glial cells, they travel in a ventral direction from the dorsal aspect of the neural tube towards the dorsal aorta. The second group of NCCs give rise to the melanocytes in the skin and they migrate later and laterally from the neural tube (Erickson, Duong & Tosney, 1992; Henion & Weston, 1997). Early studies show that the NCCs responsible for the glial cells and neurons, mainly the dorsal root ganglia (DRG) and sympathetic chain, migrate ventrolaterally and take two pathways. Some migrate from between the somites but the majority migrate into the somite in the region between the sclerotome and the dermomyotome, from where they leave later on, towards the dorsal aorta to develop the sympathetic chain and associate with newly emerging axons from the ventral root (Loring & Erickson, 1987) (Fig. 1). The migration of the NCCs through the somites is split between entering the ventral half of the somatic sclerotome and migration away through the dorsal half of the sclerotome. This is due to the expression of ephrin-B1 ligands at the caudal side of the somite which act as a repulsive cue to the NCCs when binding to their EphB2 tyrosine kinase receptors (Krull et al., 1997). Besides being influenced by the ephrin-B1, the NCCs are also affected by Sema3a and F-spondin within the somites which guide them through the correct somite region. Migration away from the somite sclerotome requires the signalling of neuregulin. At the dorsal aorta clumping of NCCs into columns is regulated by Sema3a (Young, Anderson & Anderson, 2004). Some trunk NCCs, destined to form neurons and glial cells, stop traveling ventrally towards the dorsal aorta and coalesce at the lateral aspect of the neural tube and form the dorsal root ganglia (DRG). Others keep migrating ventrally to reach the dorsal aorta and develop into the sympathetic ganglia (SG) (Loring & Erickson, 1987). According to the study by Kasemeier-Kulesa, Kulesa and Lefcort (2005), the trunk's NCCs travel through the somites towards the dorsal aorta in a chain-like method, stretching from the dorsal to the ventral end of the somite and using active filopodia in the direction of migration. Once the NCCs are outside the ventral border of the somite, they can also travel freely in the rostral and caudal directions to the nearest developing SG and aggregate to it. A subpopulation of these trunk NCCs might still retain their pluripotent and plastic abilities and for some time they can change their location from the DRG to the SG or vice versa as well a from DRG to a nearby DRG until they are eventually inhibited from doing so (Kasemeier-Kulesa et al., 2005). This inhibition can be due to their completed differentiation of sensory and sympathetic cells by factors from the bHLH genes *Ngn2* and *Mash1*, bone morphogenetic proteins (BMPs) and Wnt-1 protein (Parras et al., 2002; Lee et al., 2004; Huber, 2006). Alternatively migration may be inhibited due to an extracellular matrix (ECM) barrier that develops around the cells (Perris & Perissinotto, 2000).

A final migration of the cells occurs at the dorsolateral region of the dorsal aorta. The migration happens either in the rostral or caudal direction in order to form the paravertebral sympathetic chain, which will be then followed by a phase of rapid mitosis (Hasan, 2013). At this region of the dorsal aorta, before the final migration to form the symphatetic chain, the terminal differentiation of the aggregated NCCs occurs, to fulfil their fate to become sympathoadrenal cells. This process is signaled by BMPs released from the aortic smooth muscles (Huber, 2006).

The cellular migration in the rostral direction develops the superior cervical ganglion (SCG). Later on, the extension of axons alongside blood vessels requires ARTN signalling through both GFRalpha3 and Ret re-

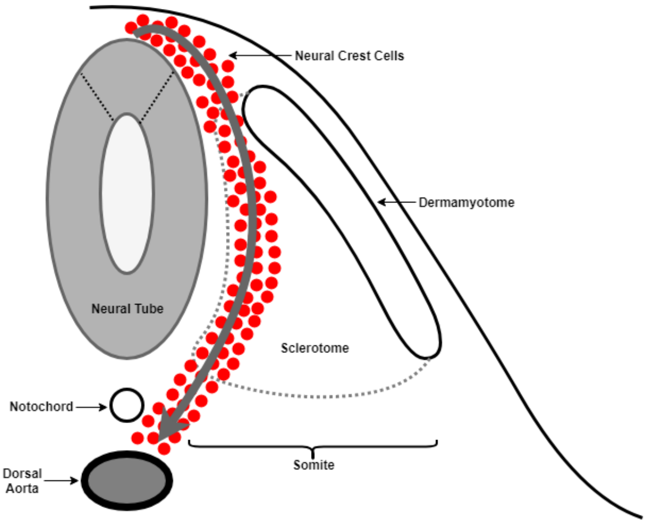

Figure 1: Neural crest cell migration through the somite and towards the dorsal aorta in a cross sectional view.

ceptors (Young et al., 2004). Within the ganglia, the cells adhere together using N-cadherins expressed by the sympathetic progenitor cells (Kasemeier-Kulesa, Bradley, Pasquale, Lefcort & Kulesa, 2006).

Within the superior cervical ganglion of studied foetal rats, axons bud from the side of the cell body, which determines the direction they are about to take. The axon does not branch within the ganglion, only branching slightly in the process of forming the peripheral nerves. The elongation process is rapid and the sympathetic targets are reached within a few days (Rubin, 1985). The sympathetic axons use the signal ligand ARTN, a member of the glial cell line-derived neurotrophic factor (GDNF) ligand family, which is produced and released by the smooth muscles of the developing vessels to find their target tissue by following the vessels. As in NCCs, ARTN acts on the receptor complex made up of both Ret and GFRalpha3 found on the developing sympathetic axon (Honma et al., 2002). The first contact between the differentiating blood vessels and the axons happens just after the formation of the primary sympathetic chain, which starts to develop in the mouse as early as day 9.5 (Enomoto et al., 2001; Wildner, Gierl, Strehle, Pla & Birchmeier, 2008).

2.2 Neurotrophic Factors as Chemoattractants in Final Target Innervation

Besides ARTN, both NT-3 and NGF proteins are required for the growth of the sympathetic axons towards the heart and away from the stellate ganglion. NGF is the prototypic member of the neurotrophin family. It has been under extensive study in the past few years and is essential for terminal sympathetic innervation of the target tissues using tropomyosin receptor kinase A (TrkA) receptors (Kuruvilla et al., 2004)

At the innervation site of the heart, target-derived NGF causes axons to become less sensitive to target-derived NT-3 and depend more on NGF. This is because NGF stimulates p75 neurotrophin receptor expression (Kuruvilla et al., 2004).

The amount of NGF synthesised by a given target tissue (including cardiac tissue) determines its final innervation density. NGF is found in higher levels in the atria than in the ventricles and also causes the expression of β_3-adrenoreceptors in cardiomyocytes especially in the left ventricle (Heumann, Korsching, Scott & Thoenen, 1984; Zhou et al., 2005). Deletion of a single copy of the NGF has been shown to result in apoptosis causing a 50% reduction in sympathetic neurons (Ieda et al., 2004). Studies on transgenic mice demonstrated that the overexpression of NGF leads to hyperinnervation, hyperplasia of ectopic cells in the stellate ganglia as well as cardiac enlargement (Hassankhani et al., 1995).

Besides development, NGF is also important for the maintenence and survival of sympathetic neurons throughout life (Ruit, Osborne, Schmidt, Johnson E. M. & Snider, 1990; Sharma et al., 2010). Mutants without both NGF and Bcl2-associated X protein (*Bax*) have normal extension of sympathetic axons along the extracardiac vasculature. However, sympathetic innervation in the heart is dramatically decreased. The simultaneous knockout of the pro-apoptotic factor *Bax* with NGF, allows neurons to survive in the absence of NGF, validating that NGF has a role in in distal cardiac sympathetic axon growth which is distinct from its impact on survival (Glebova & Ginty, 2004).

Endothelin-1 (ET-1) specifically upregulates the expression of NGF. It has been shown that both NGF expression and cardiac sympathetic innervation were reduced in mouse hearts with deficient ET-1. In such mice, the sympathetic stellate ganglia also demonstrated excessive apoptosis and neuronal loss. Cardiac-specific overexpression of NGF in these mice actually reverses sympathetic nerve retardation. These outcomes show that the ET-1/NGF pathway is critical for the sympathetic innervation of the heart (Ieda et al., 2004; Hasan, Pedchenko, Krizsan-Agbas, Baum & Smith, 2003).

2.3 Sema3a as a Chemorepellent in Cardiac Sympathetic Innervation Patterning

In contrast to neurotrophic factors such as NGF, which act as chemoattractants in cardiac nerve development, the chemorepellents in the heart which repel nerve axons and induce growth-cone collapse are still unidentified. Sema3a, a potent neural chemorepellent, is produced by the trabecular layer during early embryo development and then only by the Purkinje fibres after birth (Ieda et al., 2007; Kawasaki et al., 2002). Sema3a binds to the

Table 1: Cardiac sympathetic nervous system development.

Process	**Day**	**Species**	**Human equivalent**
NCCs migrate through the somites' ventral portion	Day 8.5–9.0 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	Week 3 Vegh et al. (2016)
NCCs migrate away from the somite and towards the dorsal aorta where they clump into colums	Day 9.0–9.5 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	Week 3-4 Vegh et al. (2016)
Terminal differentiation of NCCs at dorsal aorta before final migration	Day 9.5–10.0 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	
Final NCC migration from dorsolateral region of aorta to the caudal or rostral direction forming paravertebral sympathetic chain followed by rapid mitosis	Day 9.5–10.0 Serbedzija, Fraser and Bronner-Fraser (1990)	Mouse	
Sympathetic precursor cells reach the cervical region along the dorsal aorta and start to accumulte in a columnar fashion	Day 12 (*Day 10.5) Rubin (1985)	Rat	Week 4 Witschi (1962)
Precursor cells at the cervical region develop the superior cervical ganglia (C1–C5) and the stellate ganglia further caudally (C8)	Days 12–14 (*Day 10.5–12.5) Rubin (1985)	Rat	
First axons start to bud from cell bodies in the ganglia	Day 12 Rubin (1985)	Rat	
Enhanced and rapid mitosis of the accumulated precurser cells forming the superior cervical and the stellate ganglia	Day 14 (*Day 12.5) Rubin (1985)	Rat	Week 6 Witschi (1962)
At the developing heart, the angiogenic remodeling moves outwards to the subepicardium from the sinus venosum. VSMCs are recruited to the subepicardium and NGF is expressed to stimulate elongation of axons.	Day 13.5 Nam et al. (2013)	Mouse	Week 7 Vegh et al. (2016)
First sympathetic axons start to innervate the subepicardial layer of the developing heart and extend along the developing coronary veins in the layer using NGF secreted by the veins-associated VSMCs	Days 13.5 Nam et al. (2013)	Mouse	
Sympathetic targets reached after a rapid process of axon elongation using ARTN ligand released from vessel smooth muscle to find target (i.e. Axons follow vessels)	Day 15 Rubin (1985)	Mouse	Week 8 Otis and Brent (1954)
Sympathetic axons are present across the whole dorsal aspect of the developing heart while more axons continue to associate with the large coronary veins.	Day 15.5 Nam et al. (2013)	Mouse	
VSMCs in the myocardium during the development of the coronary arteries secretes NGF which directs the sympathetics axons to penetrate the myocardium from the epicardium. *(NGF from the epicardial veins starts to decrease at the dorsal region)* At the same time, in the epicardium, the sympathetic axons extands distally and ventrally from the dorsal aspect along the developing coronary veins in the ventral region of the developing heart.	Day 15.5–16.5 Nam et al. (2013)	Mouse	
All venous secreted NGF has ceased but arterial NGF in the myocardium was still being secreted at continue the attraction of axons into this layer.	Day 17.5 Nam et al. (2013)	Mouse	
Sensory neuron axons reach the subepicardium of the developing heart.	Day 18.5 Nam et al. (2013)	Mouse	

ARTN: artemin, NCC: neural crest cells, NGF: neurve growth factor, VSMC: vascular smooth muscle cell
*Equivalent mouse embryonal dates for comparison purposes.

transmembrane protein receptors known as neurophilin-1 and neurophilin-2, which work to aid axon guidance (Takagi et al., 1991; Kitsukawa et al., 1997).

Sema3a is strongly expressed in the developing heart at mouse embryonic day 12 and it eventually tapers down thereafter (Ieda et al., 2007). It is found in the subendocardium, but not the subepicardium, of the atria and ventricles. This pattern of distribution is the opposite to the epicardial-to-endocardial gradient of sympathetic innervation, which suggests that Sema3a acts as a negative regulator of cardiac innervation. Further evidence is provided by Sema3a knockout mice, which were shown to have a disrupted pattern of sympathetic innervation and malformation of the stellate ganglia, which extend the sympathetic nerves to the heart, along with hypertrophy of the heart muscle and dilatation of the right atrium (Behar, Golden, Mashimo, Schoen & Fishman, 1996). These demonstrate that Sema3a plays an important role by inhibiting sympathetic neural growth in the heart.

Lorentz et al. (2010) demonstrated that the subendocardium innervation of the adult p75 (NGF receptor) knockout mouse ventricles showed similar signs to mice with overexpression of Sema3a, eventually leading to spontaneous ventricular arrhythmias. This shows that as the sympathetic neurons are innervating the heart, NGF/p75 blunt the repulsive effect of Sema3a from the subendocardium. The delicate balance between NGF and Sema3a synthesised in the heart determine the correct epicardial to endocardial transmural symphatetic innervation of the ventricles (Carter, Feng & Paolocci, 2010).

2.4 The Role of Cardiac Neural Crest Cells in Cardivascular Development

Cardiac neural crest cells (CNCs), required for the complete development of the heart, are a subpopulation of the NCC, which originate from the first three somites up to the mid-otic placode in the rostral section of the embryo, corresponding to neural tube rhombomeres 6, 7 and 8 at the posterior rhombencephalon region (Kirby, Gale & Stewart, 1983; Maschhoff & Baldwin, 2000; Kirby, 2002). CNCs are essential to numerous processes during cardiac development. This includes the modifications of the pharyngeal arch arteries and the cardiac outflow tract to separate the pulmonary trunk from the ascending aorta to form two separate circulations. CNCs also form smooth muscles and pericytes of the great arteries as well as the neurons responsible for the cardiac conduction system. They also contribute to signalling for conotruncus elaboration (Brown & Baldwin, 2006). The CNCs undergo epithelial-to-mescenchyme transformation (EMT) and migrate to the third, fourth and sixth pharyngeal pouches before undergoing further migration to the developing heart, thus providing mesenchymal cells to the developing heart and large vessels (Maschhoff & Baldwin, 2000; Hamburger & Hamilton, 1992). It appears that these CNCs enter the heart both from the arterial and venous poles (Poelmann, Mikawa & Gittenberger-de Groot, 1998; Poelmann & Gittenberger-de Groot, 1999). The subpopulation entering the venous pole of the heart settle in cardiac regions related to the conduction system such as the atrioventricular (AV) node area, the retroaortic root bundle, the bundle of His, the left and right bundle branches and the right ventricular ring bundle, as well as the regions of the atrioventricular cushions. However, it seems that the CNCs do not form the final conduction system, as they then go into apoptosis and therefore fail to differentiate and survive. However, the exact timing of arrival, their apoptosis and the changes of the electropysiological properties within the heart, affect the conduction system's last phase of development. Altough the conduction system development might be affected by the CNCs, it is not a NCC derivative but a derivative from cardiac myogenic precursors (Cheng et al., 1999). This might be due to separation of the central conduction system from the myocardium by the apoptotic CNCs (Poelmann & Gittenberger-de Groot, 1999). CNCs entering the arterial pole migrate to areas related with the outflow tract and its septation, semilunar valves, smooth muscle of the great vessels and the ganglia around them (Poelmann et al., 1998). CNCs use both adhesion molecules such as integrins to interact with the ECM and molecules such as gap junctions alpha 1 connexin (Cx43) and cadherins to communicate with each other (Lo, Waldo & Kirby, 1999). CNCs also require non-canonical Wnt11 signalling in order to form filopodia and lamellipodia thus allowing migration (De Calisto, Araya, Marchant, Riaz & Mayor, 2005).

Mesenchyme derived from CNCs in the pharyngeal arches differentiate into connective tissue that stabilises the great arteries.

It also forms a sheath around the persisting aortic arch arteries, which make up the aortopulmonary septation located at the conal cushions final fusion site. They also populate the cardiac ganglia, which are entirely made up of CNC both the neurons and supporting cells (Kirby et al., 1983; Maschhoff & Baldwin, 2000; Waldo, Lo & Kirby, 1999). Other CNC-derived cells in the pharyngeal arches form the smooth muscle layer that surrounds the pharyngeal arch arteries as they remodel into the aortic arch, the ductus arteriosus and the proximal carotid arteries (Kirby & Waldo, 1995; X. Jiang, Rowitch, Soriano, McMahon & Sucov, 2000). In fact, CNC have the ability to form three types of progenitors. Firstly, stem cells (CNC-SC), which rise to smooth muscle, neurons, chondrocytes, Schwann cells, pigment cells and are capable of self renewal. In addition, CNCs also forms fate-restricted cells (CNC-RC) and smooth muscle lineage (CNC-smC), which are committed to smooth muscle formation (Sieber-Blum, 2004). However, it is still not known what is the exact role of the CNCs during the asymmetric remodelling of the aorta (Snider, Olaopa, Firulli & Conway, 2007).

Two important gene products required for the development of the heart are NT-3, belonging to the neurotrophin family of neurotrophin-4/5 (NT-4/5), NGF and brain derived neurotrophic factor (BDNF), as well as TrKC, belonging to the Trk tyrosine kinase receptors family (Sieber-Blum, 2004). NT-3 binds to TrkC and leads to recruitment, docking and phosphorylation of signal proteins, which in turn result in activation of pathways that regulate proliferation, neuronal differentiation as well as survival. (Sieber-Blum, 2004; Barbacid, 1994). Unlike other Trk receptors, TrkC is highly expressed in non-neuronal tissues including the heart and vascular smooth muscle cells (Tessarollo et al., 1993; Donovan, Hahn, Tessarollo & Hempstead, 1996). This wide expression of TrkC suggest that it might have a pleiotropic function while the embryo is developing, such as mitogenic function in cardiomyocytes during cardiac looping and ventricular trabeculation (Tessarollo et al., 1993; Lin et al., 2000).

Another important protein is the norepinephrine transporter (NET). NET is a presynaptic reuptake protein and is expressed in adult brain's neuroepithelium, DRG, spinal nerves sympathetic nervous system and the locus cereleus of the brainstem (Amara, 1995; Galli, Blakely & DeFelice, 1996). However, NETs were also found in embryos of birds and mammals, suggesting a broader functional spectrum than that in the adult organism (Ren, Pörzgen, Youn & Sieber-Blum, 2003). It is in fact expressed by NCCs during their late migration when it is in the region of the notochord. Its function is to promote norarenergic differentiation using maternal norepinephrine that crosses the placenta rather than a transporter as in the adult neurons (Rothman, Gershon & Holtzer, 1978; Zhang et al., 1997). Importantly, NETs in the embryo are not only expressed in neuronal cells but also in non-neuronal cells including the cardiovascular system, such as the heart's epicardium and myocardium including the trabeculae, the endothelium of the aorta and veins and the NCCs at the cardiac outflow tract (Ren et al., 2003).

A summary of the neural crest migration, as well as the important controling factors are shown in Fig. 2.

2.5 Branchial Arch Vessel Remodelling to Produce the Great Vessels

Before the aortic arch is remodelled to its definite left sided arch structure, the outflow tract was a single vessel that branched bilaterally and symmetrically into the third, forth and sixth aortic arches within the pharyngeal arches. The remodelling happens using a programmed process of asymmetrical obliteration and preservation of specific arches, with the third arch arteries becoming the common carotids, the fourth arch arteries forming the distal part of the brachiocephalic artery and the proximal right subclavian artery while the sixth arch arteries develop the proximal part of the pulmonary arteries and the ductus arteriosus (Snider et al., 2007). Such a remodelling process requires interaction among the pharyngeal arch arteries endothelium, the surrounding smooth muscle derived from the NCCs and the mesenchyme, as well as the endoderm (Le Lièvre & Le Douarin, 1975; Yanagisawa et al., 1998; Wendling, Dennefeld, Chambon & Mark, 2000). Studies show that the remodelling of the aortic arch depends on a threshold requirement of colonising NCCs (Stoller & Epstein, 2005).

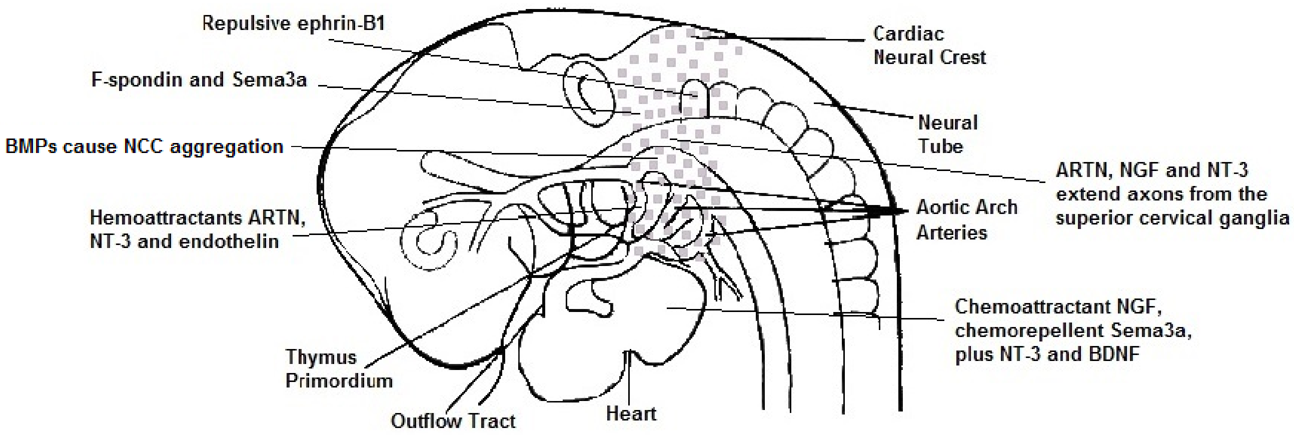

Figure 2: Neural crest cell migration and the main factors involved.

2.6 Cardiac Sympathetic Growth Occurs Along the Coronary Veins

In recent years numerous investigators have been trying to understand the different potential mechanisms of how the sympathetic nerves grow into the heart. From the anatomical congruence between sympathetic nerves and coronary vessels it is clear that sympathetic axons in the heart, like in other organs, follow blood vessels (Nam et al., 2013).

Sympathetic development in the developing heart of mice commences at embryonic day 13.5, when the axons extend along the developing large coronary veins in the dorsal subepicardium of the ventricles. By embryonic day 15.5, these axons spread throughout the dorsal surface and consequently penetrate the dorsal myocardium, while the others reach the ventral subepicardium. It has been demonstrated in many in vitro and in vivo studies that during angiogenesis and coronary vein recruitment, epicardium-derived VSMCs transiently release NGF. This directs the extension of sympathetic axons along the developing coronary veins. Later VSMCs of the developing coronary arteries in the myocardium start to secrete NGF which casues the sympathetic axons to penetrate the myocardium (Nam et al., 2013). This transient period of NGF expression may lead to the explanation as to why axons do not innervate veins despite following them during periods of remodelling.

As coronary vein remodelling is completed, the expression of NGF by the subepicardial VSMCs is downregulated, while its expression by the myocardial VSMCs commences during the period of arterial remodelling. This step leads to the extension of the sympathetic axons into the myocardial cell layer, correctly distributing throughout the myocardium before finally innervating the cells (Nam et al., 2013).

By contrast to the above model, other studies demonstrated that in the developing limb skin, the arteries align with sensory nerves and the sympathetic axons extend along the arteries from the sympathetic ganglion (Luff, 1996; Mukouyama, Shin, Britsch, Taniguchi & Anderson, 2002; Glebova & Ginty, 2005). Nam et al. (2013) provided a detailed mechanism, however it does not explain how the spatiotemporal changes in NGF expressed by the VSMCs in the coronary veins during sympathetic development is controlled. This mechanism requires precise localisation of NGF expression which is crucial to cardiac innervation. There is other research in progress regarding the involvement of other signals and cues that are present in the final target innervation of the myocardium (Ieda et al., 2007).

2.7 Growth of Cardiac Sensory/Afferent Fibres

The heart transmits sensory information to the central nervous system such as nociceptive signals through both sympathetic and parasympathetic vagal afferent fibres (Hua et al., 2004). In a study in cats, it was shown that the cardiac afferent sensory fibres from the heart passed through the stellate ganglion as the efferent fibres do and through the dorsal root ganglia ipsilaterally from C8 to T9, where the cell bodies are (Kuo, Oravitz & DeGroat, 1984). It is interesting to speculate whether in humans (as in rats below, assuming that the sensory nerves follow the sympathetics), any such sensory fibres pass back to the superior and middle cervical ganglia and enter the dorsal root ganglia at higher cervical nervel levels as this may help explain referred cardiac pain to the shoulder tip, neck and the angle of the jaw (innervated by C4, C3 and C2 dermatomes respectively). The cardiac sympathetic afferent fibres are known to be more dense between the T2 and T6 segments located within the Lissauer's tract and lamina 1 of the dorsal horn's lateral border and then extending to terminate into lamina 5 and 7 near the intermediolateral nucleus region. This uniform pattern of grey matter termination, which contains the spinothalamic fibres involved in autonomic nociception and reflexes, is also seen in other visceral sympathetic afferents in the lower thoracic and lumbar segments (Kuo et al., 1984). The vagal afferents for nociception travel and synapse in the nucleus tractus solitarius (NTS) of the medulla and go on to excite cells of the upper cervical spinothalamic tract C1–C3, which might explain the referred pain to these dermatomes (Chandler, Zhang & Foreman, 1996; Foreman, 1999).

Another study on mice hearts showed that the cardiac afferent fibres, which are found on the epicardial surface of the heart and travel through the DRG, contains the vanilloid receptor 1 (VR-1). However, these VR-1 containing neurons are scarce in the myocardium. It was found that these VR-1 containing afferent fibres are essential for the sympatho-excitatory reflex during a myocardial ischemia, which reflex is the increase of cardiac sympathetic activity when the cardiac sympathetic afferent fibers are stimulated (Zahner, Li, Chen & Pan, 2003). A study by Bennett, Dmietrieva, Priestley, Clary and McMahon (1996) found that sensory neurons in viscera express mainly calcitonin gene-related peptide (CGRP) and trkA. TrkA is sensitive to the much required factor, NGF during the development of afferent fibres (Ieda et al., 2006). Sensory neurons in the heart develop later than autonomic motor neurons. In mouse hearts, at the dorsal ventricular subepicardium, autonomic motor neurons are present by day E15.5, while sensory neurons start to appear on day E18.5. Also, the autonomic motor neurons were found to follow and interact with the coronary veins in the subepicardium during cardiac development, whilst other studies show that sensory neurons follow arteries (Nam et al., 2013; Mukouyama et al., 2002)

2.8 Laterality of Innervation and any Influence on Cardiac Pain Syndromes

It is known that the heart is supplied bilaterally by sympathetic fibres from the superior, middle, inferior cervical and the upper thoracic paravertebral sympathetic ganglia (Vegh et al., 2016). However, is there any difference in the laterality of supply of the different parts of the myocardium, and if so can this relate to patterns of ischaemic cardiac pain? A study using Diamidio Yellow retrograde tracer injected into the ventricles of rats in order to label the stomata of the postganglionic nerves in the sympathetic chain shows that the ventricles of the heart are innervated by sympathetic postganglionic neurons bilaterally, but with more left stellate cell bodies innervating the left ventricle and more right stellate cell bodies innervating the right ventricle (Pardini, Lund & Schmid, 1989). The majority of the supply is originating from the middle cervical-stellate (MC-S) ganglion complex with around 92% of labelled cells. (Stellate ganglia are a complex fusion of the lower cervical and first thoracic ganglia) Lesser supply is coming from the superior stellate ganglion and the upper thoracic ganglia. After performing unilateral MC-S ganglionectomies the investigators showed that both atria and the left ventricle are supplied bilaterally by the MC-S complex. On the other hand, the right ventricle receives most of its noradrenaline from the left MC-S ganglion complex, meaning that the left ganglion is supplying all chambers (Pardini et al., 1989).

Studies have shown that electrocardiographic (ECG) changes following unilateral right stellate ganglionectomy were similar to those following left (Yanowitz, Preston & Abildskov, 1966). However, the left stellate ganglion fibres innervate primarily the posterior ventricular surface. On the other hand, sympathetic tone is removed from the anterior surface following unilateral right stellate ganglionectomy (Yanowitz et al., 1966). The identity of the anterior surface of the heart is largely the right ventricle and right atrium and a bit of the interventricular septum and apex of the left ventricle, suggesting a distinction in innervation to left and right ventricles by left and right stellate ganglia. There is also a functional distinction of the left and right sympathetic innervation. Randall and Rohse (1956) found that left stellate stimulation produced mainly inotropic changes (mainly innervating the muscle which feels ischaemic pain), while right stellate stimulation produced both inotropic and chronotrophic changes.

Looking at these older studies, if the sensory afferent nerves from each side follow the sympathetic innervation to each individual sympathetic chain before entering the spinal cord via the dorsal nerve root, the majority of the ventricles would be supplied by nerves entering the left side of the spinal cord. Since most cardiac ischaemia occurs in the thicker left myocardium, this (as well as the considerable left stellate innervation of the right ventricle myocardium) may explain why referred pain to the left arm (lateralising pain) in myocardial infarction and ischaemia far exceeds the presentation of right arm pain. This provides interesting hypotheses for further study.

3 Conclusion

It is clear that there has been extensive research conducted on the development of the sympathetic innervation of the heart. This review highlights areas for further research, which include the exact site of synthesis and function of NGF in cardiac innervation, identification of the chemorepellants which induce cone growth collapse in the heart, and the exact role of the CNCs during the asymmetrical remodelling of the aorta. Although this study collated work based on animals, the information gathered can give a better idea and understanding of how what happens in human subjects when it comes to this complex process. The review attempts to give insights into possible explanations of referred pain distribution in clinical cardiology, based on our understanding of human embryology and anatomy.

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Research Report

Does Absence of Charismatic Species Impact the Ecotourism Potential of Central Mediterranean Islands?

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Abstract. Central Mediterranean Islands tend to be devoid of large terrestrial charismatic fauna which usually serve as target species for ecotourism. This has raised questions on the potential of ecotourism in such destinations. However it has been argued that absence of charismatic megafauna should not be considered as a limitation. Ecotours were organised on nine islands in the central Mediterranean region. Interviews and focus groups were held with participants of the ecotours and stakeholders. It has been argued that most charismatic species are marine, touting marine ecotourism as the ideal tourism product. Furthermore, rather than focusing on charismatic species, the ecotourism product on such islands should revolve around the various coastal environments and habitats and other smaller species including non-mammals, especially endemic ones thus facilitating a broader approach to conservation. Owing to the remarkable biodiversity of plant species, charismatic megaflora and plants have also been identified as important targets for ecotourism. Furthermore, due to the intense environmental pressure and limited size of protected areas the overlap with cultural, rural and adventure tourism has been suggested. In order for the ecotourism product to be more competitive archipelago tourism also referred to as island hopping is also recommended, a proposal which is supported by the presence of endemic and sub-endemic species. Results show that absence of charismatic species does not limit ecotourism development on such islands.

Keywords: Charismatic species, ecotourism, central Mediterranean, islands, conservation

Abbreviations

Marine Protected Areas = MPAs; NGOs = Non-Governmental Organisations; 3S = Sand, Sun and Sea

1 Introduction

Charismatic megafauna are arguably considered important to motivate ecotourists to visit protected areas (Skibins, Powell & Hallo, 2013). Such species tend to be terrestrial mammals and normally serve a flagship role engaging in both ecotourism and conservation campaigns (Albert, Luque & Courchamp, 2018; Lindsey, Alexander, Mills, Romañach & Woodroffe, 2007; Verissimo, Fraser, Groombridge, Bristol & MacMillan, 2009).

Mammal faunas on Mediterranean islands experienced almost complete extinction due to human colonisation (Blondel & Vigne, 1993; Schüle, 1993). Whereas humans have actively introduced new faunistic elements on Mediterranean islands (Gippoliti & Amori, 2006; Schembri, 2003) few endemic terrestrial mammals still survive in Mediterranean islands (Blondel & Vigne, 1993). One example is the shrew (*Crocidura sicula*) which is found on the islands of Sicily, Levanzo, Favignana, Marettimo, Ustica and Gozo (Sarà, 1998). Hence, terrestrial charismatic megafauna, including large mammals, are lacking from central Mediterranean islands. This has raised questions on the potential of protected areas situated on Mediterranean islands to attract ecotourists. Furthermore, owing to the lack of charismatic megafauna, the possibility of applying the concept of flagship species (used in other regions for biodiversity conservation) on islands has been described as problematic (Verissimo et al., 2009).

However, research has criticised the mammal-centric approach and focus on charismatic megafauna (Entwistle, 2000). Questions have also been raised on

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whether charismatic species are indeed a key attractor of ecotourists to protected areas and if factors other than simply charismatic species might explain attractiveness of protected areas (Hausmann et al., 2017).

Weaver (2005) distinguished between the different nature-based products, a major element of ecotourism together with interpretation and sustainability. He argues that the nature-based product ranges along a continuum. On one end, one finds a holistic approach featuring an entire ecosystem such as a forest or coral reef. This approach normally also includes landscapes (Buckley, 2013; Di Minin, Fraser, Slotow & MacMillan, 2013) and scenery (Lindsey et al., 2007) as targets for tourists. Owing to the fact that few places are devoid of human influence, the holistic approach normally also incorporates the cultural component to the nature-based focus (Weaver, 2005). On the other end one finds an elemental approach focusing on specific non-captive flora and fauna charismatic species (Weaver, 2005). Similarly, Lee, Lawton and Weaver (2013) argued that ecotourism attractions tend to focus on rather pristine ecosystems and wild endemic charismatic megafauna that inhabit such ecosystems. In these cases, ecotourism attractions go beyond charismatic species and emphasis is not only made on mammals or fauna.

Similarly, others have remarked that tourists' interest for visiting protected areas goes beyond charismatic terrestrial megafauna. In some cases, depending on the site, geological features (such as volcanoes, and cliffs) are also included as attractions (Lee et al., 2013). Even if having received less recognition than charismatic megafauna (Hall, James & Baird, 2011), a smaller number of destinations also feature charismatic megaflora (such as trees) (Lee et al., 2013; Weaver, 2005). Yet in some regions where such mega attractions are lacking, other smaller attractions have been identified to serve as flagship species confirming that nature-based attraction parameters should not necessarily focus on megafauna (Lee et al., 2013). These include less charismatic species (Buckley, 2013; Di Minin et al., 2013) including birds (Glowinski, 2008; Lindsey et al., 2007; Verissimo et al., 2009), rarer less-easily observed and/or less high-profile mammal species (Lindsey et al., 2007) such as bats (Weaver & Lawton, 2007). Reptiles and amphibians known collectively as herpetofauna have also been considered to play an important role as a pull factor for the ecotourism (Wollenberg et al., 2011). Furthermore, butterflies and dragonflies have also been regarded as charismatic microfauna which can play a flagship role in ecotourism and attract visitors to protected areas (Cannings, 2001; Harvey Lemelin, 2007). Plants such as orchids have also been identified as targets for tourists and potential flagships for conservation (Lindsey et al., 2007; Pickering & Ballantyne, 2013).

Whereas charismatic species and the impact on ecotourism have been given due importance in various regions across the world (Weaver, 2005), little attention has been given to this aspect when it comes to peripheral areas including islands such as those in the central Mediterranean region. Studies on such biodiversity in this region have mostly been conducted from the natural sciences perspective. Meanwhile, peripheral areas such as islands have been regarded as areas ideal for ecotourism purposes due to high species richness which include charismatic megafauna. These persist due to the remoteness of the areas sparing species and habits from negative environmental impacts from anthropogenic sources (Garrod & Wilson, 2004). In fact whereas emphasis in research has been made on terrestrial mammals, marine charismatic species such as whales, sharks, dolphins, turtles and seals have also been identified (Albert et al., 2018; Garrod & Wilson, 2004; Giglio, Luiz & Schiavetti, 2015).

Similarly to the arguments raised above for terrestrial environments, in the case of peripheral areas such as tourism dependent islands and archipelagos lacking charismatic fauna, other species such as seabirds have been considered to have considerable potential to act as flagship for tourism purposes. This is especially the case if such species are endemic to the islands, have a low population (or are threatened with extinction) and have unique features of special biological or behavioural interests (Verissimo et al., 2009). Ecotourism targets in such areas also include fish, marine microfauna, sea caves and other geological formations such as stacks and arches, corals and flora along with cultural attractions (Garrod & Wilson, 2004). This research aims to study if the lack of terrestrial charismatic mega-fauna impacts ecotourism in the area of study and if alternative approaches can be used to develop and practise ecotourism in the central Mediterranean region. This is of particular interest as policy makers are eyeing alternative forms of tourism to mitigate the negative impacts of mass tourism and ensure that destinations remain competitive.

2 Material and Methods

2.1 Area of Study

The area of study consists of nine islands (three archipelagos and an island) all situated in the central Mediterranean Region. These are the Pelagian Islands (comprising Lampedusa, Linosa and the islet of Lampione), the Aegadian Islands (comprising Favignana, Levanzo and Marettimo) and the Maltese Islands (comprising Malta, Gozo and Comino) along with the island of Pantelleria. The islands have extensive terrestrial and marine areas which are protected through one or more designation including regional, national and EU legislation (Protected Planet, 2018). Notwithstanding the relatively small land area, the resulting limited variety of habitats, the long history of colonisation and in the case of the Maltese islands the high population density, the islands host a high variety of biota including several endemic species and are thus considered as biodiversity hotspots (Vogiatzakis, Mannion & Pungetti, 2008).

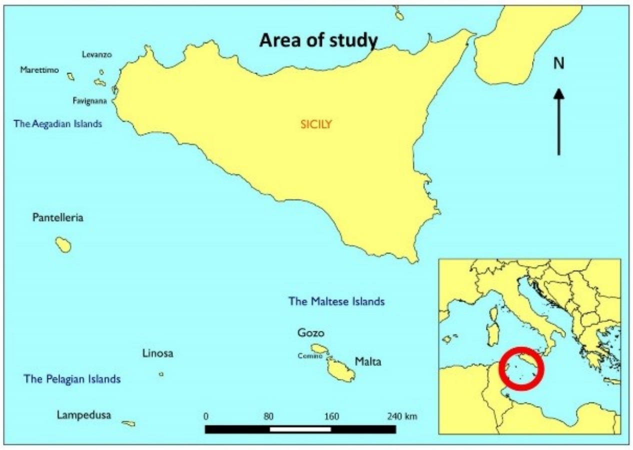

Figure 1: Central Mediterranean islands representing the area of study. Source: QGIS, 2016. Designed by Andrea Pace.

2.2 Methods

The case study approach was used in terms of research design as it provides flexibility as a multiple methodology (Jennings, 2001; Yin, 2014). The multiple case study approach was implemented whereby the same research methods were applied to the different islands under study (Yin, 2014). Research was conducted between May 2013 and July 2016 and over 240 people participated in the study. Four study visits (one on each archipelago/island) were organised to observe and familiarise oneself with the area of study.

Eventually, four ecotours were organised (one on each archipelago/island). These involved the organisation of a specific itinerary for 66 ecotourists who voluntarily accepted to participate in the trips, at their own expense. 43 ecotourists participated in one ecotour, 23 ecotourists participated in two ecotours whereas 5 ecotourists participated in three of the ecotours organised generating a total of 94 ecotourism experiences. The programme of the ecotour involved established ecotourism excursions in protected areas. Interviews and focus groups were conducted with participants of ecotours including those who participated in more than one ecotour and those who revisited the ecodestinations on their own initiative following the ecotour organised as part of the research. A non-probability sampling technique was used whereby individuals who presented themselves to the ecotours were asked to participate.

In addition, 174 in-depth and semi-structured interviews were also held with stakeholders across the entire area of study in order to obtain their views on various aspects related to ecotourism. As per Okech (2011) and Orams (1999a), ecotourism stakeholders interviewed included locals, resource users (including operators, guides, tourists), government and official agencies, non-governmental organisations (NGOs) and academics (see Table 1). Two sub-types of strategic informant sampling technique were used to recruit interviewees: expert sampling and snowball sampling. Interviews were held face to face and owing to the characteristics of islands being small-entangled communities, notes were taken instead of recording to ensure tangible information is acquired and that an adequate pool of stakeholders accept to participate. A checklist of items discussed during interviews and focus groups can be found in Annex Annex 1. Data obtained through interviews and focus groups was analysed manually through coding, sorting and looking for dominant themes.

3 Results

When asked about activities and features that are most important during a trip abroad, ecotourists interviewed referred to experiencing nature, visiting uncrowded places and increasing knowledge on wildlife. Watching wildlife including plants and animals was the least ecotourism related aspect referred to by ecotourists interviewed. The main reasons which motivated respondents to participate in the ecotours included to visit a new ecodestination (ecotourists remarked that they had not visited the central Mediterranean island in question), the fact that the tour was predominantly nature based, a competitive price of the package, the positive experience garnered from the previous ecotours and to support conservation initiatives. The desire to watch charismatic fauna also did not feature among the most popular expectations of those participating in the ecotours. In fact, the learning experience, immersing in nature and visiting a new interesting ecodestination were the most popular expectations among respondents participating in the four ecotours.

Ecoguides interviewed in the area of study expressed concern on the absence of terrestrial large charismatic species save for some introduced mammals such as red deer (*Cervus elaphus*), mouflons (*Ovis orientalis musimon*) and wild boars (*Sus scrofa scrofa*) on Marettimo. They said that this might leave an impact on the satisfaction of ecotourists. When interviewed, ecotourists visiting the Maltese Islands outlined that they observed little terrestrial fauna save for the fresh water crab (*Potamon fluviatile lanfrancoi*). However, they did not express disappointment. The observation of species which are considered as charismatic such as the fresh water crab and birds in Malta, loggerhead turtles (*Carretta*

Table 1: Distribution of stakeholders with whom interviews were held in the area of study.

Eco-destination	**Count/ Percentage**	**Stakeholders**					
		Affected locals	**Resource users**	**Academics**	**Government, agencies, politicians**	**NGOs**	**Total interviews**
Aegadian Islands	Count	10	19	2	3	4	38
	Percentage	26.30%	50%	5.30%	7.90%	10.50%	100%
Pelagian Islands	Count	2	12	3	1	5	23
	Percentage	8.70%	52.20%	13%	4.30%	21.70%	100%
Pantelleria	Count	3	12	5	8	3	31
	Percentage	9.70%	38.70%	16.10%	25.80%	9.70%	100%
Maltese Islands	Count	4	22	9	28	16	79
	Percentage	5.06%	27.80%	11.39%	35.44%	20.30%	100%
General	Count	0	1	0	1	1	3
	Percentage	0%	33.30%	0%	33.30%	33.30%	100%
Total	Count	19	66	19	41	29	174
	Percentage	10.90%	37.90%	10.90%	23.60%	16.70%	100%

caretta) and birds on Lampedusa and deer and mouflons on Marettimo did leave a positive impact on the satisfaction of ecotourists.

On the other hand, disappointment expressed by ecotourists did not refer to lack of wildlife sightings but to other factors. The predominant reasons expressed by those who felt that that the ecotour did not fulfil their expectations was related to the desire to immerse more in nature especially in Malta where the island has become very urbanised, long waiting times due for transport services and the constant need to use transport services especially on relatively bigger islands such as Pantelleria and Malta. This was followed by the desire for more marine ecotourism activities such as snorkelling, which at times were impeded by the presence of jellyfish. It was argued that too much emphasis was made on botanical aspects and that more attention needs to be placed on the marine environment and related activities. Other reasons included that aspects of the tour such as accommodation did not reflect ecotourism principles and that some islands such as Malta were too geared for mass tourism.

The majority of those who participated in the ecotours organised as part of the research had already participated in other ecotours either as part of the research or on their own initiative. The majority said that their expectations, as identified prior to the ecotour, had been fulfilled. Following the ecotour, the absolute majority of respondents said that they were willing to visit another ecodestination for a holiday including a central Mediterranean island. Furthermore, ecotourists said that they would recommend the ecodestination they had visited to their friends for their next holiday. Ecotourists also expressed willingness to participate in future ecotours or revisit the ecodestination. The high rate of satisfaction can also be confirmed by the fact that out of the 66 ecotourists who participated in the ecotour, 28 ecotourists participated in more than one ecotour and others revisited the ecodestinations on their own convincing other friends to join them.

Even if absence of wildlife sightings were not considered as a limiting factor by ecotourists, charismatic species such as bottlenose dolphins (*Tursiops truncatus*) and turtles are used as target species to promote excursions especially on the Pelagian Islands. Specific excursions which include diving are organised around the islet of Lampione to observe juvenile grey sharks (*Carcharhinus griseus*). Such excursions are often a success and ecotourists do encounter marine megafauna due to the abundance of marine life. Specific excursions to islets such as Filfla are also organised to observe avifauna which are rare or have a limited distribution such as the Mediterranean storm petrel (*Hydrobates pelagicus*) along with other marine targets such as dolphins. Shearwater boat trips are also organised along cliffs to observe Scopoli's Shearwater (*Calonectris diomedea*) 'rafting' on the water before returning to their nests.

Excursions organised normally incorporate not only target species but also visiting related venues or related interpretation centres. For example in the case of Marettimo where the endangered monk seal (*Monachus monachus*) has been spotted after a long period of time a particular approach is adopted whereby the monk seal observation centre and spots usually frequented by such species including coastal caves are visited. Meanwhile, sightings are very rare and have been limited to scientific studies. In the case of the Pelagian Islands, visits are made to the rescue centres, nesting sites and trips are also organised in an attempt to watch turtles in the wild.

Operators emphasised that excursions offered to ecotourists do not have the sole aim to observe marine species but also seek to raise awareness on the need to protect such species. In fact, one should note that failure to observe charismatic species did not leave a complete negative impact on ecotourists visiting the Pelagian Islands. Such ecotourists did not manage to see turtles in the wild and only had the chance to visit turtle nests on beaches being monitored by NGOs and observe injured turtles in rescue centres on Lampedusa and Linosa. Meanwhile, ecotourists still contributed financially towards related conservation projects.

Conservation was considered by ecotourists as an important element of ecotourism and one which influences them when choosing a destination to visit. Furthermore, it was also considered an important element of the ecotourism experience by ecotourists. Ecotourists also requested operators and NGOs involved more information on how their participation in the ecotour would support conservation initiatives including those related to flagship species such as turtles. The importance of conservation for ecotourists can be further confirmed by the dissatisfaction expressed by participants of ecotours visiting a store selling natural sponges, a traditional trade on the island of Lampedusa. Concerns have been raised by managers of Marine Protected Areas (MPAs) since ecotourism activity targeting charismatic species on Lampedusa were disregarding ethical considerations due to close encounters with such species possibly leaving an impact on their behaviour.

Various approaches were proposed by stakeholders to overcome the challenge of absence of terrestrial charismatic species (see Table 2). The first was to consider smaller, including non-mammal species such as invertebrates and flora as charismatic species which are of interest to ecotourists. In this regard emphasis was made on endemic species, species with a limited geographical distribution and species which are considered to be rare/threatened or appealing. Species reflecting one or more of these characteristics and outlined by stakeholders included the fresh water crab, the painted frog (*Discoglossus pictus*), the Mediterranean killifish (*Aphanius fasciatus*), the hedgehog (*Erinaceus algirus*), the weasel (*Mustela nivalis*), the Sandarac Gum tree (*Tetraclinis articulata*), the Maltese rock centaury (*Cheirolophus crassifolius*), orchids and birds such as the blue rock thrush (*Monticola solitarius*). NGOs, academics and ecotourism operators pointed out that entomology and herpetology excursions focusing on insects and reptiles respectively could be organised on the islands. Owing to the rich diversity of flowering plants in a confined area excursions focusing on flower gazing, ethnobotany and nature photography could be organised as an alternative. Other suggestions included to capitalise on natural aspects offered by the islands such as the scenery and the various volcanic phenomena found on the island of Linosa and more so on Pantelleria. Other lines of thought included to make less emphasis on the terrestrial environment and rather than just focus on botanical aspects put more emphasis on marine and coastal environment. Others reiterated this point on the proviso that islands had to capitalise more on the water surrounding them and the relatively bigger marine protected areas in comparison to terrestrial areas. In addition, academics explained that marine life was so rich that one could also observe a variety of species including charismatic/appealing ones in shallow water. Another approach was to develop ecotourism products that incorporate related niches that overlap with ecotourism including adventure tourism, agri/rural tourism, pescatourism, volunteering and cultural tourism. This would not only overcome the limitation of limited/absence of charismatic terrestrial fauna but also enhance the ecotourism experience per se. In another proposed approach, stakeholders including academics recommended archipelago tourism also known as island hopping to maximise the ecotourism experience and experience the different nature related opportunities provided by different islands. Such services are already being offered in the area of study. This was of particular interest due to the relation that exists between islands through the presence of sub-endemic species (species unique to a number of islands) in the area of study, case in point reptile and snails. One example is the lacertid lizard (*Podarcis filfolensis*) endemic to the Maltese and Pelagian archipelago (found on Linosa and Lampione) (Scalera et al., 2004).

Table 2: Approaches proposed for the ecotourism experience in the central Mediterranean region.

How to overcome lack of terrestrial mega-fauna
• Target smaller non-mammal species • Put more emphasis on flowering plants due to their rich biodiveristy • Capitalise on the geologic and volcanic phenomena • Give more importance to coastal and marine environments • Include overlapping niches including volunteering, adventure, cultural, agritourism/rural tourism, pescatourism • Introduce archipelago tourism/island hopping and focus on endemicity and sub-endemic species

Stakeholders outlined that activities targeting different species could be held throughout different months of the month. Whereas this was not a problem as climate on these islands was fair allowing outdoor activities to be held throughout most days of the year, one had to be organised and in some cases also plan activities according to the particular season. Furthermore, the ideal timing to target specific species had to be taken into consideration to increase likeliness of observing the target species.

Stakeholders added that owing to the relatively small size of the islands this also allowed ecotourists to visit various habitats in various protected areas and move swiftly in between terrestrial and marine sites reducing travelling time and increasing time available to immerse in nature and observe target species.

Stakeholders interviewed raised alarm on the presence of alien species and the possible impact these may have on endemic and other species which are considered as charismatic species. In addition they also expressed concern on transboundary issues in marine environments such as marine traffic in the area of study, presence of marine plastic litter, aquaculture and oil drilling that may all leave an impact on marine biodiversity including charismatic species.

4 Discussion

Irrespective of the rich biodiversity found on islands in the area of study, large terrestrial charismatic species, especially large mammals which are normally considered as top targets for ecotourism, are absent. The introduced deer, wild goats and wild boar on Marettimo are an exception. As a result, conversely to what normally happens elsewhere, ecotourism targets in the region include smaller, less charismatic species such as the fresh water crab which are at times difficult to observe either due to their behaviour, limited distribution or limited numbers.

One finds more diversity and abundance of charismatic species in coastal and marine environments. Only 3 of the 20 most charismatic species identified by Albert et al. (2018) are found in the area of study and these are all marine species (sharks, whales and dolphins). More environmental awareness and measures to reverse negative environmental impacts have also started to bear fruit. This can be confirmed by the return of the loggerhead turtle to nest on Maltese beaches in 2012, 2016 and 2018 (Anonymous, 2018, June 25) following a long absence (Deidun & Schembri, 2005). The monk seal has also been sighted on Marettimo (Donati, 2015). As a result, there is a natural drive favouring the development of marine ecotourism, ecotourism that takes place in coastal and marine environment.

The presence of charismatic species in marine environment surrounding central Mediterranean islands has been well documented in literature. The loggerhead turtle and the bottlenose dolphin are regularly found around Maltese waters (Mifsud et al., 2017). Pantelleria serves as a nursery for the loggerhead sea turtle and the white shark (*Carcharodon carcharias*). One also finds cetaceans such as the fin whale (*Balaenoptera physalus*) and the striped dolphin (*Stenella coeruleoalba*) apart from several species of rays (Margottini, 2011). In the case of the Aegadian Islands, various marine charismatic species have been identified including the monk seal, Bluefin tuna (*Thunnus thynnus thynnus*), loggerhead sea turtles, storm petrels, dolphins (*Stenella coeruleoalba* and *Tursiops truncates*), mantas, sharks (*Lamna nasus* and *Prionace glauca*) and sperm whales (*Physeter macrocephalus*) (Donati, 2016), several of which are rare on protected (Donati, 2015). The Pelagian Archipealgo is closely associated with loggerhead sea turtle nesting sites which include the Spiagga dei Conigli on Lampedusa with an area of 6000 m^2 and the Spiagga Pozzolana di Ponente on Linosa with an area of 1100 m^2 (Piovano et al., 2006).

With respect to the approach to identify smaller less charismatic species possibly non-mammals including invertebrates as ecotourism targets one should note that emphasis has been made on the need to create new ecotourism opportunities through under-appreciated, less charismatic biodiversity in protected areas (Di Minin et al., 2013). This is supported by the fact that less charismatic biodiversity has been valued by tourists as an integral aspect of nature-based experiences (Hausmann, Slotow, Fraser & Di Minin, 2016). Furthermore, this is crucial because protected areas which lack charismatics species may fail to attract ecotourists. As a result they generate less income for management purposes of the area (Goodwin & Leader-Williams, 2000; Kiss, 2004). The shifting of attention from large charismatic species to smaller species and their branding as charismatic species is also beneficial from a management and conservation perspective. This is because the narrow interest in charismatic species can lead to the underappreciation of other biodiversity (Di Minin et al., 2013; Kerley, Geach & Vial, 2003) pushing down the conservation ladder other small species leading to their poor conservation (Weaver, 2008). Thus, the absence of large mammals implies that conservation will not focus on a single species but will also have a broader perspective featuring also smaller species.

In the case of Mediterranean islands, too much importance given to large mammals can be a threat to the biodiversity in the region (Gippoliti & Amori, 2006). A classic example is the introduction of species considered to be charismatic (such as deer on Marettimo) has left tremendous negative impacts on trees and plants (Gianguzzi, Scuderi & Pasta, 2006) which also contribute to the ecotourism potential of such islands. The situation has been aggravated by some conservation measures such as stopping hunting on such species (Gippoliti & Amori, 2004).

One should keep in mind that various specialised ecotourism excursions to observe specific species including microfauna have also been reported in literature (Harvey Lemelin, 2007; Wollenberg et al., 2011). Furthermore, studies have shown that tourists with a high zoological interest are willing to pay higher prices for specialised biodiversity high-quality tours (Wollenberg et al., 2011) thus sustaining conservation initiatives of such species too. This is thus an opportunity which is being overlooked and underestimated. For instance, the Maltese archipelago hosts 10 micro-bat species (Baron, 2007) which have not garnered attention for ecotourism purposes. This could possibly be due to a failure to engage knowledgeable ecoguides who can offer interpretation in the field. This also explained the emphasis made of botanical trips which led to some ecotourists expressing some disappointment on the lack of attention given to marine environments even if these have interesting attractions such as maërl beds and rare coral reefs (Margottini, 2011). In this regard, one should note that interpretation is a fundamental element of ecotourism (Weaver & Lawton, 2007). Meanwhile, whereas in the islands under Italian jurisdiction the possibility of ecoguides specialising in nature-based excursions exist, no such provisions are allowed under Maltese Law. As a result, operators need to engage not only an official tourist guide holding a license by the national authority but also an expert in the field making operations unfeasible.

In the case of the Maltese Islands national species have been designated on the basis of a number of criteria including that species are charismatic and serve as a symbol to raise environment awareness thus serving as flagship species. These include the blue rock thrush as the national bird, the Mediterranean Killifish as the national fish, the Maltese freshwater crab as the national invertebrate, the Maltese rock-centaury as the national plant and the Sandarac Gum tree as the national tree (DOI, 2018). This implies that even if charismatic megafauna are lacking, the possibility of applying the concept of flagship species in the area of study is still possible.

The identification of endemic species including smaller non-mammals as charismatic species also reflects findings of other research in the field. It has been found that endemism, which is relatively high on islands and archipelagos, can play a vital role to influence visitors to contribute financially for conservation purposes and for ecotourism purposes (Verissimo et al., 2009).

The emphasis made on flora as a way to overcome the limited presence of large terrestrial charismatic fauna comes as no surprise. In fact, the Mediterranean Region is considered to be unique due to richness in species and the high rate of endemicity found in vascular plants (Gippoliti & Amori, 2006). In the Maltese Islands, over 2000 species of terrestrial plants have been recorded to date (Schembri, 2003). Furthermore, the archipelago also supports a number of strictly endemic species (including 23 plant species) and other species (including some 20 species) which are sub-endemic to the Maltese and circum-Sicilian islands (Lanfranco et al., 2013). In addition, one finds charismatic plant species which tend to garner attention including the 12 species of orchids, some being rare whilst others being endemic (Cutajar et al., 2017). In the case of the Aegadian Islands Marettimo hosts no less than 500 plant species (Gianguzzi et al., 2006) and some nine endemic plant species can also be found in the archipelago (Pasta & La Mantia, 2013). Flora on the Pelagian islands is considered of exceptional interest and includes 21 strictly endemic plants (Pasta, La Mantia & Rühl, 2012; Pasta & La Mantia, 2013). Pantelleria is characterized by abundant plant species richness (approximately 600 species), with the presence of six endemic plant species (Gianguzzi, 1999; Gianguzzi, Cusimano, Cuttanaro & Romano, 2013; Pasta & La Mantia, 2013).

The approach of seeking other ecotourism targets beyond large terrestrial charismatic species is also beneficial to overcome seasonality experienced in the area of study especially on smaller islands. Butler (1994) distinguished between natural and institutionalised seasonality. In the case of natural seasonality, which is influenced by climate, this can be easily overcome in the area of study, as the climate of the islands makes such seasonality less pronounced allowing several outdoor activities to be held throughout the entire year. Furthermore, the peak of certain activities such as bird watching and flower gazing do not fall in the traditional tourism season while the season of the ecotourism activity such as dolphin watching extends well beyond the tourism season. In the case of institutionalised seasonality which originates due to lifestyle such as work and education commitments one should keep in mind that ecotourism tends to attract older tourists, whose holidays are not tied by work or school holidays (Garrod & Wilson, 2004). This implies that diversification of ecotourism opportunities is crucial to ensure that such islands can also target this segment of tourists off-season and mitigate seasonality.

On several islands in the area of study Sand, Sun and Sea (3S) tourism and coastal-based tourism are the predominant form of tourism. This has also led to a scenario whereby extensive parts of the coast have been either developed or face anthropogenic disturbance (Deidun, 2010). Sandy beaches in particular are highly sought after and thus such habitats experience tremendous human pressure (Deidun, Azzopardi, Saliba & Schembri, 2003). The diversification of ecotourism targets and the resulting possibility to practise such form of tourism beyond coastal areas is thus an opportunity to provide other tourism opportunities and ease the pressure from sandy beaches and coastal environments.

One should note that most participants of the ecotours had already participated in other ecotours and visited other protected areas including those in destinations synonymous with the “big 5”. This might explain why ecotourists did not underline the absence of large charismatic species as a major limitation or leaving a negative impact on their satisfaction. This is because research has shown that tourists who already had previous experience in visiting protected areas are more likely to appreciate and support initiatives that promote a broader biodiversity experience which goes beyond charismatic species only (Giglio et al., 2015; Hausmann et al., 2016). Meanwhile one should not limit marketing to experienced ecotourists. Hausmann et al. (2016) argue that while experienced tourists tend to be the target group of tourists in protected areas lacking charismatic species, less experienced tourists had preferences which were not restricted to such species. Thus, they could still be attracted to such sites by focusing on biodiversity related activities related to less charismatic species and landscapes, accessibility of protected areas, the opportunity to avoid crowds and the overlap with adventure and cultural aspects.

Alien species are considered as a threat to habitats and their biota and can cause significant changes not only in marine environments (Wallentinus & Nyberg, 2007) but also in freshwater ecosystems. The spread of alien species can also leave an impact on charismatic species. A classic example is the impact left following the release and extensive spread of the red swamp crayfish (*Procambarus clarkii*) in Maltese watercourses leaving an impact on the indigenous Fresh water crab (Deidun et al., 2018) which is considered as a charismatic species.

Lack of wildlife sightings do not necessarily influence the satisfaction of overall ecotourism experience (Orams, 1999b). There have been times where target species were not observed during the excursion, but ecotourists were still enticed by the species and related conservation projects to the extent that they also contributed financially to support the protection of the species (Deemer, 2014). Meanwhile there have also been times where lack of wildlife sightings did contribute to cause dissatisfaction among ecotourists (Lawton, 2012; Muloin, 1998). Thus limiting ecotourism experiences on target species is risky since no matter how large the abundance of species is, observing target species during excursions is not always successful. This is in fact also emphasised by operators ahead of excursions (EcoMarine Malta, 2018). Hence, the approach of revolving ecotourism experiences around the broader aspect of biodiversity that goes beyond solely targeting charismatic species is also advantageous from this perspective.

Whereas according to Garrod and Wilson (2004) the strict competition in the ecotourism market makes it challenging to attract repeat visitors, a number of ecotourists participating in the ecotours revisited the ecodestinations convincing other friends to join them. This together with other results such as the high level of satisfaction of ecotourists, willingness to participate in similar ecotours on other Mediterranean islands and willingness to recommend the destination to friends confirms the potential of ecotourism in the region irrespective of the absence of large charismatic species.

Various concerns have been raised by stakeholders on the negative impact on charismatic species including those in marine environment due to anthropic impacts. Such concerns concord with research conducted in the Pelagian Islands confirming that the increased and intense traffic of boats during the summer period (especially between May and October) leads to accidental collisions (Prazzi, Nicolini, Piovano & Giacoma, 2010). This also causes disturbance to the bottlenose dolphin which in return causes their displacement from coastal areas (La Manna, Manghi, Pavan, Lo Mascolo & Sarà, 2013, 2014). In addition, prolonged and close contact of humans with charismatic species can leave detrimental impacts (Weaver, 2005). Failure to ensure sustainability of fisheries and the good health of the seas is considered to be a major flaw in Mediterranean marine governance leading to drastic impacts on charismatic species (RAC/SPA, 2013).

5 Conclusion

This research has shed light on lack of charismatic species and its impact on ecotourism potential on islands, an aspect which has not received any attention in the central Mediterranean region. It has been confirmed that the absence of charismatic species is not a detrimental issue hindering ecotourism potential. On the other hand various other approaches being adopted or proposed have conservation benefits including giving due attention to the broader biodiversity rather than focusing on few charismatic megafauna. Results have marketing implications especially since nature-based tourism is growing world-wide with more and more tourists visiting protected areas. Marketing efforts need to focus on both experienced and inexperienced ecotourists

(Balmford et al., 2015). Yet different packages and research on ecotourist profiling might be necessary. In this regard, more studies on profiling of ecotourists in the region and which are currently lacking are required.

In 1987, Schembri, Lanfranco, Farrugia, Schembri and Sultana pointed out that whereas quality/quantity arguments on tourism had been ongoing and even if other countries were benefiting from a tourism product based on nature, Malta had failed to tap into this niche. Diamantis (2000) argues that ecotourism in Mediterranean Islands was still in its infancy hinting at the lack of demand. Meanwhile, whereas tourism in the Mediterranean is strong, new trends in tourists' interests are shifting from 3S tourism (Vogiatzakis et al., 2008). This implies that new tourism products such as ecotourism need to be further explored. Meanwhile the situation reported by Diamantis (2000) did not change much. Yet, one cannot blame this on the absence of charismatic species. Other challenges need to be overcome including island connectivity, lack of innovative ecotourism products and lack of services such as interpretation. The area of study is also impacted by lack of promotion of the islands as ecodestinations with too much focus being made by policy makers and operators on mass tourism. Lack of management of protected areas due to lack of funding, extensive detrimental environmental impact and lack of environmental awareness among locals and operators were other challenges identified by stakeholders.

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Annex 1 Checklist of issues raised during interviews and focus groups

- Activities and features that are most important during an ecotour
- Motivations to participate in an ecotours
- Presence of large charismatic species
- Absence of terrestrial large charismatic species and impact on ecotourism experience
- Absence of wildlife sightings as a limiting factor
- Causes of disappointment during ecotours
- Fulfilment of expectations identified prior to the ecotours
- Willingness to recommend the ecodestination visited to others
- Aspects incorporated in excursions organised
- Aims of ecotourism excursions beyond target species
- Species that can serve as flagship ecotourism targets
- Challenges being faced and opportunities for ecotourism development in area of study
- Approaches to overcome the challenge of absence of terrestrial charismatic species

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News Article

The 36th European Seismological Commission General Assembly

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In Trieste, during the 35th General Assembly of the European Seismological Commission (ESC), the Department of Geosciences at the University of Malta was chosen to host the 36th General Assembly (Fig. 1). The city of Valletta won the bid among competition from three other European cities. The event took place at the Mediterranean Conference Centre (Fig. 2) from the 2–7 September 2018 and was preceded by a one-week Young Seismologists Training Course (YSTC; Fig. 3).

The ESC is a commission within the International Association for Seismology and Physics of the Earth's Interior, whose General Assembly takes place every two years. It is primarily a meeting ground for academics and researchers working in the field of seismology and related applications, including applied and social aspects of seismology, such as earthquake hazard and risk, earthquake engineering, education and awareness, and risk prevention.

Figure 1: Speakers addressing the Assembly at the opening ceremony. From left to right: Prof. Ina Cecić, ESC president; Dr Sebastiano D'Amico, Chairperson of the Local Organising Committee and ESC Vice-President; Prof. Alfred Vella, Rector of the University of Malta; the Hon. Dr Michael Farrugia, Minister for Home Affairs and National Security; Prof. Pauline Galea, Head of the Department of Geosciences; Prof. Stefano Parolai, ESC Secretary General. H.E. Marie Louise Coleiro Preca, President of Malta, delivered a video-recorded message.

More than 800 seismologists from Europe and other parts of the world gathered to present their latest research and engage in discussions about the theory, applications and implications of earthquakes and their effects. This year's ESC had a record participation and was deemed a great success for the number of high-level presentations, grouped into 42 separate sessions (494 oral presentations and 438 posters) and a daily poster session in the Sacra Infermeria Hall. This was one of the largest conferences ever hosted by the University of Malta. The scientific sessions covered themes such as seismic hazard assessment, induced and triggered seismicity, seismic tomography, historical seismology and macro seismology, the physics of earthquake sources, tsunami hazard, seismic risk to cultural heritage sites, earthquake risk, communication and mitigation, earthquake engineering, and many others. Internationally renowned experts in various fields delivered keynote lectures every morning, which included a wide range of topics. Keynote lectures were as follows:

- Prof. Pauline Galea (Department of Geosciences, University of Malta, Malta): Doing seismology on a small island – history, problems and opportunities.
- Dr Oona Scotti (Institut de Radioprotection et de Surete Nucleaire, France): Modelling fault systems in seismic hazard assessment: challenges ahead.
- Prof. Keith Koper (University of Utah, USA): Using CTBTO international monitoring system seismic arrays for basic science.
- Jaroslava Plomerova (Czech Academy of Sciences, Czech Republic): Upper mantle discontinuities – anisotropic view on the lithosphere-asthenosphere system. (Inge Lehmann Distinguished Award Lecture).
- Prof. Atilla Ansal (Özyeğin University, Istanbul, Turkey): Uncertainties in site-specific response analysis.

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Figure 2: The halls at the Mediterranean Conference Centre. Top: The Sacra Infermeria hosting the poster sessions and exhibitors stand. Bottom left: La Cassiere Hall during one of the oral sessions. Bottom right: La Valette Hall during the closing session.

Figure 3: Participants of the Peter Bormann Young Seismologist Training Course of 2018 at the Malta University Residence.

- Dr Alessandro Amato (Istituto Nazionale di Geofisica e Vulcanologia, Italy): Early warning systems for earthquakes and tsunamis - some reflections on their physical, social and legal impact.
- Mitsuyuki Hoshiba (Meteorological Research Institute, JMA, Japan): Ten years' experience of nationwide earthquake early warning in Japan.

5 students were awarded for best poster and oral presentation and the IASPEI Early Career Award was also presented. During the week preceding the Assembly, the Peter Bormann Young Seismologist Training Course was held for about 50 international participants (Fig. 2) at the Malta University Residence in Ħal Lija. This course was intended for postgraduate students of seismology and early career scientists. The course theme was Array Seismology; lectures and practical sessions were delivered by an international faculty, which included staff from the Comprehensive Test Ban Treaty Organization (CTBTO) in Vienna, who sponsored a

number of African students to attend the course and the General Assembly. The course exposed the students to the theory and practice of seismic arrays at a wide range of spatial scales, under the tutorship of a number of experts in the field. This topic had not been addressed previously in the preceding editions of the YSTC. The Assembly social dinner was held on Wednesday 5th September at a tourist Resort Qawra, along the northern coast of Malta (Fig. 4).

Figure 4: The social dinner in Qawra attend by many participants.

During the ESC Council Meeting the titular members voted to hold the next General Assembly in 2020 in Corfu, Greece.

Acknowledgements

We would like to express our warmest gratitude to all who contributed to the organization and success of this event: the entire Local Organising Committee (Fig. 5), the international scientific committee, ESC-Executive Committee, and organisers of special sessions and other parallel events, without whose enthusiasm, support, and devotion it would not have been possible to make such an event happen. A special thanks goes also to the invited speakers, conveners, and authors for their excellent work and interest in the conference. Finally, we are glad that the Young Seismologist Training Course provided a unique opportunity for the students to learn and exchange ideas about "Array Seismology". Our sincere thanks goes to all the lecturers, the European Geosciences Union and CTBTO for making this Training Course a big success. Finally, we would like to also thank the Embassy of United States of America in Malta, the Embassy of Italy in Malta and the Italian Institute of Culture for providing grants to support students to attend the General Assembly.

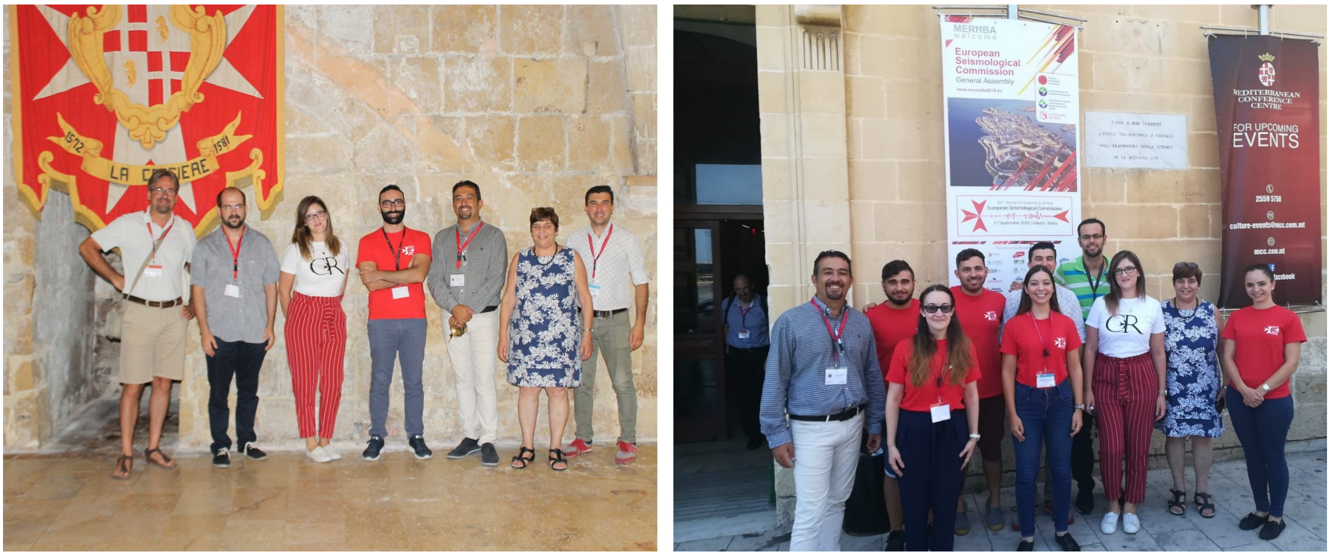

Figure 5: Left: Local Organizing Committee: (from left to right) Damiano Pesaresi, George Bozionelos, Daniela Farrugia, Emanuele Colica, Sebastiano D'Amico, Pauline Galea, Matthew R. Agius. Right: Local Organizing Committee together with volunteers helping during the event.

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Table of Contents

Articles