University of BRISTOL

BILT

STUDENT RESEARCH JOURNAL

ISSUE 7

HIGHLIGHTS MAGAZINE

Welcome

This year's Student Research Journal received contributions from 42 students across 9 subject areas and was supported by an editorial board of 18 student editors and 27 peer reviewers.

This highlights magazine brings you 9 of the best articles from the full journal highlight outstanding papers from each subject area, covering a range of topics, methodologies and approaches.

Papers were selected by subject editors and peer reviewers and these pieces showcase the breadth, originality, and academic quality of this year's research. All are a testament to the incredibly talents student researchers we have here at the University of Bristol.

We hope you enjoy discovering and learning more about subjects both familiar and novel!

E*mma Segal and Megan Stannett*
(Co-Editors-in-Chief 2025-26)

Scan the QR code to read the full 7th edition of BILT Student Research Journal

Meet the Co-Editors-in-Chief

Emma Segal



Emma Segal recently graduated from the University with an MSci in Medical Microbiology. Her research interests lie in infectious disease biology, particularly host–pathogen interactions, immunology and antimicrobial resistance. Her MSci research investigated how Staphylococcus aureus evades the human complement system, building on previous independent research experience during her degree. Alongside her studies, Emma has worked with BILT on projects aimed at improving the student experience. As Co-Editor-in-Chief of the Student Research Journal, she has enjoyed managing the editorial process and helping bring student research to a wider audience. She is particularly proud to have given students' work a platform while creating opportunities for others to gain editorial experience.

Megan Stannett



Megan Stannett recently graduated from the University with an MSci in Cancer Biology and Immunology. Her research focused on the biomechanical properties of childhood leukaemia, using novel 3D disease-modelling approaches to better understand the cancer microenvironment and identify potential therapeutic targets. Alongside her studies, Megan contributed to student-led initiatives across the University, including working as a student ambassador and writing for Epigram. As Co-Editor-in-Chief of the Student Research Journal, she has particularly valued creating opportunities for students to share their research beyond their degree programmes. She is passionate about cultivating an inclusive and supportive research community in which students can build confidence, develop their skills and engage with research across disciplines.

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Student Research Conference

On February 18th, the BILT Student Research Conference brought together students, academics and staff from across the University of Bristol for an inspiring afternoon dedicated to celebrating student research and academic curiosity.
Organised by BILT Student Fellows and Student Research Journal Co-Editors-in-Chief Emma Segal and Megan Stannett, the conference created a space for students to share their work, learn about research pathways, and engage with the wider academic community.



The event opened with a thought-provoking address from Professor Alvin Birdi, Associate Pro Vice Chancellor (Education Innovation and Enhancement), who reflected on the importance of research-rich learning and the role universities can play in empowering students to see themselves as researchers.



Following the opening address, attendees heard from an engaging panel of researchers and educators in the “Getting into Research” panel discussion. The panel brought together academics and postgraduate researchers from across the university to discuss their research journeys, the challenges they have faced along the way, and their advice for students considering research careers. The conversation highlighted the diverse paths into research and emphasised the importance of curiosity, resilience and collaboration.

Student Research Conference

A major highlight of the afternoon was the student research presentations, where students showcased their projects across a wide range of disciplines. Presentations were evaluated by academic judges from across the university, who carefully considered the quality of research, communication and engagement. Congratulations to our presentation prize winners:

- Jocasta Cochrane – Faculty of Health and Life Sciences – 'Exploring the β-lactamases of the Psychrophilic World'
- Luka Kadrikj – Faculty of Science and Engineering – 'Exploring Surface Chemistry through CSEHI'
- Theadora Gallie – Faculty of Arts, Law and Social Sciences – 'Barriers and Facilitators to the Engagement of Care Leavers with Health Services'

In addition to the presentations, students also displayed a fantastic range of research posters, giving attendees the opportunity to explore projects in an informal setting and speak directly with student researchers. The best poster awarded to:

- Natasha Davis

Alongside the research presentations, the conference also featured an "Ask the Editors" session led by members of the BILT Student Research Journal editorial team. Editors shared insights into the journal's publication process, offered guidance for prospective authors, and answered questions about how students can develop their research into publishable work.

The event concluded with closing reflections and prize giving from Hannah Grist, Head of CREATE, who celebrated the impressive work presented throughout the afternoon and highlighted the importance of initiatives that support student research.

Events such as the Student Research Conference play an important role in fostering a research-rich culture at the University of Bristol. By providing opportunities for students to present their work, connect with academics and explore pathways into research, the conference helps inspire the next generation of researchers.

Interested in taking part in the next Student Research Conference? Email us **bilt-info@bristol.ac.uk** for more information about how you can take part.

Working Across Generations: Perceptions of Young–Senior Co-Entrepreneurship

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Meet the Author

Khushi Agarwal is a graduate of the University of Bristol's School of Management, holding an MSc in Management (Entrepreneurship & Innovation). Her work centres on entrepreneurship, innovation, and intergenerational collaboration, with a particular interest in ventures that create meaningful social value alongside commercial success. Drawn to questions of how ideas emerge and how businesses can address societal challenges, she is especially interested in topics related to well-being, ageing, and quality of life. Outside academia, she enjoys listening to music while taking long walks and observing the world around her, often finding inspiration in everyday conversations, experiences, and human behaviour.

Keywords: Intergenerational co-entrepreneurship, Young–senior co-entrepreneurship, Stakeholder theory, Thematic analysis, Venture co-founding.

Abstract

This study explores the feasibility of intergenerational co-entrepreneurship between young adults (20–34) and retired professionals (60+), globally. It is motivated by the increasing participation of both groups in entrepreneurship, and the growing interest in collaboration across generations, often presented as a complementary model combining youthful innovation with senior experience. However, limited research explores how these groups understand the realities of co-founding a venture. To address this, the study draws on stakeholder theory and treats both groups as internal stakeholders. It adopts a qualitative, exploratory approach using 20 semi-structured interviews, analysed thematically to examine participants' motivations, expectations, and concerns. The findings show that while intergenerational (young-senior) co-entrepreneurship offers significant potential, its complementarity is not automatic. Its feasibility depends on how partners distribute authority, build trust, align decision-making pace and risk orientation, and manage capacity constraints. Additionally, participants highlighted value beyond financial outcomes, including social purpose, knowledge exchange, and personal development. Overall, the study reframes intergenerational co-entrepreneurship as a relational, contingent model and extends stakeholder theory by highlighting internal power dynamics within entrepreneurial teams. The findings offer practical insights for entrepreneurs, support organisations, and policymakers designing sustainable intergenerational ventures.

Introduction

Entrepreneurship is increasingly pursued across generations. (Li et al., 2020; Murnieks and Cardon, 2023). For young adults, it is often linked to autonomy, innovation, and meaningful work, while shaped by insecure labour markets, unequal access to finance, and limited professional networks (Sitoula, 2015; Elkayaly and Fahim, 2021). Reflected in the AXA UK (2024) report that 60% of young people aspire to be self-employed by age 30, indicating strong and growing entrepreneurial ambition.

Entrepreneurship has also increased among seniors, often associating it with longer working lives, ageing populations, and the desire to remain active after retirement (Mouraviev and Avramenko, 2020; Management Editorial Team, 2023; Kanama et al., 2025). In the UK, around 991,000 individuals aged 60+ were self-employed in 2023 (Martin, 2024). While seniors bring experience, knowledge, and networks, they may also face ageism, health constraints, and digital challenges (Martin and Welsch, 2019; Dent, 2020; Boerner, Fritz and Frick, 2025).

The research in these domains highlight potential complementarity. Younger entrepreneurs are often seen as energetic, creative, and digitally skilled, whereas older adults are associated with experience, credibility, and strategic judgment

(Perez-Encinas et al., 2021a; Perez-Encinas et al., 2021b; Watkins and Omilion-Hodges, 2025). This has led to growing interest in intergenerational collaboration. Studies and real-world cases suggest that age-diverse teams can create value. For example, John and Mark Cronin's enterprise demonstrates how a father's expertise can enhance his son's vision, providing a model for family-based intergenerational collaboration (Berger, 2018; Cronin and Cronin, 2021). Additional cases, such as OneClick.chat, founded by Dillon Myers and Alan Gibson, and Chip Conley's involvement at Airbnb, illustrate how younger and older partners may contribute distinct but valuable forms of knowledge (Hilgers, 2016; Hannon, 2019; Conley, 2018).

However, studies on intergenerational co-founding remains limited. Research on youth and senior entrepreneurship is largely separate, while intergenerational studies often focus on mentoring or workplace interaction rather than shared venture creation (Sitoula, 2015; Dent, 2020; Watkins and Omilion-Hodges, 2025). Even when discussed, it typically presented success cases or outcomes, with little focus on partner coordination (Hannon, 2019; Perez-Encinas et al., 2021a). This is important, as theoretical complementarity may not translate easily into real collaboration.

Existing literature also points to potential tensions. For instance, younger founders may lack experience, stability, and legitimacy, while older partners may prefer slower communication, structured routines, and lower-risk strategies (Polat and Yılmaz, 2020; Elkayaly and Fahim, 2021; Mustafa, 2024; Manoj, 2025). Further complicated by stereotypes such as, older adults being seen as resistant to change and younger individuals as impulsive. Thus, influencing both internal relationships and external evaluations (Lyons and Kuron, 2014; Stanford Report, 2024; Boerner, Fritz and Frick, 2025). However, the co-founding perceptions still remains underexplored.

Therefore, the article examines how young adults (20–34) and retired professionals (60+) perceive the feasibility and value of co-founding a venture. The study uses stakeholder theory to examine young–senior co-entrepreneurship as an internal stakeholder relationship, rather than only as a combination of age-based traits or entrepreneurial motivations. Stakeholder theory has commonly been used to understand the wider network of individuals and groups connected to a firm, including customers, suppliers, employees, investors and communities, and to challenge the narrow view that business should prioritise shareholders alone (Freeman, 1984; Freeman et al., 2010). Building on this, Ramoglou, Zyglidopoulos and Papadopoulou (2023) argue that entrepreneurial opportunities are actualised not only through market demand, but also through the involvement of stakeholders at centre-stage and non-economic considerations. This lens is useful here because a young–senior venture does not become feasible simply because two individuals possess different resources or motivations; it depends on whether the co-founders, as internal stakeholders, can align their interests,

expectations, values and forms of contribution within the venture. While motivation, human capital, entrepreneurial identity and life-course perspectives help explain why an individual may enter entrepreneurship (Elder, 1998; Shane, Locke and Collins, 2003; Fauchart and Gruber, 2011; Unger et al., 2011), they are less able to capture the co-founder dynamics. Adopting a global perspective, the study captures diverse contexts of ageing and entrepreneurship, offering insights for entrepreneurs, support organisations, and policymakers.

Accordingly, this research contributes to the underexplored field of intergenerational co-entrepreneurship, here termed young–senior co-entrepreneurship, by reconceptualising co-founders as internal stakeholders and examining whether such ventures create value beyond financial outcomes, including learning, social contribution, personal development and post-retirement engagement.

The remainder of this paper is structured as follows: Section 2 outlines the research design and methodology. Section 3 presents the key findings. Section 4 discusses these findings in relation to existing literature and stakeholder theory. Section 5 concludes with the main insights, contributions, and directions for future research.

Methodology

Research Design and Sample

This study adopts a qualitative, exploratory design to examine how younger and older adults perceive intergenerational co-entrepreneurship. A qualitative approach is appropriate as the research focuses on experiences, expectations, and relational dynamics that cannot be meaningfully captured through quantitative measures (Bell, Harley and Bryman, 2022).

Data was collected through 20 semi-structured interviews with two groups: young adults (20–34) and retired professionals (60+). The youth range reflects GEM's 18–34 category (Schøtt, Kew and Cheraghi, 2015), excluding those under 20 as decision-making maturity stabilises after this stage (Cauffman and Steinberg, 2000). While this strengthens the reliability of responses, it excludes younger aspirants whose views may differ. Similarly, the 60+ group captures the post-retirement stage (Statista, 2023; see Appendix 1 for the Statista graph), avoiding the tendency to merge seniors with mid-career adults, which can obscure distinct motivations (Kautonen, 2013).

Participants were selected using purposive sampling to include individuals with exposure to entrepreneurship and intergenerational interaction. This ensured responses were grounded in experience rather than abstract opinion. The sample included participants from India, the United Kingdom, the United States, China, Germany, Thailand, and Georgia, offering diverse perspectives. Details of participants, including anonymised codes, age, country, sampling method, and interview length, are presented in Table 1 to ensure transparency while maintaining confidentiality.

Younger Adult Participants (Aged 20–34)				
Code Name	Age	Country of Origin	Sampling Technique	Interview Length
Y1	25	India	Self - selection	52 minutes
Y2	24	Germany	Self - selection	32 minutes
Y3	23	India	Purposive	40 minutes
Y4	26	Thailand	Purposive	37 minutes
Y5	24	United Kingdom	Purposive	50 minutes
Y6	26	Georgia	Purposive	52 minutes
Y7	21	India	Purposive	32 minutes
Y8	23	Thailand	Purposive	50 minutes
Y9	24	China	Purposive	31 minutes
Y10	33	India	Purposive	58 minutes
Retired Professional Participants (Aged 60 and above)				
Code Name	Age	Country of Origin	Sampling Technique	Interview Length
R1	60	United Kingdom	Self - selection	45 minutes
R2	63	United Kingdom	Self - selection	36 minutes
R3	60	India	Purposive	31 minutes
R4	69	India	Purposive	30 minutes
R5	77	India	Purposive	35 minutes
R6	72	India	Purposive	40 minutes
R7	61	India	Purposive	32 minutes
R8	62	United States	Self - selection + Quota	44 minutes
R9	62	United Kingdom	Purposive + Quota	45 minutes
R10	67	India	Purposive	30 minutes

Table 1: Participant Identifier Table

Data Collection and Analysis

Data was collected through semi-structured interviews conducted online or in person, depending on availability. This allowed consistency while giving participants flexibility to elaborate. Interviews lasted 30–60 minutes, were recorded with consent, and transcribed for analysis. Conducted between July and August 2025, the study adopts a cross-sectional design to capture current perceptions rather than changes over time (Saunders, Lewis and Thornhill, 2023).

The interview guide focused on themes such as trust, role expectations, decision-making, and perceived value in intergenerational co-entrepreneurship. Open-ended questions encouraged reflection, with follow-up prompts used where necessary (see Appendix 3).

Data was analysed using thematic analysis following Braun and Clarke (2006). Each transcript was read several times to build familiarity before being manually analysed in Microsoft Word by highlighting key excerpts and adding initial codes via comments, which were then grouped into sub-themes and refined into

broader themes, as shown in Appendix 2. Coding was mainly inductive, allowing patterns to emerge from interview responses, and was guided by concepts like value co-creation and stakeholder dynamics (Freeman et al., 2010), which served as sensitising lenses to guide interpretation without restriction.

Ethical procedures were followed throughout. Participation was voluntary, informed consent was obtained, and all data was anonymised.

This methodology has limitations. Purposive sampling and a small sample limit generalisability and may introduce bias. Although participants came from multiple countries, cultural differences were not examined in depth. Translation from Hindi to English, single-researcher analysis and uneven gender representation may also have shaped the findings.

Findings

This section presents findings from interviews with young adults (20–34) and retired professionals (60+). Participants are coded as R (retired professionals) and Y (young adults).

Understanding and Self-Perception of Entrepreneurship

Participants described entrepreneurship differently across generations. Retirees framed it practically, linking it to self-employment and financial security:

"It simply means being self-employed and it could be from small to big level" — R3

Whereas, younger participants emphasised ownership, responsibility, and value creation:

"You're also responsible for everything… if you create an impact, if you create value, you might as well create it for yourself." – Y2

Differences also appeared in perceived capacity. Retirees highlighted limits related to health and lifestyle, while younger individuals stressed stamina:

"We can work for 15 or 16 hours at a stretch… we are at the peak of our energy," — Y1

However, some younger participants also recognised constraints around experience and financial readiness. Motivations reflected these differences: younger participants focused on independence and fulfilment, while retirees emphasised continued income and applying prior experience. Both groups saw preparation as essential, though younger participants linked it to work experience and retirees to clear planning.

Motivators for Intergenerational Collaboration

Participants consistently highlighted the value of combining generational strengths. Retirees emphasised the energy and ideas of young people, while younger participants valued the experience and strategic perspective of older partners. As one noted,

"Two different brains from two different cultures, and age diversity… could

contribute more rather than… the same age group, same experience." – R6

while another reflected,

"I don't see myself having that level of vision… I'm good with planning."– Y1

Collaboration was also viewed as a space for mutual learning. Younger participants expected guidance alongside opportunities to contribute digital skills, while older participants described the exchange as reciprocal:

"Both sides have learned from me. I've learned also from them… we learned together." — R2

Shared values further shaped collaboration. Participants stressed that alignment in purpose, particularly beyond financial outcomes, was central to sustaining partnerships:

"You have to create job opportunities… make contributions to society." — Y9

Intergenerational ventures were also seen as generating broader societal benefits, including knowledge exchange, innovation, and continued engagement for retirees.

Barriers and Tensions in Collaboration

Despite recognising benefits, participants identified tensions around working style, risk and capacity. Retirees often viewed younger partners as impatient, while younger participants perceived older collaborators as slower:

"*Younger generation… work fast… but older people… think multiple times before doing things… you need to find the middle ground."* — Y4

Risk orientation reflected this divide. Older participants emphasised caution and risk aversion, whereas younger individuals appeared more open to experimentation and failure, supporting research linking youth entrepreneurship with greater risk-taking and older entrepreneurship with more cautious strategies (Perez-Encinas et al., 2021a). As one retiree observed:

"When you get older, you get more cautious, more risk averse. And the thing about youngsters is that they are just like, 'I'm just going to do it. If it goes wrong, we'll do something else." — R2

Stereotypes shaped expectations but did not eliminate willingness to collaborate. Practical constraints, particularly time and energy, also emerged:

"you can maybe work 18 hours a day… sometimes in an older generation… you can't expect that." — Y10

These findings suggest that capacity is not only self-perceived but also a structural constraint influencing collaboration.

Collaboration Dynamics

Participants described collaboration around roles, decision-making, and communication. Role division often followed generational lines, with younger participants focusing on execution and older partners on strategy and finance:

"I would like to do the marketing part by myself… I would pass the finance management and the sourcing to the older partner" — Y2

"I would trust the older individual with the decision making… I'll probably take the execution part." — Y10

Older participants similarly emphasised planning and oversight:

"Ideation… strategic planning… operations… I can take these, and I would pass social media marketing to the younger partner." — R8

Decision-making preferences differed, with retirees favouring structured processes and younger participants prioritising efficiency. Trust and communication also emerged as central.

"Trust. … trust is something which builds a core foundation. … communication, it needs to be crystal clear, communication gaps can dent your business in the long run." — Y1

While some noted that age differences could create misunderstandings, others emphasised that trust outweighed age:

"I wouldn't consider age a factor at all, really; what matters is suitability and trustworthiness, not their date of birth." — R3

Overall, collaboration dynamics depended on role clarity, shared authority and transparent communication.

Enablers and Conditions for Successful Ventures

Participants identified enabling conditions related to skills, mindset and environment. Six younger participants emphasised interpersonal skills such as communication, empathy and patience:

"Communication… and empathy… you understand where they come from, and you don't take something as a personal offence." — Y6

Retired participants stressed structure, clarity, and accountability:

"writing things down and being able to remember them and then discuss them in the group and say, we said this, and we went wrong… CRMs… recording our conversation with clients… what actually was said rather than what you remember was said." — R2

Mentorship and reciprocal learning were also valued. Additionally, R8 critiqued the education system, arguing that *"there's too much of an emphasis on business studies… critical thinking skills have become lost,"* suggesting that interpersonal and reflective skills are essential for sustaining such ventures.

Beyond individual skills, participants highlighted supportive environments:

"doesn't matter what the age group is… it totally depends on the workspace environment," — Y7

While R8 argued *"companies don't grow without diversity of thought,"* underlining

that organisational climate and value alignment matter more than age alone.

Overall, successful intergenerational ventures rely on a balance of relational skills, structured practices, and supportive environments.

Therefore, the findings suggest that while intergenerational collaboration offers clear complementary strengths, its effectiveness depends on how differences in roles, pace, and expectations are managed. The following section interprets these findings in relation to existing literature and stakeholder theory.

Discussion

The findings challenge the assumption that intergenerational entrepreneurship operates as a straightforward combination of complementarities and barriers. Instead, collaboration is shaped by ongoing negotiation across roles, trust, tempo, and capacity.

Role Differentiation and Uneven Authority

The issue in intergenerational co-founding is not role differentiation itself, since venture requires division of work, but the basis on which roles and authority are assigned. In the findings, roles were often divided through age-based assumptions, with younger partners closer to execution and older partners closer to strategy and decision-making. While this reflects the complementary strengths noted in earlier research (Ensari, 2017; Perez-Encinas et al., 2021a), it also shows how complementarity becomes unequal when age is treated as a shortcut for competence.

Such arrangements may shift the partnership from co-founding towards a mentor–mentee relationship. Younger partners may feel obliged to follow senior decisions because of experience, hierarchy, or respect, while older partners may become concentrated points of authority. Over time, this could create frustration, weaken open disagreement, or make decision-making dependent on one side's judgement. Ramoglou, Zyglidopoulos and Papadopoulou (2023) argue that stakeholders are not passive recipients of entrepreneurial action. Therefore, both partners can be understood as internal stakeholders whose cooperation, interests and expectations shape whether the venture functions effectively. Therefore, stable young–senior co-entrepreneurship may require roles to be based on expertise, flexibility to revise responsibilities, and checks and balances that preserve the partnership.

Trust as a Central Coordinating Mechanism

Trust determines whether stereotypes in young–senior co-entrepreneurship remain barriers or become manageable differences. Both groups expressed assumptions about one another, with younger partners associated with speed and impatience, and older partners with caution or rigidity. However, these perceptions did not necessarily prevent collaboration. Where trust existed, participants were more willing to interpret differences as learning rather than as a reason to avoid partnership.

Co-founding requires delegation of both

work and authority. Without trust, partners may hesitate to rely on each other's judgment, leading to repeated checking, overlapping effort, slower coordination and wasted resources. Trust therefore functions as relational governance, helping partners cooperate when every decision, responsibility or conflict cannot be controlled through formal rules alone (Mayer, Davis and Schoorman, 1995; Poppo and Zenger, 2002). From a stakeholder theory perspective, this reflects the need to manage relationships for shared value rather than allowing one group's assumptions to dominate the other (Freeman et al., 2010).

Prior familiarity may reduce stereotypes because some trust may already exist before the venture begins. Where partners are unfamiliar, clear agreements, defined responsibilities, and transparent decision processes can reduce uncertainty without replacing interpersonal trust. Therefore, trust becomes more than a personal preference; it is a practical condition for delegation, coordination and long-term partnership stability.

Temporal Differences as a Structural Constraint

Differences in decision-making pace show that young–senior co-founding depends not only on shared goals, but also on compatible expectations about how quickly business decisions should be made and acted upon. Younger participants often associated venture work with speed and momentum, while older participants emphasised deliberation and risk checking. This should not be treated as a fixed age rule, but as a practical difference shaped by life-stage experiences and working habits.

Pace matters because it shapes venture functioning. A slower decision process may seem responsible to one partner but frustrating to another, while quick action may appear entrepreneurial to one and careless to the other. Such differences can create coordination friction, with younger partners seeing opportunities as lost through over-caution and older partners attributing mistakes to haste.

This extends stakeholder theory by showing that internal stakeholders bring expectations about the rhythm of action, not only resources, interests and values. Building on Ramoglou, Zyglidopoulos and Papadopoulou's (2023) non-economic considerations, working pace can be one condition that shapes opportunity actualisation. Young–senior ventures may therefore need early agreement on decision timelines, risk thresholds and independent authority, so speed and caution balance rather than become sources of blame.

Capacity Constraints and the Limits of Complementarity

Capacity is not an individual limitation in young–senior co-entrepreneurship; it becomes a shared condition shaping what the partnership can sustain. Complementarity suggests that one partner's strength can offset the other's weakness, but this only works when differences in availability, energy, digital responsiveness and commitment are openly managed. Otherwise, the same differences that make the partnership

attractive can produce misalignment in workload, communication and expectations.

This develops stakeholder theory by showing that internal stakeholders influence opportunity not only through their resources, but also through the constraints they bring into the venture. A partner's limited availability, slower responsiveness or different boundaries between work and personal life may quietly define the pace and scope of the business. Equally, the intergenerational learning exchange may create value, but only if the benefits outweigh the coordination effort required to maintain it. Building on Ramoglou, Zyglidopoulos and Papadopoulou's (2023) view that opportunity actualisation depends on stakeholder conditions, young–senior ventures therefore require early clarity on capacity, commitment and fallback arrangements. Complementarity is most likely to become productive only when differences can be absorbed without weakening the venture.

Implications

The central theoretical insight is that co-founders shape venture feasibility not only through the resources they bring in, but also through the constraints they introduce into the partnership. This extends stakeholder theory by showing that young-senior co-entrepreneurship depends on managing internal stakeholder conditions and relations, not complementarity alone.

Practically, this suggests that young and senior co-founders should assess the partnership, not only the business idea, before committing to a venture. This includes clarifying autonomy, decision rights, responsibilities and handling of disagreements. Furthermore, support organisations should move beyond age-basis matchmaking by helping potential partners test their fit through guided conversations, scenario-based exercises or short collaboration trials. Incubators and accelerators can support this through mixed-age founder programmes that could combine mentoring with shared decision-making practice. For policymakers, the findings suggest that targeted initiatives can make intergenerational entrepreneurship operationally feasible, rather than treating it only as a response to youth employment or ageing workforce challenges.

Limitations

As this study focuses on perceptions of young–senior co-entrepreneurship, its findings should be understood as identifying perceived conditions for feasibility rather than measuring venture outcomes. This does not reduce the value of the study, instead, it highlights the early-stage expectations, concerns and relational conditions that may shape whether such partnerships are attempted and sustained. Future research could build on this by examining active young-senior ventures over time.

Conclusion

This study examined how young adults and retired professionals perceive the feasibility of intergenerational co-entrepreneurship. Drawing on stakeholder theory and qualitative evidence from 20

interviews, the findings show that such collaboration offers clear potential but cannot be assumed to work simply because each generation brings different strengths. Thus, its viability depends on how co-founders organise their relationship.

The study further demonstrates that value in intergenerational ventures extends beyond financial outcomes to include social, emotional, and developmental benefits. By positioning co-founders as internal stakeholders, it reframes intergenerational entrepreneurship as a relational process in which entrepreneurial potential becomes viable only when expectations, contributions and responsibilities are made workable in practice.

These insights highlight practical implications for entrepreneurs, support organisations, and policymakers. Intergenerational ventures require structured support mechanisms, such as matchmaking platforms, training, and institutional guidance, to translate potential complementarities into sustainable collaboration and to address broader challenges related to ageing and youth employment.

However, the study has limitations. As this study focuses on perceptions rather than active ventures, its findings should be interpreted as perceived conditions for feasibility rather than measured venture outcomes. The small, purposively selected sample and uneven gender representation also limit generalisability and the diversity of perspectives captured.

Future research could build on this work through larger, more gender-balanced and culturally diverse samples, as well as longitudinal or observational studies examining how young–senior partnerships develop over time. Further studies could also explore how targeted support mechanisms enable sustainable intergenerational ventures.

Student Feedback Literacy from a Critical Perspective: A Neurodivergence-Affirming Review

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Meet the Author

Nathaniel is a final year Biology MSci student. In his final year, he has focused on improving the educational experiences of neurodivergent people. Drawing empathy from his own experience and a passion for social justice, he has worked to identify the implicit discrimination in education systems. He believes in the universal design of education, working to proactively remove biases and develop a learning environment that works for everyone. Nathaniel now works as England and Wales Coordinator of the Plant-Based Universities campaign, working to make university catering affordable, inclusive, and sustainable.

Keywords: Neurodiversity, Feedback Literacy, Evaluative Judgement, Managing Affect, Inclusive Education

Abstract

A feedback literate student possesses the competencies necessary to make effective use of feedback. The framework of student feedback literacy has informed institutional interventions to improve student progress in higher education. However, current conceptualisation of student feedback literacy is yet to consider minority perspectives, including those of neurodivergent people. It is therefore unclear how minoritised students' experiences with developing feedback literacy differ. Synthesising neurodivergent students' perspectives with sociocultural and political understandings of feedback, this critical review analyses implicit power imbalances, discrimination, and normative expectations in the feedback literacy framework. The review focuses on the conception of making judgements and managing affect, critiquing the implicit objective conception of evaluative judgement and individualisation of responsibility for affective response. The review proposes two concepts in the student experience of feedback literacy: Evaluative Masking as a behaviour suppressing authentic academic expression, and Constructive Defensiveness as a misunderstood but valid response to feedback. Future research directions are noted to facilitate reconceptualisation of the framework to create a neurodivergent-affirming understanding of student feedback literacy. This review lays the basis for future minority perspectives to be incorporated into feedback literacy, and the adoption of practices that will make developing student feedback literacy more accessible and equitable.

Introduction

Undergraduate students remain unsatisfied by the feedback they receive from markers on assessments, and this acts as a barrier to student progress (Boud & Molloy, 2013). Alongside improving the content of feedback, improving student uptake is crucial. One recent conceptual advance considering this is the development of the student feedback literacy framework, which recognises core competencies of a learner who effectively uses feedback (Carless & Boud, 2018). For effective action-taking: a student must appreciate the feedback process, make suitable judgements on quality, and manage affect in the feedback process.

However, minority perspectives on feedback are lacking, with homogeneity of cohorts assumed even in studies examining learner sociocultural context (Xu & Carless, 2017). In not recognising these perspectives, the feedback process can be unsupportive to specific needs, reducing psychological safety.

Incorporating neurodiverse perspectives on feedback is critical. Given the breadth of neurodivergence – incorporating ADHD, autism, dyslexia and mental health conditions, it is likely experiences of feedback are heterogeneous. However, across these cognitive profiles neurodivergent students report poor experiences in higher education (McDermott & Mosley, 2023). There is increasing recognition that institutional policy must be built with neurodivergent needs centred (Kennedy et al., 2025), and this should include feedback processes,

which can be designed based on neurodivergent-affirming conceptions.
This review critiques feedback literacy from a neurodivergence-affirming perspective. It aims to synthesise literature from pedagogy and neurodiversity studies to critique assumptions and biases underpinning the current framework. This theoretical conceptualisation will support inclusive principles in feedback research and implementation. The review covers two competencies associated with feedback literacy as described by Carless & Boud (2018) – Evaluative Judgement and Managing Affect. Each is analysed for conflicts with broader concepts in pedagogy and inclusivity studies. The effect of these conflicts on the neurodivergent student's learning is explored, alongside future research and policy directions.

Evaluative Judgement

Improving evaluative judgement facilitates actioning on feedback through refinement of work according to internal judgements (Boud & Carless, 2018). In considering the current barriers, scholars have recommended more opportunities for self-evaluation and deeper engagement with marking criteria. While opportunities for understanding criteria are likely to benefit neurodivergent students significantly (Bhandari & Rainford, 2023), it is important to recognise the unique barriers faced by neurodivergent students (Kennedy et al., 2025). Development of evaluative judgement requires the acquisition of tacit knowledge (Carless & Boud, 2018). This is often conveyed poorly to neurodivergent students, especially in more subjective subjects where markers often disagree (Bloxham et al., 2016). Moreover, neurodivergent students may possess special interests that reshape their judgement of quality (Boven, 2018) Taking neurodivergence as a diverse but valid sociocultural context, this section therefore aims to identify how current understandings of evaluative judgement may take an overly narrow view of its development and presentation.

Implicit Objectivisation of Quality

Despite the conceptualisation of feedback literacy as socioculturally grounded (Gravett, 2022), research often treats evaluative judgement as an objective skill (e.g. Tai et al., 2018). However, this risks sidelining accepting alternative but valid evaluations of work. Evaluative judgement is built on the quality against which it is practiced. However, instead of challenging how quality came to be conceptualised, many trust consensus views (Sadler, 2013). Feedback literacy as conceptualised implicitly trusts the consensus, and focuses on calibration and accuracy over developing an individual academic perspective and expression of quality (Carless & Boud, 2018).

Conceptualisation of quality as objective has shaped empirical methods. For example, Ibarra-Saiz et al. (2020) measured project management students' evaluative judgements relative to the consensus-based QUASS metric, rather than accepting the multiple valid styles of project management. Implicitly, this quantification frames minority perspectives as deficient, even when well-justified.

Although accuracy framing is beginning to

be critiqued (Fernandez-Toro & Duensing, 4 2021), this is yet to influence the conception of feedback literacy or be applied to disciplines traditionally viewed as more standards-based. In failing to search for alternative perspectives on quality, research is yet to understand how identity influences university students' work. This may curtail the ideas generation and epistemic insights expressed by many neurodivergent students (White & Shah, 2011; Dror, 2022). We may therefore recognise that without active consideration, neurodivergent perspectives are at risk of being overlooked.

Dialogue and Identity

If we accept evaluative judgement as identity-based, discussion is critical to develop broader appreciations of judgements This aligns with the increased interest in dialogue in feedback (Steen-Utheim et al., 2017). Dialogue can challenge the teacher-student hierarchy, improving trust and encouraging feedback-seeking behaviour (Price et al., 2011). Further, in challenging a consumption-based unidirectional view of feedback, we recognise dialogue as an opportunity for self-reflection and academic transformation. However, for truly open and non-hierarchical dialogue, efforts should be made to encourage constructive disagreement and bidirectional uptake of insights. Often, dialogue spaces can silence full expression of student voice (Hookway, 2012). More open dialogue is an opportunity to develop evaluative judgement in students through encouraging justification of their perspectives. This could provide space for dialectics, creating perspectives of quality reflective of both academic and student perspectives. More developed academic literacies have been more successful in actively challenging top-down standardisation in favour of actively seeking different student manifestations (Lea & Street 1998). If feedback literacy research followed this approach, dialogue becomes a bidirectional space to explore quality, and to genuinely deconstruct the hierarchies that reinforce unjust conceptions of quality (Hookway, 2012). This makes dialogue an incredibly powerful way to recognise the often missed contributions of neurodivergent people to the academic space and exploring expressions that need greater recognition (Bailey, 2023).

Calibration and Conformity

By creating psychologically safe dialogue spaces, we can ensure students feel confident voicing their perspective (Gibbs, 2006). However, communication of standards as objective and fixed may implicitly undermine trust, even when aimed at justifying institutional policies. Such a cognitive environment rewards conformity, not transgression, which while useful in some practical concepts, may inhibit expression of a student's academic voice (Hookway, 2012). Safer environments would more strongly challenge conformity and reward unique academic expressions. Conformity in subjective academic settings rewards safety over risk-taking. An objectivity-based environment allows many students develop confidence in their ability to calibrate to standards (Little et al., 2023). Yet when neurodivergent perspectives are not validated, they may feel less confident than peers

(Cai & Richdale, 2015; Buckley et al., 2024), including when they are shown to be no different in ability (Mahak et al., 2025). There may therefore exist an implicit pressure for students to develop internal feedback that suppresses under-recognised cognitive skills. This is particularly relevant for neurodivergent students (White & Shah, 2011; Milton, 2014). In dialogue and peer settings, their strengths may be under-recognised, and therefore unvoiced. This implicitly reinforces the neurodivergent insecurity that their cognitive profile is deficient (Cai & Richdale, 2015), rather than a potential strength to be shared. To open itself to true dialogue, research and practice must therefore actively work to avoid creation of cognitive environments that pressure conformity. One way researchers have started to recognise more nuanced expressions of student judgement, even in corrective settings, is through greater appreciation of the role of metacognition (Kolovelonis, 2023; Clark et al., 2025). Metacognitive students, aware of both the quality of work and thought processes behind it, may more clearly distinguish between when feedback reflects epistemic injustice (Fricker, 2007).

Evaluative Masking

Neurodivergent students will be familiar with having to suppress their behaviours to conform to societal standards, known as masking. There is growing recognition that 6 masking can be applied beyond social interaction to viewpoints and perspectives (Chapman et al., 2022). Perhaps a neurodivergent student's judgement process can be perceived not as evaluative judgement but as evaluative masking – suppression of academic voice in fear it would be poorly received (Boven, 2018). Evaluative masking may suppress exploration of focused interests, which could critically harm neurodivergent student's motivation (Milton & Sims, 2016). Intense interests and pursuits clash with a system that often considers undergraduate hyperfocus a deficit (Hua et al., 2012). The masking student is pressured to broaden or abandon their interests, harming their wellbeing (Grove et al., 2018). Though not yet applied to evaluative judgement, the significant toll of masking in higher education is increasingly recognised (Syharat, 2023). This calls for the redesigning of standards to accept focused interests as valid, and as an opportunity to motivate neurodivergent students (Boven, 2018).

Managing Affect – A Critical Lens

Carless and Boud (2018) identify management of affect as a core competency of feedback-literate students. However, given the inherent sociocultural placing of affective response and management, considerably little literature focuses on what constitutes constructive affective response. This is particularly noticeable in contrasting the attention given to teacher affect (Carless & Winstone, 2023). Given the increased proximity education researchers have with educators, this likely represents an empathy gap that creates an inherent theory-practice rift in understandings of affect. Recognising the emotional and political factors underpinning feedback, this passage examines how under-exploration of affect in feedback may implicitly reinforce normative expectations distant from neurodivergent experience. It moves past viewing affective response as

a study skill as traditionally theorised (Carless & Boud, 2018) and suggests that "poor affect management" (Winstone & Carless, 2019) itself is not the barrier to improvement, but poor institutional support for diverse affective needs.

Management of Affect may be Disproportionally Relevant for Neurodivergent Students

One factor potentially explaining the under-exploration of management of affect is that it is generally not seen as a barrier to feedback literacy (Winstone & Carless, 7 2020). This is justified by student surveys that show typically only 10% of respondents view report challenges in managing affect. These learners are often dismissed as "low achieving", with the implicit idea that these learners are less capable of developing feedback literacy competencies more generally (Winstone & Carless, 2020). Little attention has been paid as to how non-response bias or feelings of shame in these optional surveys may shape the low proportion of students reporting affect as a barrier. Moreover, from a critical pedagogy angle, it is crucial to remember that those 10% of students represent a systemic failure, not an individual deficit. Minoritised groups often face greater rejection sensitivity to feedback (London et al., 2012), this is particularly relevant for neurodivergent students where rejection sensitivity stems from both trauma-based and neurobiological sources (Müller & Pikó, 2024). When we synthesise these psychological sensitivities with the negative impact on wellbeing of higher education of neurodivergent students (McDermott & Mosley, 2023; Kennedy et al., 2025), we recognise the disproportionate difficulty neurodivergent students face to present idealised affective responses. Negative emotional responses to feedback may thus not be universally seen as opposition to improvement as traditionally viewed (Winstone & Carless, 2019), but as a valid reaction rooted in social and contextual factors. The theory that neurodivergent students may experience stronger affective responses to feedback aligns with neurodivergent students' voices. Neurodivergent students generally find higher education involves a great deal of emotional labour (Bhandari & Rainford, 2023, Jackson et al., 2017), including to feedback they perceive as insensitive (Syharat et al., 2023). One could therefore argue that affective expectations can be implicitly ableist, expecting neurodivergent students to present emotional responses that misrepresent the pressures they face. A neurodivergent student can face the stress of masking their feelings, or the social and academic rejection from refusing to suppress their emotional response (Chapman et al, 2022).

Identifying Institutional Responsibility

Recognising affective response as sociopolitical, we understand that individuals may have limited autonomy to independently shape healthy affective expression to 8 feedback. Yet this is not fully recognised in current feedback literacy literature, where little attention is paid to how institutions can aid students in managing affect in feedback (Carless and Boud, 2018). Instead, conceptions have implicitly responsibilised the self, reflecting the prevailing neoliberal attitude on emotional regulation common in Western society (Trnka & Trundle, 2014). This is reflective in the exemplification of students

independently managing their own affect in response to negative feedback (Winstone & Carless, 2019) through students whose survey responses fit idealised conceptions of reflectiveness and affective management (Shields, 2015). This is an inherent extrapolation: to assume that one student's emotional cognition is reflective of what all should aim towards. From this normative perspective, alternative management of affect may be viewed as a deficit. This strongly contrasts with views on affect from neurodivergent students and the wider student body: as reflective of cognitive differences and not as an individual deficits to address (Molloy et al., 2020; Bhandari & Rainford, 2023; Syharat et al., 2023). This aligns much more closely with the sociocultural situation of feedback literacy that scholars often cite (Nieminen & Carless, 2023). The rift between theorisation and student perspective and experience indicates that individualisation of affect has failed to develop student feedback literacy. This can threaten the creation of psychologically safe spaces (Carless & Young, 2024). Therefore, before we begin to consider how affective scaffolding can be universally designed, we must first explicitly and thoughtfully consider the institutional responsibility of affective support in feedback.

Institutional Responsibility and Identity Although management of affect is still generally viewed as a skill over a sociopolitical construct, some authors do accept that institutions bear some responsibility to support affect. For example, Xu and Carless (2017) praise the consideration of cultural perceptions and teacher-student relationship in mediating anxieties in the feedback process for Chinese students. This recognition of best practice is positive, but generalised the challenges to those faced across the entire cohort, clashing with evidence that neurodivergent students face not only intensified challenges to the neurotypical cohort, but also different challenges (Kennedy et al., 2025). Authors acknowledge the cultural differences between Chinese and Anglosphere classrooms 9 (Xu & Carless, 2017) but do not extend this to the cognitive and cultural heterogeneity in Western institutions. As secondary education becomes more inclusive, more neurodivergent students attempt academic degrees (ACDET, n.d.), increasing the diversity of lecture theatres. Therefore, we may not only view the institution as responsible for whole-cohort wellbeing, but also for proactively supporting individual needs, aligning with universal design principles.

Without recognising the different affective needs in the classroom, instructors risk implicitly reinforcing deficit framings, a stigmatisation increasingly recognised as prevalent in education (Jense van Rensburg & Liang, 2025). This stigmatisation reduces neurodivergent students' confidence in asking for support (Cai & Richdale, 2015), reducing feedback-seeking and support-seeking behaviours. This is in contrast to an expressed desire of neurodivergent students to feel more confident in these elements (Syharat et al., 2023). Future work must therefore move beyond identity-blind empirical studies and policies (Little et al., 2023), and centre these as contextual factors reshaping a learner's affective needs.

It cannot be fully theorised how neurodivergent students' affective management processes can be fully supported and accommodated in feedback, as empirical research is entirely lacking. However, the first step for theory and practice is a conceptual shift from a deficit-based to an emotionally-affirming understanding of the affective challenges of neurodivergence in education and feedback (Rowney-Smith et al., 2024; Kroll et al., 2025). This extends work that already recognises feedback literacy's manifestation could look different for disabled students (Nieminen et al., 2022) but moves to specifically recognise and root out additional challenges related to identity, discrimination and rejection sensitivity. Framed this way, we can begin open conversations with neurodivergent students to understand their experiences, and adapt feedback policy following inclusive best practice in tertiary education (Bhandari & Rainford, 2023). Relational aspects also likely shape neurodivergent affective management as they do with students more generally (Ellis, 2010; Xu & Carless, 2017), and increased emphasis on relations in feedback, particularly between peers who share identities, may share and reduce emotional labour (Simonsmeier et al., 2020). Together, this recognises that the institution's responsibility is not only to calm whole-cohort fears, but to proactively prepare feedback spaces to be inclusive of neurodivergent and minoritised student needs.

Discrimination and Affective Response

When we acknowledge both the wider discriminatory nature of society and its manifestation in normative standards of quality, we recognise that educational practices can be reflective of broader discrimination (Tannock, 2007). This leads to the appreciation that higher education can be built for neurotypical academics and students (McDermott & Mosley, 2023). From this, the poor mental health of neurodivergent students begins to make sense (Kuder et al., 2021). Analogising to more researched minoritises, such as LGBTQ+ students, we can predict significant improvements in wellbeing when an inclusive institutional culture is created (Huang & Fan, 2024). As well as viewing feedback as a current contributor to discrimination, we can therefore recognise it as a future mitigator.

If feedback reinforces discrimination, negative emotions to feedback may be seen as not only understandable but justified. Instead of reflecting misunderstandings of feedback (Carless & Boud, 2018), they reflect the empirically-backed and personally frustrating undermining of neurodivergent ability (Bailey, 2023; Marcotte, 2023; Beagan et al., 2025). Given neurodivergent people are often justice sensitive, it may be particularly frustrating that their manifestation of academic ability is so underformulated. Even in critical literature, there is a gap in recognising neurodivergent minority stress compared to the academic stresses recognised in other minorities (Vaccaro et al., 2021). Only from neurodivergent individuals themselves is there any discussion of the distress and discomfort created by the strands of discrimination running through higher education (Botha, 2021; Butcher & Lane, 2024). This can make feedback inherently personal while reducing

autonomy in the development process, which together can make developing a growth mindset exceedingly difficult (Westby, 2020). Thus, at the root of what is considered poor affective response may be systemic discrimination.

Reframing Defensiveness

When we recognise affective response as influenced by discrimination, educators can appreciate the importance of critical creating compassionate feedback spaces in mitigating distressing affective responses. However, given that societal discrimination for neurodivergent students is deeply embedded, reducing institutional guilt may not absolve responsibility for institutions to work with negative affective 11 reactions. We may therefore decide to design systems that can work with emotional reactions typically framed as negative. One particularly negatively-framed response is defensiveness. Defensiveness is often considered entirely maladaptive (Rudolph et al., 2007), or even antithetical to reflectiveness (Schwartzman, 2007). However, this may limit our appreciation of defensiveness as a contextual response. We know from empirical research that defensiveness to critical feedback is elevated in women (London et al., 2012), students of colour (Cohen et al., 1999), and likely in neurodivergent students (Bhandari & Rainford, 2023). Moreover, it is often ignored that when evidence-based, justified defensiveness is built on self-reflection. Viewing defensiveness as a skill, a form of self-reflection, and a natural inclination of rejection-sensitive learners (Babinski et al., 2019), it may therefore be valuable to engage with defensiveness openly.

Reframing defensiveness as something to work with may not only be helpful in reshaping a deficit framing of emotional responses that is damaging for neurodivergent students (Kennedy et al., 2025), but also in extending the dialogic interpretation of feedback (Carless & Chan, 2016) to include engaging with Constructive Defensiveness. Constructive defensiveness to feedback includes a student's meta-cognition of injustice within feedback, and may present a powerful opportunity for self-advocacy that increases workplace readiness in environments where anti-neurodivergent discrimination is still commonplace (McDermott & Mosley, 2023). Constructive engagement with defensiveness could involve listening to student perspectives and providing opportunities for further engagement for those who feel strongly: a potentially critical and underexplored method of driving increased feedback literacy and improving institutional practice. Institutions can reconceptualise processes in which educators and students communicate on feedback from improving student concordance with fixed expectations (Westby, 2020), to a bidirectional and dialogic practice that recognises not only recognises an exchange in ideas of quality, but also of emotional perspective (Lea & Street, 1998).

Conclusion

This review examined an underexplored proposition - that neurodivergence may shape student feedback literacy. Owing to gaps in direct evidence, the review provided conceptual directions through synthesis of other research fields. Through this, it has laid the foundations for empirical research incorporating

neurodivergent perspectives on assessment and feedback. A key theme throughout the review was the normative expectations that arise when identity is unconsidered. It is critical that future studies centre identities such as neurodivergence when examining student experiences with feedback. Incorporation of neurodivergent perspectives will begin to address a fundamental problem in feedback literature – the gap between theory and practice. Engaging in more radical co-construction of feedback frameworks and practice will increase empathy and broaden our perspective on the sociocultural construction of feedback literacy. Such engagement recognises that manifestation of feedback literacy is shaped by learner context. This may help influence the development of psychologically safe feedback environments, opening the feedback space for bidirectional dialogue.

The development of inclusive and socioculturally contextual feedback literacy presents an opportunity to close attainment gaps for minoritised students. By moving away from a study skills conception, we recognise feedback literacy as the development of a learner aware of their position, their areas for continuous development, and of justice in the feedback process. Through reimagining feedback literacy through a critical pedagogical lens, this review has recognised that feedback literacy is inherently political, built on power relations, and an opportunity for developing critical consciousness. Whether we engage with further critical analysis and incorporation of traditionally marginalised perspectives may determine if feedback literacy policies unintentionally perpetuate educational inequality or can contribute to equity in higher education.

Is the Paris Agreement Enough? Human Rights and State Obligations in the Climate Crisis

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Keywords: Climate change, Paris Agreement, human rights, general principles of international law, obligation of states in respect of climate change.

Abstract

Climate change presents profound challenges not only to the environment but also to the protection of fundamental human rights, raising critical questions about the scope of State's legal obligations under international law. This article argues that compliance with the Paris Agreement alone is insufficient to discharge State's broader human rights obligation in addressing climate change. While some States contend that the climate treaty regime constitutes the exclusive legal framework governing climate obligations this article demonstrate that such a position misconstrues the relationship between treaty law and general principles of international law. It contends that principles such as a due diligence, prevention of transboundary harm, and the obligation not to cause significant environmental damage to continue to apply alongside the climate treaty regime. Drawing on jurisprudence from the International Court of Justice, regional human rights courts, and United Nations bodies, the article highlights the growing recognition that climate change directly threatens rights including the right to life, territorial integrity, and self-determination. Ultimately, the article concludes that climate treaties operate within, rather than replace, the wider framework of international law, requiring States to adopt comprehensive measures that safeguard both environment and human rights.

As climate change continues to pose an existential threat to both the environment and human well-being, it has triggered a contentious international debate over the scope of state's legal obligations in responding to its impacts. In the Advisory proceedings of *Obligations of States in respect of Climate Change*[1], Germany argued that compliance with the Paris Agreement is sufficient to fulfil state's human rights obligations related to climate change. The argument is central on the belief that the Paris Agreement, with its legally binding elements regarding mitigation, adaptation and finance, is enough to provide a comprehensive framework for addressing climate change, and thus sufficient to cover human rights obligations [2] However, this position is flawed, as it disregards the additional obligations imposed by the general principles of international law (GPIL) in addressing climate change under the realm of international law and human rights.

This article will argue that fulfilling obligations under the Paris Agreement does not suffice for States to meet their obligations under human rights conventions in the context of climate change. It will first argue that the Paris Agreement should not be treated as the exclusive legal framework for climate action. It will then argue that state's climate obligations extend beyond the Paris Agreement, particularly under GPIL, which imposes duties such as due diligence, the prevention principle and the obligation not to cause significant transboundary harm. Lastly, it will examine the right to territorial integrity and the right

of self-determination, highlighting the increasing recognition of climate change's impact on the enjoyment of fundamental human rights. Finally, the article will conclude by demonstrating that a narrow reliance on the Paris Agreement is insufficient to discharge state's broader legal obligations in combatting climate change and safeguarding human rights.

I. The Paris Agreement is not the primary legal framework for climate change

In international law, it is common that more than one rule addresses a specific issue; such parallelism occurs frequently. [3] Lindroos (2005) argues when multiple legal norms apply to the same issue, the principle of *lex specialis* serves to resolve any potential conflicts by giving priority to the more specific rule over the general one[4].

In this context, Germany argued that the Paris Agreement and the United Nations Framework on Climate Change Convention (UNFCCC)—as specialized legal instruments on climate change—should take precedence over broader human rights obligations when addressing climate-related issues [5]. Germany maintained that any potential normative conflict should be resolved by giving priority to the more specific legal framework [6]. Similarly, Australia argued that principles outside the treaty regime do not supplant or impose obligations upon States in relation to climate change [7]. Bangladesh[8] even contended that the UNFCCC regime should, on the basis of the *lex specialis* principle, displace, limit or modify what are considered to be the *leges generals* in these proceedings, i.e. important GPIL such as prevention of significant transboundary harm, the due diligence principle and the prevention of harm.

However, these arguments are misleading as the application of *lex specialis* is not automatic. States cannot invoke it merely because two or more legal regimes address the same subject matter. For *lex specialis* to apply, two key elements must be met; there must be an actual inconsistency between the two provisions[9]; or a clearly expressed intention that one is meant to exclude the other. As clarified by the International Law Commission; " it is not enough that the same subject matter is dealt with by two provisions; there must be some actual inconsistency between them, or else a discernible intention that one provision is to exclude the other"[10].

In relation to the first element, there is no inherent inconsistency between the GPIL, such as the prevention of transboundary harm, prevention of significant harm to the environment, due diligence and, on the other hand, the treaty regime addressing climate change. A state party to the climate treaty regime can fully comply with its obligations under that regime while simultaneously fulfilling the broader principles of GPIL[11]. It is not a matter of conflict but rather of complementarity, as the climate change legal regime seeks to prevent environmental harm that crosses borders, aligning with international legal obligations to prevent such damage [12]. Thus, these principles and the climate change regime are not only compatible

but are harmoniously applied together.

This was echoed by Egypt:
"the existence of treaties dealing with the subject of climate change does not preclude the application of the general rules of international law" [13].

However, Kuwait[14] argued that applying general principles such as transboundary harm could impose additional duties not expressly agreed to within the climate treaties, thereby violating the principle of State consent in treaty law.

Nevertheless, this counterargument fails because it mischaracterizes the relationships between climate treaties and GPIL. Under Article 31(3) (c) of the Vienna Convention on the Law of Treaties, treaties must be interpreted in light of relevant rules of law, unless explicitly excluded—which the Paris Agreement does not do. Moreover, Meguro (2020) argues, the notion that climate should be addressed exclusively through the treaty regime ignores the multidimensional nature of the crisis [15]. Climate change affects not only the environment but also human rights, sovereignty and global peace [16]. Climate treaties do not exist in a legal vacuum—they operate within the wider matrix of international law and must be interpreted as part of an integrated system of obligations [17].

This point was raised by the Cook Islands:
"the UNFCCC, including the Paris Agreement, is not a *lex specialis* regime that regulates all climate change action, and singularity provides for all of State's obligation in respect of climate change, and legal consequences under State's obligations, to the exclusion of all general rules of international law" [18].

Thus, the climate change treaty regime does not function as *lex specialis* in a manner that would displace or limit State's obligations under GPIL. Instead, the climate regime exists within the overarching framework of international law, complementing rather than overriding its general principles.

The second requirement–that there must be a clear intent to exclude GPIL —is plainly unmet. Nowhere in the UNFCCC, Kyoto Protocol or Paris Agreement is there any explicit provision that displaces core principles of GPIL [19]. International treaties cannot be assumed to implicitly override fundamental principles of customary international law (CIL) without express provisions to that effect. As Thirlway aptly described:

"if a treaty provides that the parties, in their mutual relations, shall not be obliged to do something which CIL would otherwise have required of them, it is trite law that the obligation so set aside does not form part of the law between the parties. On the other hand, where the treaty is silent, CIL continues to apply" [20].

This position is not theoretical—it is supported by International Court of Justice (ICJ) jurisprudence. In *Construction of a Road in Costa Rica along the San Juan River,* Nicaragua argued that the 1858 Costa Rica-Nicaragua Treaty of Limits[21] constituted *lex specialis* concerning the obligation to consult under CIL [22].

However, the Court rejected the claim that a treaty could displace procedural obligations under CIL merely by containing some relevant provisions. It ruled that:

"the fact that the 1858 Treaty may contain limited obligations concerning notification or consultation in specific situations does not exclude any other procedural obligations with regard to transboundary harm which may exist in treaty or customary international law"[23].

Likewise, in ELSI, the Court refused to accept that a fundamental principle of CIL could be silently waived, reaffirming that any departure must be clear and express. The Chamber rejected the notion of silent derogation of CIL, holding that it was:

"unable to accept that an important principle of customary international law should be held to have been tacitly dispenses with, in the absence of any words making clear an intention to do so"[24].

Therefore, there is no discernible inconsistency between the climate change treaty regime and the relevant GPIL. Both legal frameworks operate in parallel, without contradicting each other, and there is no provision in either body of law that suggests any inherent conflict. Thus, it cannot be argued that the climate change treaties exempt States parties from obligations that would otherwise be required under GPIL, nor is there any indication that the treaties were designed to exclude the application of GPIL.

II. Additional Climate Obligations extend beyond the Paris Agreement

Since it has been established that there is neither an actual inconsistency between the Paris Agreement and the relevant GPIL, nor a clear intention within the treaty text to exclude the application of broader norms, it follows that the principle of *lex specialis* does not preclude the operation of GPIL. If the Paris Agreement was the only source of obligations to combat climate change then any State that is not party to the climate change regime— or which has withdrawn from it— would have no legal obligations to protect the climate from anthropogenic greenhouse gas (GHG) emissions [25]. To limit these obligations solely to treaty law is to ignore the broader framework of international law, which not only reinforces these duties but also establishes accountability for States that fail to act.

a. Duty of Due diligence

The first obligation beyond the Paris Agreement, in fulfilling human rights obligations, is the duty of due diligence. The duty requires states to take all reasonable steps and measures to prevent significant harm to other states or the environment [26]. States are obligated to mitigate and remedy human rights impacts of climate change, including those arising from business activities within their territory, or jurisdiction [27].

The Office of the High Commissioner for Human Rights (OHRHR) emphasised this;
"The obligations of States in the context of climate change and other environmental harms extend to all rights-holders and to

harm that occurs both inside and beyond boundaries [28].

The *Pulp Mills* on the River Uruguay (Argentina v. Uruguay), clarified this;

"It is 'every State's obligation not to allow knowingly its territory to be used for acts contrary to the rights of other States"[29].

However, Australia contended that due diligence is a non-self-standing legal obligation but merely a standard of conduct that applies within the framework of other substantive obligations [30]. In circumstances where a State is required to achieve a particular outcome, due diligence is restrained in directing how the State should act to meet that goal [31]. The Seabed Disputes Chamber of ITLOS supports this, stating that an obligation 'to ensure' 'is not an obligation to achieve… rather, it is an obligation to deploy adequate means, to exercise best possible efforts, to do the utmost, to obtain this result [32].

However, this interpretation is flawed. To suggest that due diligence only exists in relation to other obligations undermines its preventive function. The *Pulps* case affirmed that States have an ongoing duty to act with care and vigilance to prevent transboundary harm- regardless of whether a specific result is ultimately achieved [33]. Framing due diligence merely as a standard of effort ignores its legal weight as a proactive obligation and dilutes the States' responsibility to take meaningful action.

Fulfilling due diligence is important for upholding human rights. The link between due diligence and human rights was proclaimed by the UN Resolution 76/300 which;

"Reaffirm[ed] that States have the obligations to respect, protect and promote human rights, including in all actions undertaken to address environmental challenges, and to take measures to protect the human rights of all"[34].

Similarly, in its commentary on Article 6 of the International Covenant on Civil and Political Rights (ICCPR), which protects the right to life, the United Nations Human Rights Committee emphasized that States must take legislative and other measures to:

"ensure that all activities taking place in whole or in part within their territory and in other places subject to their jurisdiction, but having a direct and reasonably foreseeable impact on the right to life of individuals outside their territory…are consistent with article 6"

In *Cáceres et al v Paraguay*, the UN Human Rights Committee held that Paraguay violated Articles 6 of the ICCPR. This was because of its failure to impose environmental laws regulating the usage of poisonous agrochemicals, which led to the contamination of drinking water and food resources. The contamination resulted in the author's hospitalization and the unexplained death of Mr. Cacerres. Thus, Paraguay's failure to act amounted to a breach of its obligations [35]. Likewise, in *Poma Poma v Peru*, the UN Human

Rights Committee found a violation of Article 27 (rights of minority groups to preserve their cultural, religious and linguistic identity) of ICCPR. Peru's water operations produced environmental harm that disrupted the author's traditional livelihood of llama herding. No prior consultation was conducted with the author or her indigenous community. The lack of participation led the Committee to find Peru in breach of its human rights obligations. Both examples demonstrate the importance of due diligence in upholding human rights and for the safety of communities.

Although the duty of due diligence is not explicitly stated in Resolution 76/300, the obligation to protect and take measures to uphold human rights plainly aligns with its core principles. Resolution 76/300 aligns with various human rights conventions that recognize environmental protection as a human right such as the Additional Protocol to the American Convention on Human Rights in the area of Economic, Social, and Cultural Rights [36], the Charter of Fundamental Rights of the European Union[37], the Arab Charter on Human Rights [38] and the African Charter on Human and Peoples' Rights[39]. These instruments uphold that states have obligations beyond the Paris Agreement, comprising both negative obligations (to refrain from harming the environment) and positive obligations (to take proactive measures for its protection) in environmental governance.

b. Duty of Prevention

The next obligation is the prevention of transboundary harm to the environment. The prevention principle is outlined in Principle 21 of the 1972 Stockholm Declaration on the Human Environment[40] and Principle 2 of the 1992 Rio Declaration on Environment and Development.

The Duty of Prevention principle is one of general application. As Kiss (1989) observed, the ICJ in *Corfu Channel*[41] established a general principle of international law: every state has an obligation not to knowingly allow its territory to be used in a manner that violates the rights of other states [42]. It obliges States 'to exercise best possible efforts, to do the utmost'[43] to prevent activities which take place in its territory, or in any area under its jurisdiction, from causing significant harm to other States.

Importantly, the *Trail Smelter*'s declaration —that a State has no right to permit activities within its borders that cause serious injury to another, especially when such harm is proven by clear and convincing evidence[44]—serves as a compelling precedent affirming the human right obligation to prevent transboundary environmental harm under international law.

However, Australia disputed that the prevention principle, usually applied to localized transboundary harm, cannot be directly applied to climate change due to its complex aggregate nature [45]. Unlike conventional pollution cases, climate change concerns emissions from multiple sources over time, presenting causation and attribution difficult [46].

This reasoning, while recognizing the complexity of climate change, incorrectly assumes that the diffuse nature of harm excuses States from their human rights obligations. Complexity does not negate responsibility. The prevention principle is a cornerstone of GPIL and has evolved to address environmental threats— including those with far-reaching cumulative impacts. The ICJ has repeatedly affirmed States duty to prevent transboundary harm, regardless of whether it arises from a single source or a global phenomenon [47]. Accepting Australia's argument would create a legal vacuum in which States could evade responsibility for contributing to climate change simply because it is a collective problem.

The European Court of Human Rights (ECtHR) has further affirmed the prevention duty as a human rights obligation by interpreting provisions of the European Convention on Human Rights (ECHR). The Court acknowledged that states must ensure a healthy environment as part of their duty to uphold the right to life (Article 2) and the right to private and family life (Article 8). In *Tatar v Romania* [48] , the Court set out a "positive obligation to take all reasonable and suitable measures to protect the rights [of the applicants to a private life]" which entails "the paramount duty to put in place a legislative and administrative framework for the effective prevention of damage to the environment and to human health"[49]. Similarly, in *Duarte Agostinho and Others v Portugal* [50] , the applicants argue that Portugal's recurring forest fires since 2017 are a direct consequence of climate change, causing them harm and posing serious health risks. They claim that the 33 respondent states have violated Articles 2 and 8 of the ECHR by failing to take adequate climate action, as outlined in the 2015 Paris Agreement. This case was brought before the Grand Chamber, which had not yet issued its judgement at the time of writing. Thus, the growing body of jurisprudence underscores the prevention duty as a human rights obligation in addressing environmental harm.

Environmental harm can have severe impacts on human rights, hindering the enjoyment of the human rights [51]. Thus, the prevention principle becomes essential in safeguarding human rights. The ECtHR has affirmed this principle, acknowledging that environmental degradation can threaten the rights to life[52], respect for private and family life[53], and property[54]. Accordingly, the prevention duty provides a robust legal basis for safeguarding human rights to individuals both within and beyond their borders.

c. Obligation not to cause significant transboundary harm

Another obligation is the duty to not cause significant transboundary harm. A state's jurisdiction rests on its effective control over activities conducted within its territory or authority. It is responsible for preventing those activities from causing harm beyond its borders, particularly when such harm affects the rights of individuals in other states [55]. The Inter-American Court of Human Rights (IACtHR) clarified this;
'States have an obligation to avoid

transboundary environmental damage that can affect the human rights of individuals outside their territory'[56].

In *Carême v. France* [57] the ECtHR reaffirmed the duty not to cause transboundary harm as part of France's positive obligations under the ECHR[58]. The applicant alleged that France has taken insufficient steps to prevent climate change and this failure entailed a violation of Article 2 and Article 8 under the Convention [59]. While the Court did not find a violation in this case because the Claimant's residence was located in an area subject to flooding by 2040[60], the Court recognised the duty not to cause significant transboundary harm, reflecting the growing recognition that environmental protections are integral to fulfilling human rights obligations.

Thus, the Courts have indicated that there are certain activities that involve significant risks to the health of the individual, and therefore, States have a specific obligation to regulate them such as the introduction of monitoring and oversight mechanisms [61].

III. The Right to Territorial Integrity (TI) and The Right to Self-Determination

Every State is entitled to be respected for its TI—"respect for territorial sovereignty is an essential pillar of international relations" [62]. As the Court has affirmed, "the respect of territorial integrity is an important part of the international legal order and is enshrined in the Charter of the United Nations." [63]. Respect for sovereignty not only affirms a State's rights over its own territory but also imposes a corollary duty: no State may use its territory, or permit its use, to carry out acts that infringe upon the rights of other States. This obligation is intrinsic to the very notion of sovereignty, for one State cannot invoke its sovereign rights to undermine the sovereignty of another. Sovereignty, therefore, entails a duty to ensure that the rights of other States—particularly their TI— are safeguarded within one's own jurisdiction [64].

The duty to respect a State's territorial sovereignty is not confined to its land territory alone. As the Court in *Military and Paramilitary Activities in and against Nicaragua* affirmed, "the principle of respect for State sovereignty"—enshrined in Article 2(1) of the UN Charter—entails an obligation on all States to "respect the territorial sovereignty of others" [65]. This obligation extends beyond land to encompass a State's "internal waters, territorial sea, and the airspace above its territory"[66].

However, Australia argued that TI, focused on preserving state sovereignty and borders, is unrelated to human rights obligations in addressing climate change, which are grounded in individual protections like the rights to life and health [67]. However, this view overlooks how transboundary climate impacts can directly threaten the TI of vulnerable states and the rights of their people. As affirmed in ICJ jurisprudence[68], no state may use its territory in a way that causes harm to others. Climate change does just that, undermining both TI and human rights, such as the rights to self-determination.

Thus, the two are inherently connected in today's global climate reality.

The right to TI is intrinsically linked to the right to self-determination, describing it as a "corollary" and a "key element in the exercise" of that right under international law [69]. It is through the peremptory norm of general international law [70] that people are entitled to freely determine their political states and pursue their economic, social and cultural development [71]. The right to self-determination is a fundamental human right enshrined in Article 1 common to the UN Charter, ICCPR [72] and the International Covenant on Economic, Social and Cultural Rights (ICESCR)[73].

Every State, under the principle of self-determination is obligated to refrain from 'any action which would dismember or impair, totally or in part, the territorial integrity or political unity of sovereign and independent States conducting themselves in compliance with the principle of equal rights and self-determination of peoples'[74].

In 2009, the United Nations Human Rights Council recognized the right to self-determination as one of the rights most vulnerable to the impacts of climate change. Unlike most individual human rights, self-determination—as enshrined in Common Article 1 of both the ICCPR and ICESCR— is a collective right. It imposes obligations not just on states towards individuals within their territory, but also their conduct toward other states[75], making it a right with corresponding inter-state duties. Additionally, the right under Common Article 1 of the Covenants holds a distinct position, preceding individual rights listed in Part II of both covenants. Unlike those individual rights, Article 1 is not constrained by the territorial limitations set out in Article 1. Instead, it applies beyond a State's own territory, without restriction. As the court has affirmed, the obligations under this provision extend to the actions of a State wherever it implements authority, emphasising its broader extraterritorial reach [76].

However, the U.S. disputed extending the right to self-determination to climate change, contending there is no historical foundation for observing GHG emissions as violating this right—especially not in relation to peoples outside a state's own borders [77]. However, the U.S. misses the legal recognition of this right. The Court has constantly declared that this right is an obligation *erga omnes* [78], and the International Law Commission has recognized its standing as a norm of *jus cogens* [79]. Accordingly, all States—despite of whether they are party to any particular treaty—are obliged to respect and guarantee the right, with no derogations allowed. That is so regardless of whether and when a State has signed and ratified any related human rights treaty.

Conclusion

In conclusion, while the Paris Agreement plays a crucial role in global climate governance, it cannot be regarded as the sole legal framework governing states' obligation in addressing climate change and fulfilling human rights obligations. GPIL imposes further duties such as due

diligence, the prevention principle and the obligation not to cause significant transboundary harm. Climate change creates direct threats to basic human rights such as the right to territorial integrity and to the right of self-determination for vulnerable communities whose livelihoods are threatened. This highlights the need for state action beyond the Paris Agreement. Depending exclusively on the Paris Agreement to fulfil climate-related human rights obligations would not only undermine legal accountability but also weaken well-established principles of international law. A thorough approach that incorporates GPIL, human rights law and environmental principles is necessary to safeguarding that states wholly encounter their obligations in tackling climate change and safeguarding fundamental human rights.

The Effect of Dominance Rank on Anemone Use in a High-Density Captive Population of the Common Clownfish Amphiprion ocellaris

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Meet the Author

Jonny Magnay graduated from the School of Biological Sciences in 2025 and has since begun a Postgraduate Research degree at the University of Glasgow. His research interests lie in applied marine ecology, with a focus on understanding how anthropogenic stressors influence ecological interactions, and on developing novel, evidence-based approaches to mitigate human impacts and support ecosystem restoration. Through his research, he aims to contribute to the conservation and recovery of marine habitats while advancing the understanding of our impacts on the natural world.

Keywords: *Amphiprion ocellaris*, clownfish, dominance hierarchy, resource use, animal welfare

Abstract

In socially hierarchical species, social dominance often mediates access to resources. Clownfish conform to this trend, with host anemones a contested resource. However, the persistence of this relationship in captivity remains unknown. Our study investigates the relationship between dominance status and anemone use in a high-density captive population of the common clownfish (*Amphiprion ocellaris*). Using continuous focal sampling, we recorded dyadic agonistic interactions and time spent in anemones during 20-minute periods, inferring a dominance hierarchy using David's scores. Opposing our predictions, high ranking individuals exhibited significantly lower anemone use than low ranking, but not medium ranking individuals. Potential explanations include trade-offs between dominance related aggression and resource use, and captive conditions making anemones act predominantly as refuges from conspecific aggression. Our findings suggest potential implications of housing captive *A. ocellaris* at high densities, informing improvements to welfare. Future research should repeat our methodology across low- and high-density captive populations to disentangle our explanations, using video recordings to avoid activity related confounds.

Introduction

Whilst clownfish social hierarchies and anemone symbioses have been widely explored, the relationship between dominance status and anemone use in captivity has received little attention. This remains true in the common clownfish (*Amphiprion ocellaris*), a small protandrous hermaphrodite [1] native to the Indo-Pacific Ocean [2] where it forms a symbiotic relationship with sea anemones [3], maintaining a strict size-based social hierarchy consisting of a dominant female, a breeding male, and up to 3 subordinates [1]. Clownfish subordinates queue for breeding positions [4], remaining sexually immature to prevent eviction from the host anemone by the dominant pair [5]. Avoiding eviction is crucial, as host anemones provide clownfish with services including protection from predators [6], access to food, external parasite removal [7], and egg protection [2]. In this way, anemones act as an essential resource, often coming under intense competition [8,9]. In most socially hierarchical species, access to such localised resources is mediated by social dominance, with higher-ranking individuals gaining priority access [10]. Although research into this association in clownfish is limited, current evidence suggests that they comply with this trend. For example, Chen and Hsieh [11] found dominance mediated shelter use in *A. ocellaris*, and Huebner et al.[12] showed that larger *Amphiprion bicinctus* monopolise species of anemone providing greater protection from predators13, restricting subordinates to less preferred species.

Despite the recent popularity of these species in aquaria [14], no studies have assessed the relationship between clownfish dominance status and anemone

use in captivity. Captive fish are often housed in confined conditions at unnaturally high densities [15], potentially increasing stress and disrupting ecological processes [16]. Understanding whether natural behaviours persist under such conditions is therefore essential for improving the management and welfare of captive clownfish. In this study, we investigate the association between dominance status and anemone use in a single high-density captive population of *A. ocellaris*, with a preliminary aim of inferring the dominance hierarchy. We hypothesise that the proportion of time spent in an anemone will increase with dominance rank. This prediction is based on the idea that anemones act as localised resources which will be controlled by more dominant individuals.

Methodology

(a) Experimental setup

This study received ethical approval from the UK Animal Welfare and Ethical Review Body (AWERB) under reference code UIN-24-069. Observations took place in a 690l display tank at Bristol Aquarium, England, 4 days per-week between the 7th October and 22nd November 2024, with a preceding pilot day. The tank housed 18 unrelated *A. ocellaris* (11 orange and 7 melanistic morphs) originating from captive breeding programmes and public donations, all acclimated for over a year, minimising the effects of observer presence. Semi-natural conditions were replicated using rocks, sand, 10-12 Entacmea quadricolor anemones which propagated during our study, and a mixed-species assemblage of fish and invertebrates (Appendix 1). Whilst *E. quadricolor* is not a natural host for *A. ocellaris* [17], this pairing is often used in behavioural studies (e.g. Wong et al. [18]), where symbioses form due to the absence of host anemones (but see Nguyen et al. [19]). Environmental conditions were maintained at 25°C, 89-96% dissolved oxygen content, and 35PPT salinity.

(b) Sampling focal individuals

Prior to beginning data collection, we identified all observable clownfish and assigned each a unique identifier based on individual stripe patterns, excluding an individual observed only 9 times and first identified after 2 weeks of sampling. Each day, 7 focal individuals were randomly selected from the total (with replacement) using the app 'Random Number Generator' by UXPAPPS-LTD [20], controlling for sampling bias. A pilot day ensured competency with fish identification and sampling methods.

(c) Recording behavioural parameters

Observations took place between 10am-2pm to control for temporal variation in activity, a trait observed in *A. ocellaris* [17], with start times varied between 10-10.30am to minimise confounding effects of the aquarium's routine. Although controlled for through random sampling, observations were paused for 5 minutes during feeding and cleaning events to minimize disturbance. To assess anemone use and infer a dominance hierarchy, we recorded 2 behavioural parameters simultaneously for each focal individual: (i) The duration of each contact with an anemone, excluding contact under 3 seconds due to observational constraints.

'Out-of-sight' conditions were accounted for as all anemones remained in view for the study duration; (ii) The outcome (winner and loser) and partner ID of dyadic agonistic interactions, using an ethogram developed from preliminary observations and published literature (Table 1). Agonistic interactions were only recorded when an aggressive or threatening provocation elicited a submissive response [11], to avoid misidentification of conflict outcomes and agonistic partners. Whilst some studies consider clownfish 'shaking' behaviours a submissive response11, there is ambiguity regarding their role as a dominant or submissive behaviour [2], so these were excluded from analysis.

After randomly allocating investigators to each parameter to control for investigator bias, we utilized continuous focal sampling by direct observation to measure both parameters during 20-minute observation periods, facilitating detailed recordings beyond that achievable using less targeted methods such as scan sampling. Whilst video recordings are often preferred as they allow for longer sampling periods and more detailed behavioural assessments [22,23], the public setting of our study rendered direct observations a more suitable alternative. Inter-observer reliability was assessed using joint observations of both parameters. To remedy the lack of consideration for chance agreements in percentage agreement24, we instead used the R package "psych" [25] to apply an unweighted Cohen's kappa [26] to the nominal dominance parameter (k=0.97, 95% CI[0.92-1], n=68), and an intra-class correlation coefficient ICC(2,1) to the continuous anemone use parameter (ICC=1, 95% CI[1-1], n=97), both indicating excellent agreement.

(d) Statistical analyses

Statistical analyses were performed using R (v4.2.1). To infer a potential dominance hierarchy, we calculated normalised David's scores (normDS) using the package "EloRating" [27], using the Dij method (de Vries' correction [28]) to account for imbalances in dyadic interaction frequencies, controlling for the influence of spatial proximity and behavioural variation. NormDS were chosen because they consider an individual's success over all dyadic interactions and have been widely used in social animals [29,30]. We subsequently assessed hierarchy steepness (strength) by calculating the slope of a regression line fitted to a plot of normDS as a function of rank, as proposed by de Vries, Stevens and Vervaecke [31]. Additionally, we assessed hierarchy linearity by using the "aniDom" package [32] to calculate triangle transitivity (Ttri), the most reliable method when not all dyadic outcomes are known [33], suiting our data. As suggested by Sánchez-Tójar, Schroeder and Farine [34], we assessed hierarchy uncertainty and resolution by calculating Spearman's rank correlations between 2 halves of the dataset using the package 'aniDom' [32], and the ratio of interactions to individuals sampled, with rs=0.5 and 10-20 interactions/individual the recommended minimum for each respective metric.

To test the relationship between dominance rank and anemone use, the

Category	Behaviour	Description
Aggressive	Chasing	Aggressor rapidly approaches recipient for > 1 body length
	Lunging	Aggressor rapidly approaches recipient for < 1 body length
	Biting	Aggressor bites recipient
Threatening	Face-to-face swimming	Aggressor and recipient swim synchronously face-to-face at < 1 body length
	Side-by-side swimming	Aggressor and recipient swim synchronously side-by-side at < 1 body length
Submissive	Fleeing	Recipient rapidly retreats from aggressor for > 1 body length
	Flinching	Recipient rapidly retreats from aggressor for < 1 body length

Table 1: Ethogram of agonistic and submissive behaviours of the common clownfish *Amphiprion ocellaris*. Adapted from Iwata and Manbo [21], Chen and Hsieh [11], and preliminary observations. A focal individual may act as either the aggressor or recipient across interactions.

range of normDS values was divided into low, medium, and high categories of equal width, and individuals were assigned a category according to their normDS score. The percentage time spent in an anemone was then calculated for each 20-minute sample. Due to substantial variation in our data, we used non-parametric tests to reduce the impact of outliers, testing for differences in median anemone use between the dominance categories using a 2-tailed Kruskal-Wallis test with a 5% significance level, followed by pairwise comparisons using Wilcox rank sum test with a Benjamini-Hochberg correction for multiple testing.

Results

Over 21 days of experimentation, we collected 136 data points for the anemone use parameter and recorded 1386 dyadic Each fish was randomly sampled between 6 and 11 times.

(a) Social dominance hierarchy

NormDS ranged between 4.18-12.22 and were different for all individuals, revealing the presence of a dominance hierarchy (Figure 1). The hierarchy was only of low to moderate steepness (0.39; Figure 1) but had a high transitivity which was significantly different from random (Ttri=0.88, $p<0.001$). Uncertainty was low (rs=0.80, 95% CI[0.63-0.93]), indicating high reliability, and a high ratio of 81.53 interactions to individuals sampled showed sufficient sampling effort. Low ranks received the most aggression per individual (195 instances) followed by high (44) medium (29) ranks.

(b) Anemone use

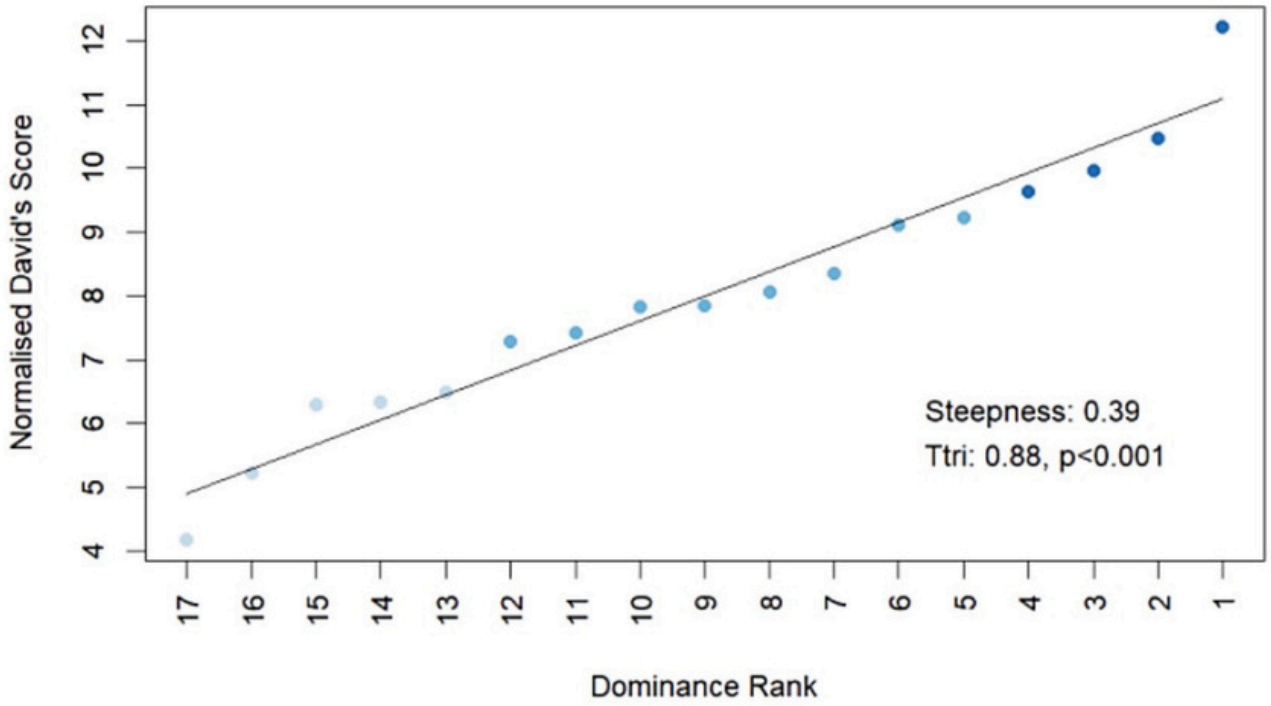


Figure 1: Normalised David's scores and associated dominance rank, showing overall steepness and linearity. Nodes are coloured according to dominance category (low, medium, high), and a regression line y = 0.39 x + 4.51 depicts overall hierarchy steepness.

The population had a low median anemone use ($\tilde{x}$ =16.33%) with moderate variation (IQR[3.10-32.69]), and 8.09% of data falling as statistical outliers. The low, medium, and high ranks contained 5, 8, and 4 individuals respectively. Low ranking fish had the highest median and largest variability in anemone use ($\tilde{x}$ =27.17%, IQR[17.73- 62.33]) followed by the medium ($\tilde{x}$ =12.29%, IQR[1.06-33.29]) and high ($\tilde{x}$ =8.38%, IQR[1.83- 18.31]) ranks. We found a significant difference in median anemone use between the dominance categories ($\chi 2$ =20.02, df=2, $p<0.0001$; Figure 2), with pairwise comparisons revealing significantly higher anemone use in the low rank than the medium or high rank (Pairwise; low vs medium: $p<0.01$; low vs high: $p<0.0001$; Figure 2). However, no significant difference was found between medium and high ranks (Pairwise; medium vs high: p =0.230; Figure 2).

Discussion

To explore how anemone use may be influenced by clownfish dominance status in a high-density captive population, we inferred a dominance hierarchy and tested for differences in anemone use between low, medium, and high-ranking individuals, predicting a positive association due to greater social dominance granting priority access to these localised resources.

(a) Dominance hierarchy

The distinct David's scores and high linearity of our study population imply a single linear dominance hierarchy across all 17 individuals (Figure 1), with observations suggesting it largely follows the natural size-based structure [35], although future research could test this experimentally by measuring clownfish size. However, it was larger and

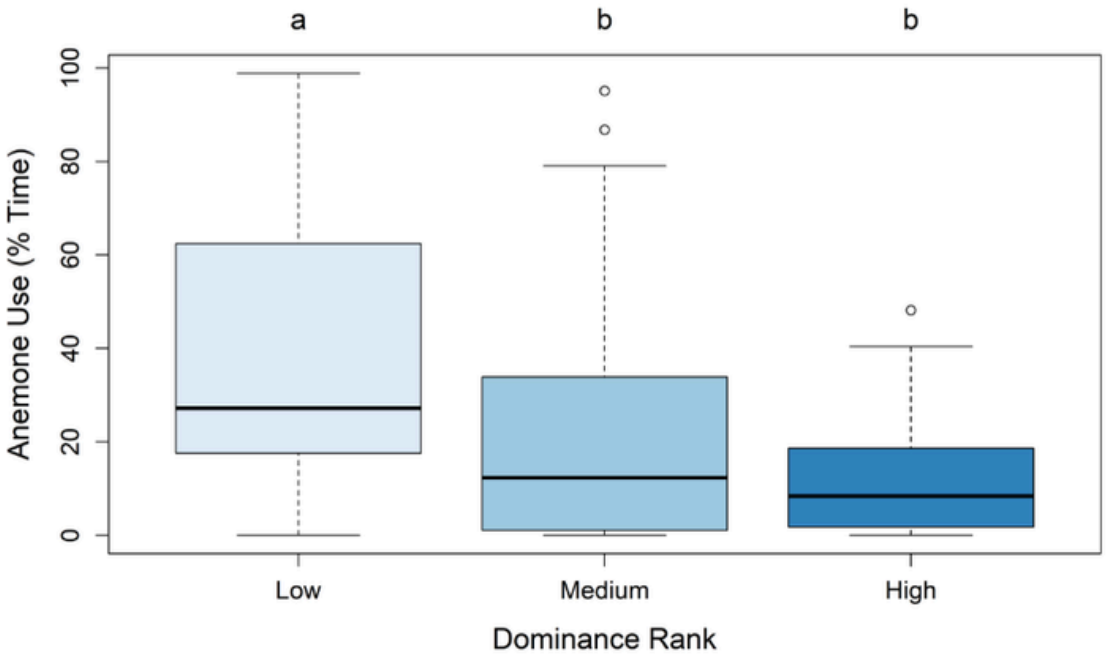


Figure 2: Percentage of time spent in an anemone by clownfish of different dominance ranks. Different letters above boxes denote significant differences between groups ($p < 0.05$), whereas shared letters indicate no significant difference.

moderately weaker than wild clownfish hierarchies [1,35], likely due to the high-density captive environment. In the wild, clownfish reject arrivals when their anemone reaches capacity [36], regulating group size and maintaining steep, size-based distances between ranks which reduce conflict [35]. In captivity, new arrivals cannot disperse, increasing population density and potentially reducing inter-rank steepness. Supporting this, we observed a high frequency of agonistic interactions, a trait often used to identify weak hierarchies [22], although this may instead be explained by greater encounter frequencies in larger populations [37].

We controlled for variation in dyadic interaction frequency by incorporating de Vries' correction in our David's score calculation, but technical limitations prevented us from accounting for acoustic dominance signals, an important regulator of clownfish hierarchies [38]. Despite this, our uncertainty and resolution metrics confirm that our methods were sufficient to replicate the present hierarchy, making it a suitable foundation for investigating the effects of dominance status on anemone use in our population.

(b) Anemone use

Opposing our predictions, low ranking individuals showed significantly higher anemone use than both medium and high ranks (Figure 2). This contrasts with previous research finding that high dominance grants priority resource use in *A. ocellaris* [11] as well as a range of other socially hierarchical species (crayfish [39], chickens [40], primates [10,41]. There is little research agreeing with our findings; although interestingly, our results align with a study of crayfish, which, using a larger population than Martin and

Moore [39], observed higher shelter-use in less dominant individuals[42].

By comparing these studies, we can infer a potential explanation for our results; group-size dependent trade-offs between dominance-related aggression and resource use. Such trade-offs have been found in *A. ocellaris* [43], where in small groups, high ranking individuals expend little effort reinforcing their dominance, allowing them to dominate localised resources, as observed by Chen and Hsieh [11]. In larger groups, however, greater competition may lead high ranking individuals to perform higher frequencies of dominance-reinforcing aggression [42], trading-off resource use, and leaving low-ranks better able to exploit resources, potentially contributing to the high anemone use among low-ranking individuals in our study. Opposing this idea, a study of *Amphiprion clarkii* found no link between group size and average aggression rates [37]. However, they did not consider rank-specific changes in aggression, limiting the applicability of their results.

Another possible explanation for our findings is the removal of ecological pressures including predation risk and food scarcity in captivity, which may reduce predator avoidance and competitive behaviours [44] and minimise the reliance of clownfish on their host anemone's ecological services [7,9]. This effect might be enhanced by our use of the non-host anemone *E. quadricolor* which can offer lower fitness benefits than natural hosts [17]. Under these reduced ecological pressures, anemones may instead act predominantly as refuges from conspecific aggression, similar to shelter-use in other captive organisms [45]. Our results support these ideas, as low-ranking individuals both received the most aggression and spent the most time in anemones, and medium and high ranks were similarly low in both measures (Figure 2). To disentangle this explanation from that regarding trade-offs between aggression and resource use, our methodology should be replicated using both low- and high-density captive *A. ocellaris* populations under the same conditions. If our findings are primarily driven by group-size-dependent trade-offs, we would expect dominant individuals to exhibit the greatest anemone use in small groups, whereas larger groups should replicate our observations. Due to our use of a single clownfish population, caution is needed when extrapolating these results to other captive populations of *A. ocellaris*. However, as a general recommendation, aquaria should ensure clownfish are housed at low population densities with sufficient shelter, preferably in the form of natural host anemone species. This should minimise aggression and reinforce natural behaviours, a key aspect of maintaining welfare [46].

Although our data exhibited substantial variation, the presence of outliers did not affect the significance of our results, reinforcing the robustness of our statistical methodology. Despite this, these outliers remain responsible for the greater variation observed in low-ranking fish, hence ecological explanations for this trend are not discussed. Both sampling and investigator bias were controlled for,

as were factors relating to fluctuating environmental conditions, spatial and temporal variation, observer presence, and the aquarium's routine. Whilst Hussey and LaPlante [43] reported differences in aggression-maintenance trade-offs between orange and melanistic *A. ocellaris* morphs, their small sample size (n=3) introduces confounds of individual variation in behaviour, a trait present in A. ocellaris and related species [18,47], so our use of both morphs is unlikely to detract from our results. However, our use of a 3 second threshold for recording anemone use due to observational constraints may have underestimated the anemone use of highly active individuals. If activity increases with dominance rank in *A. ocellaris*, as observed in laboratory mice [48], this may prove confounding. Videorecording-based observations are recommended for future studies to minimise these confounds.

Conclusion

Our study explored the relationship between dominance status and anemone use in captive *A. ocellaris*. We inferred a single linear dominance hierarchy, allowing assessments of dominance-mediated anemone use. Contrary to our predictions, high ranking individuals spent significantly less time in anemones than low ranking, but not medium ranking individuals. This may reflect group-size-dependent trade-offs between dominance-related aggression and resource use, as well as reduced ecological benefits provided by anemones in captivity where they might instead act as refuges from conspecific aggression. These findings suggest potential implications of housing *A. ocellaris* under high-density captive conditions, hence we recommend aquaria maintain low population densities with many host anemones to encourage natural behaviours and improve welfare. Our study serves as a foundation for research seeking to better understand how clownfish dominance status interacts with other behavioural traits in captive environments. Future research should repeat our methodology across multiple low- and high-density captive A. ocellaris populations to disentangle our explanations, using video recordings to avoid the activity-related confounds which may limit our study.

Numerical modelling of Fresnel and Fraunhofer diffraction using Gaussian quadrature and Monte Carlo integration methods.

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Keywords: orbital mechanics, numerical simulation, RK45 method, three-body problem, energy conservation.

Abstract

This study presents a numerical simulation of circular and elliptical orbital motion in the Earth-Moon and Earth-Moon-probe systems. The model is based on Newtonian mechanics and uses the RK45 method to solve the resulting system of first-order differential equations. The simulations are evaluated using Keplerian orbital behaviour and the principle of energy conservation to assess numerical accuracy. Relative total energy conservation errors of order for the Earth-Moon system and between and for the Earth-Moon-probe system indicate strong numerical stability within the simplified model where only the gravitational field of the Earth, Moon and probe are considered. These results support the suitability of RK45 for approximating orbital trajectories in idealised gravitational systems, while highlighting the need for further benchmarking against real astrodynamical data.

Introduction

The rapid advancement of computational methods and numerical analysis has provided state-of-the-art approach to classical astrodynamical problems.

Numerical simulation is an important tool in orbital mechanics because many gravitational systems cannot be fully solved using analytical methods. While ideal two-body orbital motion can be understood through Newton's laws of gravitation and Keplerian orbits, more complex systems involving additional bodies often require numerical methods to approximate their motion [1]. The use of numerical methods is particularly relevant in astrodynamics because they allow orbital stability and spacecraft motion to be studied as initial value problems.

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Here, this system is studied with simplifications of the complexity of the real-world physics, by ignoring spatial complexity and perturbations from other planetary bodies. The current study intentionally adopts a simplified Newtonian model in order to prioritise reproducibility and accessibility within an undergraduate computational physics context.

In this study, the application of the adaptive Runge-Kutta (RK45) numerical method [2] in simulating the Earth-Moon and the Earth-Moon-probe system is investigated. Both circular and elliptical orbital simulations are considered,

allowing the results to be compared with expected Keplerian behaviour.
The primary objective of this study is to rigorously evaluate the accuracy of an accessible RK45 simulation in reproducing orbital motion features under designated physical assumptions. The accuracy of the model is assessed using the conservation of total mechanical energy. The paper demonstrates both the usefulness and limitations of RK45 integration for simplified orbital modelling, with potential exploration of machine-learning-assisted optimisation modelling techniques for trajectory analysis while remaining ground in established physical simulations.

Methodology

Theoretical framework

The simulation is based on Newtonian gravity, which provides an appropriate approximation for the studied systems at the level of accuracy considered in this project. Physical assumptions considered in the simulations include, relativistic effects are neglected because orbital velocities are small compared with speed of light. This is because the purpose is to study the numerical behaviour of a simplified orbital motion. Additionally, all bodies are treated as physical point masses, thus the gravitational interaction is independent of the shape or the volume of the masses and depends only on their masses and relative positions.
Orbital motions are governed by Newton's law of gravitation. For two-point masses A and B that are separated by a distance are subject to a force that attracts each other which is proportional to the inverse square of , i.e. $\frac{1}{r^2}$ [3].

here is the wavelength, is the wavenumber, is the distance between the aperture and the screen and the integral is limited to the dimensions of the aperture at which diffraction occurs.

While Fresnel diffraction happens at a finite distance to the screen (near-field), Fraunhofer diffraction happens at an infinite (large enough) distance to the screen (far-field). Therefore, Fresnel and Fraunhofer diffraction share the same physical nature, but operate under different regimes dictated by the combination of propagation distance and aperture dimensions.

Since the integrand in equation (1) is complex, the numerical calculation requires the real and imaginary parts to be evaluated separately and the equation to compute becomes:

$$F_{AB} = \mathrm{G}\frac{M_A M_B}{r^2} \qquad (1)$$

where G = $6.674 m^3 kg^{-1} s^{-2}$ is the gravitational constant and M_A and M_B are the masses of A and B.

To use this equation in the simulation, it is required to combine it with Newton's second law and break it down into cartesian components to find the differential form of the equations of motion. The position *r* , velocity *v* and acceleration *a* are related by derivatives and antiderivatives, thus the following expressions for an Earth-Moon system is shown as follows [4]:

$$\dot{r_x} = v_x; \qquad \dot{r_y} = v_y$$

$$\ddot{r_x} = \dot{v_x} = -\frac{MGr_x}{\left(r_x^2 + r_y^2\right)^{3/2}}; \tag{2}$$

$$\ddot{r_y} = \dot{v_y} = -\frac{MGr_y}{\left(r_x^2 + r_y^2\right)^{3/2}}$$

For the Earth-Moon-probe system, the probe is affected by the gravitational fields of both Earth and Moon and can be obtained by applying the superposition principle [4].

$$\dot{r_x} = v_x; \qquad \dot{r_y} = v_y$$

$$\ddot{r_x} = \dot{v_x} = -\frac{MGr_x}{\left(r_x^2 + r_y^2\right)^{3/2}} - \frac{M'Gr_x'}{\left(r'^2_x + r'^2_y\right)^{3/2}}; \tag{3}$$

$$\ddot{r_y} = \dot{v_y} = -\frac{MGr_y}{\left(r_x^2 + r_y^2\right)^{3/2}} - \frac{M'Gr_y'}{\left(r'^2_x + r'^2_y\right)^{3/2}}$$

The Moon is still modelled as moving under Earth's gravitational field, while the gravitational effect of the probe on the Earth and Moon is neglected because the probe mass is much smaller than the other masses. This reduces the complexity of the model.

Orbits are generated when the forces on the body that is orbiting around the centre mass are in equilibrium such that either the circular or elliptical motion is maintained [3]. From this principle the velocity at which a body of mass *M* has to orbit for a known distance *r* can be found.

$$v_{orbit} = \sqrt{\frac{GM}{r}} \tag{4}$$

The above equation models a circular orbit, while for an elliptical orbit, the orbital velocity depends on the magnitude of the semi-major axis *a* which is half the longest diameter and the eccentricity *e* which is an adimensional quantity that measures the deviation of the orbit from circularity [5]. The elliptical orbit is not uniform as the circular orbit, therefore the velocity and the distance travelled by the mass is neither uniform nor the orbital velocity is given as follows:

$$v_{orbit} = \sqrt{GM\left(\frac{2}{r} - \frac{1}{a}\right)} \tag{5}$$

The principle of the conservation of energy serves as a test for evaluating the numerical accuracy of the simulation. The principle states that if no external work is done on the system and no energy is dissipated through non-conservative interactions, then the sum of the kinetic energy E_K and the potential energy E_P is the total mechanical energy E_T and this is constant [6].

For the Earth-Moon system, the total energy is:

$$E_T = E_K + E_P = \frac{1}{2}mv^2 - \frac{GMm}{r} \tag{6}$$

For the Earth-Moon-probe system, the total energy is:

$$E_T = \frac{1}{2}M_m v_m^2 + \frac{1}{2}M_p v_p^2 - \frac{GM_eM_m}{r_m} - \frac{GM_eM_p}{r_p} - \frac{GM_mM_p}{r_{pm}} \tag{7}$$

In an ideal conservative system, these total energies should remain constant. Therefore, fluctuations in total energy over the simulation provide a measure of numerical error and indicate the stability of the RK45 method.

Magnitude	Symbol	Value
Gravitational constant	G	$6.674 \times 10^{-11} \mathrm{m^3\ kg^{-1}\ s^{-2}}$
Mass of Earth	M_e	5.972×10^{24} kg
Mass of Moon	M_m	7.348×10^{22} kg
Mass of probe	M_p	9.00×10^{2} kg
Earth-Moon distance	d_{Earth_Moon}	3.844×10^{8} m
Velocity of Moon	v_{0_Moon}	1.018×10^{3} m s^{-1}
Perigee distance	$d_{perigee}$	3.564×10^{8} m
Apogee distance	d_{apogee}	4.067×10^{8} m
Semi-major axis	$semimajor_axis$	3.815×10^{8} m

TABLE 1: Fundamental physical constants with their values used in the simulation. The gravitational constant is taken from [7] and lunar quantities are taken from [8].

Simulation setup

The simulation was designed to model two simplified gravitational systems, the Earth-Moon system and the Earth-Moon-probe system. The equations of motion were formulated from equations (2) & (3). The initial velocities were calculated using equations (4) & (5).

The physical constants and parameters used in the simulation are the gravitational constant, the masses of Earth, Moon and probe, the mean Earth-Moon distance and the perigee and apogee distances used for the elliptical orbit. These values are kept constant throughout the simulations.

For the Earth-Moon system, the initial position of the Moon was set along the positive *x*-axis, with its initial velocity in the positive *y*-direction. For the Earth-Moon-probe system, the Moon was initialised in the same way, while the probe was placed at an initial distance from the Moon specified by the user. Its initial velocity with respect to the stationary Earth was then automatically calculated based on the specified value.

To employ the RK45 method, differential equations of the system are gathered in a function and implemented through "solve_ivp" from Python's "SciPy" library. For the Earth-Moon system, the state vector contained the Moon's two position components and two velocity components. For the Earth-Moon-probe system, the state vector was extended to include the position and velocity components of both the Moon and the probe.

The logic of "solve_ivp" is based on computing numerical integration of the ODE system with the RK45 with steps taken using the fifth-order accurate formula to control the error of every iteration [2]. This makes RK45 suitable for the present project because the solution may vary over the trajectory of the masses with

System	Orbit type	Simulation time (s)	Initial Moon-probe distance (m)
Earth-Moon	Circular	2.5×10^6	Not applicable
	Elliptical	2.5×10^6	Not applicable
Earth-Moon-probe	Circular	2.5×10^6	5.0×10^6
	Elliptical	2.5×10^6	5.0×10^6
	Circular (detailed)	1.0×10^6	5.0×10^6
	Elliptical (detailed)	1.0×10^6	5.0×10^6

TABLE 2. Simulation parameters used to generate the trajectory and energy figures for the Earth-Moon and Earth-Moon-probe systems. General view simulations modelled one lunar orbital period, while detailed view simulations modelled a part of one period.

respect to the time variable evenly spaced over the chosen simulation intervals, which allows the orbital trajectories and energy evolution to be plotted consistently.
Additionally, the numerical tolerances with absolute tolerance given by *atol* = 10^{-12} and relative tolerance *rtol* = 10^{-9} for the Earth-Moon system, while for the Earth-Moon-probe system, tolerances were chosen as *atol* = 10^{-10} and *rtol* = 10^{-8}. Tighter tolerances generally improve numerical accuracy but increase computational cost and thus, balance is required.

The total energy of the systems was calculated in accordance with the energy equations presented for diagnostic purposes of the numerical accuracy of the method.

Then the absolute energy error was calculated as the difference between the maximum and minimum total energy over the simulation interval,

$$\delta E = |E_{max} - E_{min}| \quad (8)$$

The relative error was calculated using the initial total energy as the reference value.

$$\epsilon = \left|\frac{E_{max} - E_{min}}{E_0}\right| \quad (9)$$

This quantity was used as the main magnitude for evaluation because absolute error may become very large for astronomical systems.
The full Python implementation, including function definitions, plotting scripts, parameter definitions and figure generation code, is available in the accompanying GitHub repository [9].

Results

The parameters used to generate the results presented in this work are displayed in Table 2.

Earth-Moon system simulations

The first simulation modelled the orbit of Moon around the Earth at a fixed point under Newtonian mechanics.

For the circular case, the Moon followed a stable orbit with constant radius over the chosen simulation interval, which is consistent with the initial condition where the Moon was given the circular orbital velocity corresponding to the Earth-Moon distance.

The energy plot showed that the kinetic, potential and total mechanical energies remained approximately constant throughout the simulation.

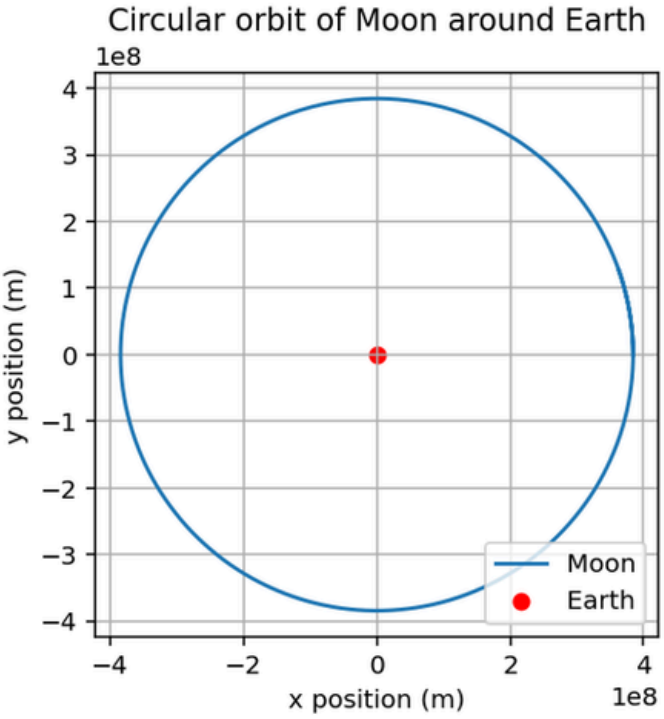


FIG 1. This figure illustrates the circular orbit of the Moon around Earth under the effects of the Earth's gravitational field, where the shape of the motion describes a circle.

The simulated elliptical trajectory showed a varying Earth-Moon separation, with varying kinetic and potential energy and approximately constant total energy.

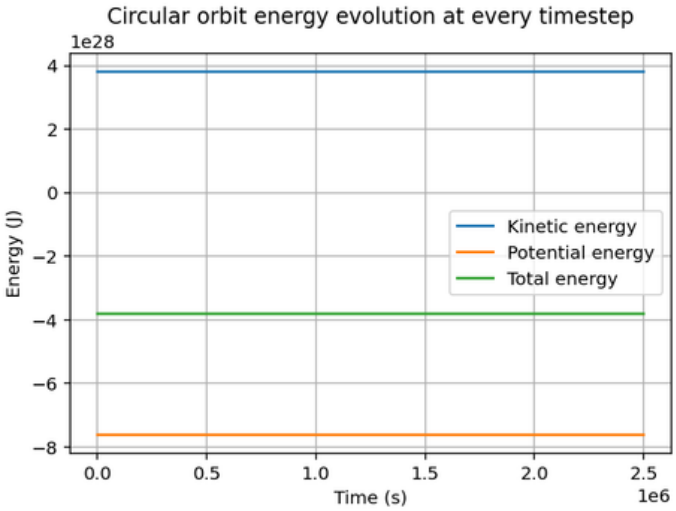


FIG 2. This figure illustrates the energy evolution of the Earth-Moon system for a circular simulation; the three energies show approximately constant values throughout the domain.

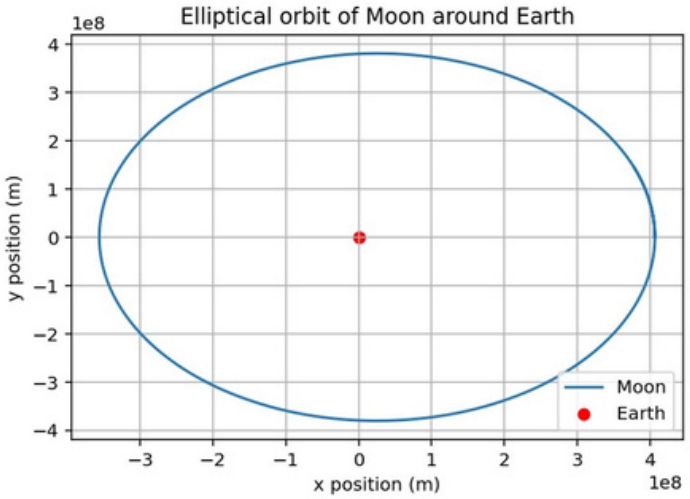


FIG 3. This figure illustrates the elliptical orbit of the Moon around Earth under the effects of the Earth's gravitational field, where the shape of the motion describes an ellipse.

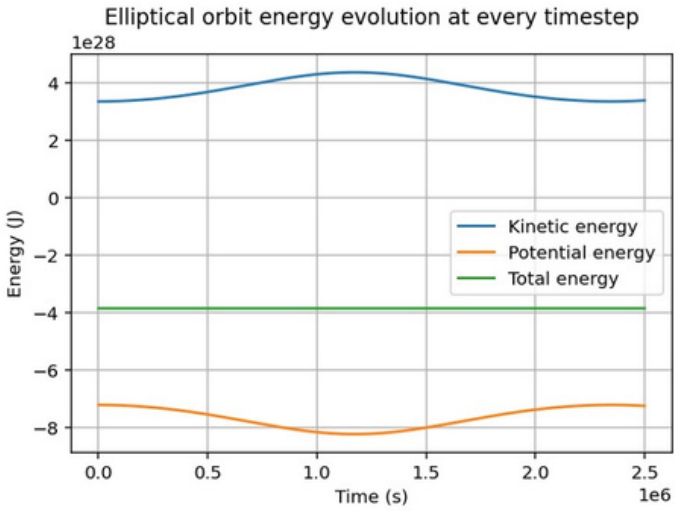


FIG 4. This figure illustrates the energy evolution of the Earth-Moon system for an elliptical simulation; kinetic and potential energies show maxima and minima approximately at half-time simulation and total energy is approximately constant.

Earth-Moon-probe system simulations

The second simulation modelled the orbit of a probe orbiting around the non-stationary Moon with respect to the stationary Earth. The trajectory plots showed that the probe's motion was influenced by both the Moon and Earth.

In the elliptical orbital simulation, since Moon is moving with respect to Earth, the probe is therefore affected by the gravitational field of both Earth and Moon. The kinetic and potential energy of the system displayed a similar change compared to the elliptical case of the Earth-Moon system, while total energy remained approximately constant.

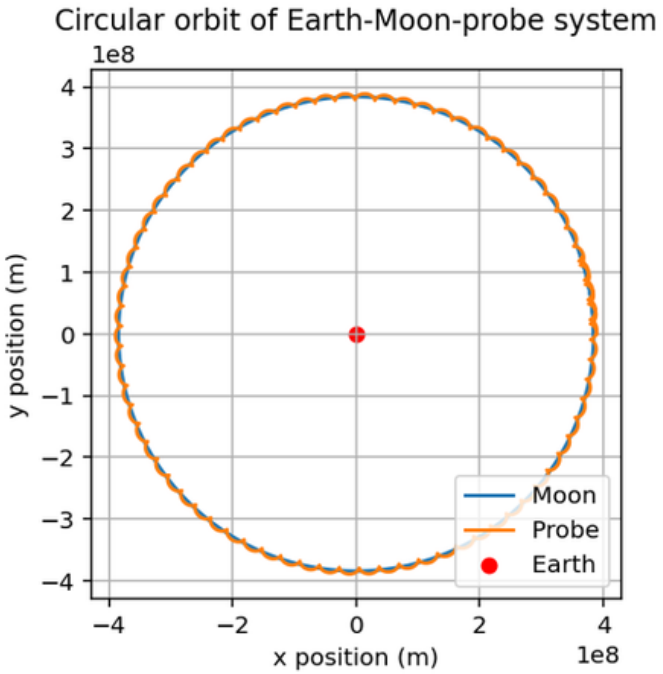


FIG 5. This figure illustrates the circular orbit of the Earth-Moon-probe system under the effects of the Earth's and Moon's gravitational field, where the shape of the motion describes a circle with local oscillatory motion.

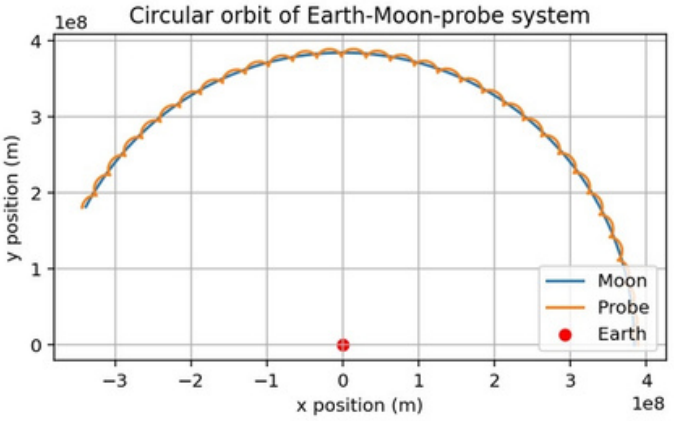


FIG 6. This figure illustrates a detailed view of the circular orbit of the Earth-Moon-probe system under the effects of the Earth's and Moon's gravitational field, where the shape of the motion describes a circle with local oscillatory motion.

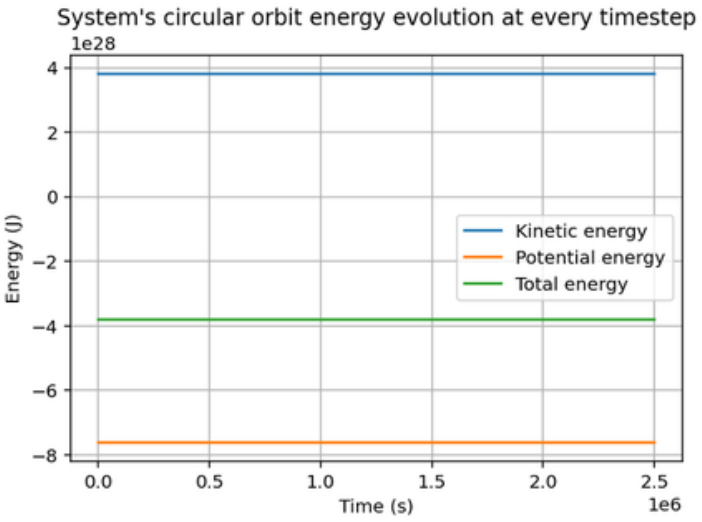


FIG 7. This figure illustrates the energy evolution of the Earth-Moon-probe system for a circular simulation; the three energies show approximately constant values throughout the domain.

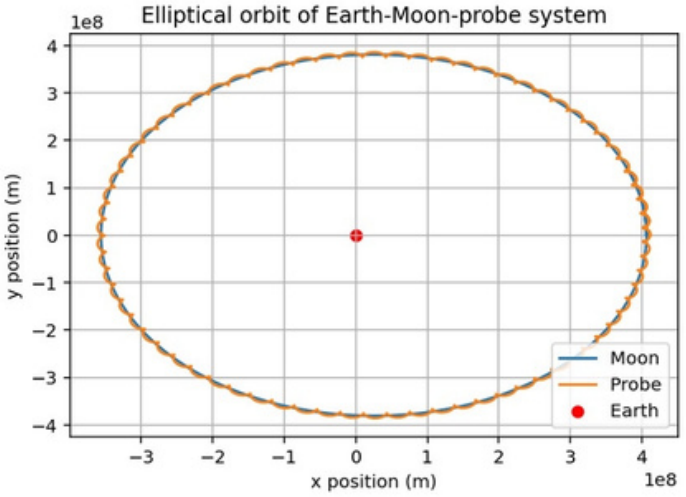


FIG 8. This figure illustrates a general overview of elliptical orbit of the Earth-Moon-probe system under the effects of the Earth's and Moon's gravitational field; the shape of the motion describes an ellipse with local oscillations.

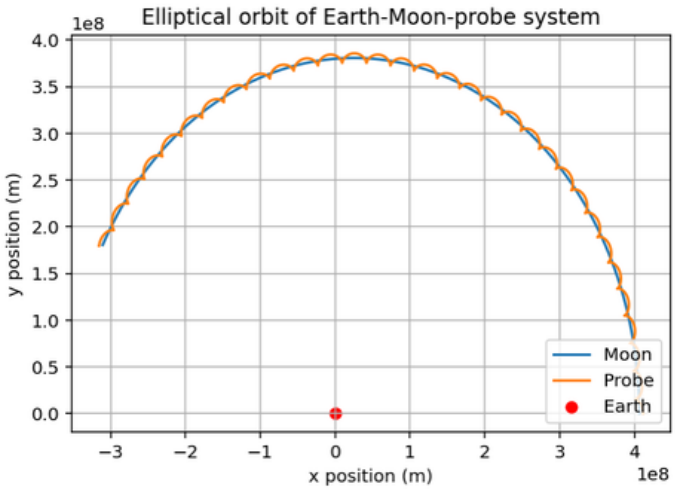


FIG 9. This figure illustrates the elliptical orbit of the Earth-Moon-probe system under the effects of the Earth's and Moon's gravitational field, where the shape of the motion describes an ellipse with local oscillations.

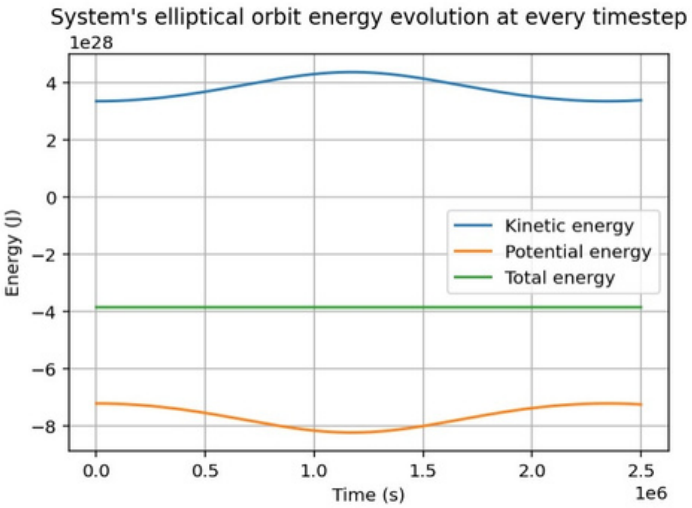


FIG 10. This figure illustrates the energy evolution of the Earth-Moon-probe system for an elliptical simulation; the kinetic and potential energies show maxima and minima approximately at half-time simulation and total energy is approximately constant.

System	Orbit type	Absolute error	Relative error
Earth-Moon	Circular	6.786×10^{19}	1.781×10^{-9}
	Elliptical	6.707×10^{19}	1.748×10^{-9}
Earth-Moon-probe	Circular	7.037×10^{13}	2.536×10^{-23}
	Elliptical	4.442×10^{14}	3.246×10^{-21}
	Circular (detailed)	8.796×10^{13}	2.242×10^{-23}
	Elliptical (detailed)	1.451×10^{13}	3.029×10^{-21}

TABLE 3. Absolute and relative total mechanical energy errors of Earth-Moon and Earth-Moon-probe systems

Absolute and relative errors

By diagnosing energy conservation using the absolute and relative errors, the numerical stability and error of the RK45 method can be evaluated. Table 3 shows the values calculated.

Discussion

The present project is a computational modelling study in the Newtonian mechanics and the celestial bodies using the numerical method of RK45 to estimate solutions to first order ODEs systems of Earth-Moon orbit and Earth-Moon-probe orbit. The project combines physical theory such as Kepler's laws and Newton's gravitational law as the theoretical foundation and numerical analysis to provide an insightful visualisation of the physical laws into the real world.

In the Earth-Moon simulations, a visualisation of a two-body system with both circular and elliptical orbits is shown in FIG 1 and FIG 3. The simulations show that RK45 preserves the expected qualitative behaviour of circular and elliptical Keplerian orbital motion while maintaining small total energy conservation errors under the chosen tolerance settings. From the energy graphs, it is observed that FIG 2 presents a straight line for all three energy lines within the given time domain.

Therefore, equation (6) implies that the relationship between energies and mechanical states are as follows:

$$E_K \propto v^2; \qquad E_P \propto -\frac{1}{r} \tag{10}$$

Hence, these results indicate that the velocity and distance of the Moon with respect to the Earth remain approximately constant in accordance with equation (10). Likewise, in the elliptical orbit FIG 3 describes an ellipse and FIG 4 presents some variations in kinetic and potential energy, but no big fluctuations are observed in the total energy within the domain. FIG 4 presents a maximum in kinetic energy when simulation is at approximately $t_{simulation}/2$ and the potential presents a minimum at the same time. This point is found when the position of the Moon is zero again in FIG 3, which is the perigee, these phenomena are in accordance with Kepler's laws describing the elliptical orbit.

Therefore, it is observed that the energies in both orbits obey the energy conservation in equation (6) and the results match the physical principles of circular orbits, where the Moon should orbit at constant velocity and distance around the Earth and the elliptical orbit, where the velocity reaches the maximum at the perigee and minimum at the apogee.

In contrast, the results generated in the Earth-Moon-probe simulations, rather obey the energy conservation law stated in equation (7). The probe alone is not an isolated two-body system because it moves in the gravitational field of the Earth-Moon system. Therefore, the total Earth-Moon-probe energy is the appropriate conservation law.

The absolute error obtained ranged from orders of 10^{13} *J* to 10^{19} *J*, which is due to the astrodynamical scale of the problem studied. These errors are not visually apparent in the energy plots because the absolute energy scale is very large. For this reason, the relative error is the more meaningful diagnostic for numerical conservation.

The relative error for both simulations is shown in TABLE 3, have order of magnitudes of 10^{-9} and 10^{-21} ~ 10^{-23} for Earth-Moon and Earth-Moon-probe systems, respectively. These results indicate strong internal numerical consistency for the simplified Newtonian systems studied and the conservation of total system energy suggests that the RK45 method remained stable for both cases.

The simulated trajectories were also consistent with the expected qualitative behaviour predicted by Kepler's laws. In circular orbits, the radius remained approximately constant, whereas the elliptical simulations showed periodic variation in orbital distance and velocity between perigee and apogee, in agreement with equation (5).

However, it is important to note that small numerical errors should not be interpreted as evidence that the simulation reproduces the real Earth-Moon system with the same level of accuracy, as the model contains several simplifying assumptions. For example, Earth was stationary rather than orbiting around Sun. Thus, external perturbations from Sun's and other planets' gravitational field were neglected. These simplifications reduce the physical realism but make the system computationally easier to analyse. In order to reflect the reality, all factors mentioned above have to be considered in the simulation and energy conservation laws also become more complex.

Compared with professional astrodynamical simulations, the present model is intentionally simplified. For high-fidelity trajectory prediction, professional astrodynamical simulations often use ephemeris data, adaptive multi-body integrators and perturbative corrections [10].

The Earth-Moon-probe system studied here is closely related to the restricted three-body problem, where the probe has negligible mass compared with Earth and Moon and its gravitational influence can

be ignored. Numerical approaches are necessary because the problem does not admit simple closed-form analytical solutions [1].

However, many high-fidelity approaches are designed for long-term applications, mission analysis or specialised stability analysis. Therefore, the present study addresses a different problem: it provides an accessible and reproducible RK45-based Python model that connects fundamental physical laws with orbital mechanics.

Hence, results suggest that the RK45 method is a suitable and numerically stable method to simulate lunar orbits and three-body systems. However, the is tolerance-dependent which means that the execution time of the programme depends on the tolerance chosen. Therefore, suitable tolerance values must be chosen to balance numerical accuracy and computational cost [11].

In this specific project, the tolerance values have been chosen to ensure high quality in the results obtained, but such accuracy is probably not needed [12], and how to determine the optimal balance between accuracy and computational performance can be further studied. Future work could investigate whether machine-learning-assisted models or optimisation algorithms can reduce the cost of repeated trajectory evaluations [13].

Machine-learning-assisted optimisation algorithms could potentially reduce the computational cost of repeated trajectory evaluations, subject to careful validation against established physics-based numerical simulations.

Finally, this study also highlights the educational value of computational modelling in physics, it connects analytical mechanics with numerical methods, allowing conservation laws and astrodynamics to be explored computationally.

Conclusion

In conclusion, the RK45 numerical method has been implemented using Python to perform modelling of circular and elliptical orbital motions of astronomical bodies based on the principles of Kepler's laws and Newton's laws. In addition, the principle of conservation of energy suggests strong numerical stability under the chosen tolerance settings, with relative errors of order 10^{-9} for the Earth-Moon system and between 10^{-21} and 10^{-23} for the Earth-Moon-probe system under the chosen tolerance settings.

Although the simulations rely on fundamental simplifying assumptions — such as a stationary Earth and two-dimensional point-mass trajectories — the model serves as an effective computational representation for foundational orbital dynamics.

Despite these limitations, the project demonstrates the usefulness of RK45 integration for accessible and reproducible orbital simulations in computational physics. Future work could extend the model by introducing physically realistic factors combined with

existing ephemeris data. Moreover, machine-learning-assisted optimisation modelling approaches may provide opportunities to accelerate repeated trajectory evaluations while remaining grounded in established physical simulations.

Beyond Transcription: The Mechanistic Functions of RNA in Double-Strand Break Repair

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Keywords: DNA, RNA, Molecular Genetics, DNA repair, Molecular Biology

Abstract

Double-strand breaks represent a severe form of DNA damage, with the potential to threaten genomic integrity and cell survival. Traditional models of double-strand break repair focus on protein-centric mechanisms, however, increasing evidence suggests that various RNA species directly influence the double-strand break repair response through complex scaffolding, localisation, and regulatory modulation of repair factors. This review synthesises the mechanistic roles which RNAs are shown to take in the DNA damage response. Evidence is presented showing long non-coding RNAs acting as molecular scaffolds, facilitating the recruitment and stabilisation of repair complexes. Small non-coding RNAs enhance repair efficiency by enabling sequence-specific localisation and modulation of repair factors, particularly through RNA-guided protein complexes at double-strand break-proximal chromatin. The dual roles of DNA-RNA hybrids are also discussed, with them contributing to both R-loop resolution and end-joining pathway choice, while in some contexts also obstructing repair complex assembly and access to DNA ends. Despite these advances, mechanistic questions remain regarding the precise molecular interactions, 3D-structures, and *in vivo* dynamics of RNA-mediated double-strand break repair complexes. This highlights the need for advanced structural and cellular analyses, with potential implications for understanding genome maintenance and developing novel therapeutics.

Introduction

Double-strand breaks (DSBs) are one of the most severe forms of DNA damage, and can be caused by radiation damage, DNA replication issues and oxidative stress [1,2]. Unrepaired DSBs can lead to chromosomal rearrangement, cancers, and cell death [3]. Therefore, accurate and efficient DSB repair is essential for maintaining genomic integrity.

There are two major, conserved, DSB repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR)[4]. Both pathways result in DSB repair, however, the mechanisms by which they operate are fundamentally different (Figure 1). NHEJ does not use a DNA template to assist in repair, instead, DNA ends are recognised and aligned by the repair complex with any incompatible overhangs being trimmed into blunt ends, which are ligated [5]. Due to the lack of a template, NHEJ is error-prone and can produce small insertions and deletions, called indels. Alternative end-joining pathways may also be employed, one being microhomology-mediated end joining (MMEJ), which relies on short regions of homology to align DNA ends [6]. Conversely, HR requires a template, usually a sister chromatid formed during the S and G2 stages of the cell cycle [7]. Due to the template, the repaired DNA is an exact copy of the original and is mostly error free.

Historically, RNA was shown to be a passive intermediary, assisting in protein synthesis as a template (mRNA) or as part of the translation machinery (ribosomal RNA and tRNA) [8]. However, recent

studies show RNA playing a prominent role in the DNA damage response (DDR). In the context of DSB repair, RNA molecules have been shown to act as scaffolds for repair proteins, form DNA-RNA hybrids at break sites and guide repair protein recruitment [9–11].

These new findings both complement and challenge the classical, protein-centric, models for DSB repair, and have wide-ranging consequences including cancer therapeutics [12]. This review adopts a mechanistic framework to synthesise current evidence for RNA-mediated DSB repair, characterising the multiple ways in which RNA molecules interact with both DNA and protein complexes to influence pathway choice and repair efficiency.

Long non-coding RNAs (lncRNAs) in DSB repair

A class of RNA that has received increasing attention in recent years are lncRNAs - non-protein coding RNAs over 200 bp in length [13]. They serve various functions within the cell, from influencing gene expression to epigenetic regulation. Notably, lncRNAs also function as molecular scaffolds for protein complexes, including DSB repair complexes, aiding in the recruitment and positioning of protein subunits in both HR and NHEJ scenarios. One example of this is HOTAIRM1 [9], which plays prominent roles in chromatin organisation and cell differentiation [14,15]. HOTAIRM1 also interacts with several RNA-binding proteins, acting as a molecular scaffold to promote repair complex assembly.

Upon induction of DSBs via laser microirradiation, HOTAIRM1 was observed

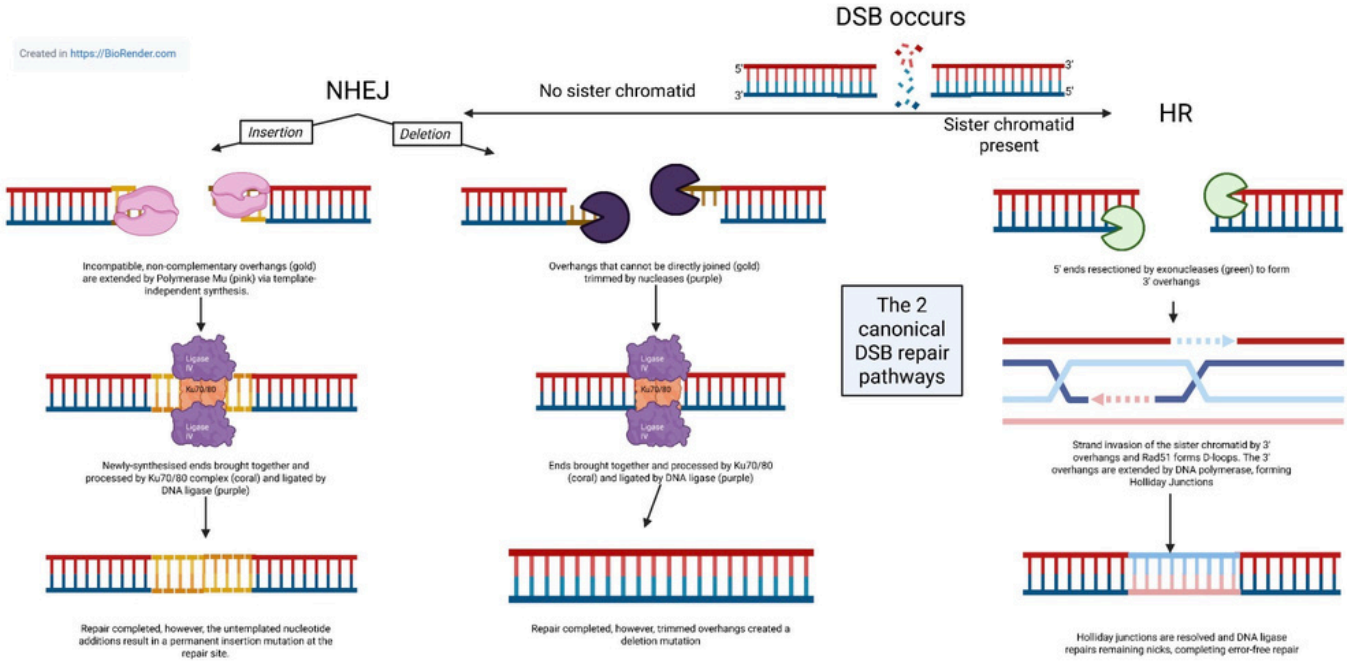


Figure 1 : Overview of canonical double-strand break repair pathways (NHEJ and HR); the NHEJ diagram shows both the formation of insertions and deletion mutations. HR repairs DSBs using a homologous DNA sequence, resulting in high-fidelity repair and an accurate restoration of the original sequence.

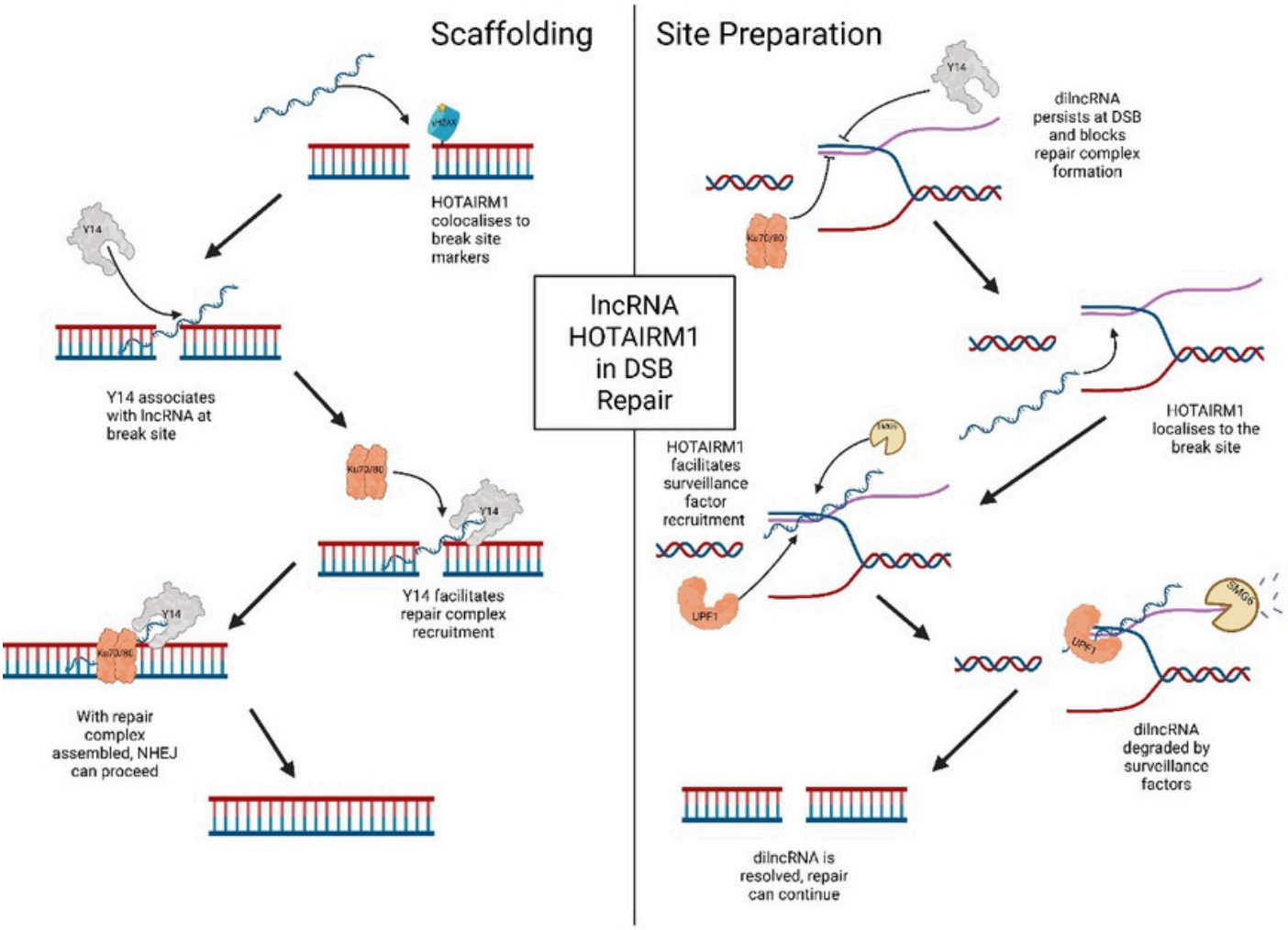


Figure 2: The mechanistic roles of HOTAIRM1 in scaffolding and repair site preparation, showing specific targeting to DSB sites and active repair complex and dilncRNA resolvase recruitment.

Upon induction of DSBs via laser microirradiation, HOTAIRM1 was observed through fluorescence microscopy to accumulate at break sites, colocalising with canonical DNA damage markers, including γH2AX (a phosphorylated histone variant) and p53-binding protein 1 (53BP1) [16,17]. Upon colocalisation, HOTAIRM1 facilitates the recruitment of exon-junction complex (EJC) protein Y14 [18], forming an RNA-protein bridge which promotes the continued assembly of repair factors, such as KU70/80, to the break site. This scaffolding activity facilitates effective end alignment and ligation, increasing repair efficiency. Knockdown of HOTAIRM1 by antisense oligonucleotides conferred delayed recruitment of NHEJ repair factors, confirming HOTAIRM1's significant role in repair complex stabilisation [9].

In addition to facilitating repair complex formation, HOTAIRM1 also recruits RNA surveillance factors during NHEJ. Double-strand breaks form damage-induced long non-coding RNAs (dilncRNAs) which aid in early NHEJ, acting as both RNA-protein bridges and DNA-RNA hybrids to help recruit repair factors [19]. While dilncRNAs

play a significant role in initiating DSB repair, their accumulation must be tightly regulated as persistent dilncRNAs can interfere with repair. HOTAIRM1 mediates the recruitment of surveillance factors UPF1 and SMG6 to DSB break sites, which target persistent dilncRNAs for degradation and clear the break site to allow for further complex recruitment. siRNA knockdown of UPF1, SMG6 and HOTAIRM1 reduced NHEJ efficiency [9], supporting a second role for HOTAIRM1 at break sites via surveillance factor recruitment and demonstrating its dual role in NHEJ (Figure 2).

NHEJ is not the only repair pathway influenced by lncRNA expression. Increasing evidence shows that lncRNAs also play a role in homologous recombination, with one being BGL3 [20]. Following DSB induction, PARP1 recruits BGL3 through its DNA binding domain, localising BGL3 to the break site. BGL3 then associates with the BRCA1/BARD1 complex, forming an RNA-protein bridge which stabilises the repair factors at the break site. This association promotes the stable recruitment of downstream effectors, such as HP1γ and Rad51, enabling efficient end resection and repair. siRNA knockdown of BGL3 led to an increase in γH2AX retention, indicating impaired repair functionality [20]. Although HOTAIRM1 and BGL3 function in different repair contexts, the conservation of lncRNA scaffolding between NHEJ and HR highlights the broader importance of RNA in DSB repair.

While enhanced repair efficiency is generally beneficial for the maintenance of genomic integrity, hyperactive repair can be problematic. For example, the lncRNA-EME1 acts as a molecular scaffold, facilitating the recruitment of BRCA1 to the break site and enhancing HR efficiency [21]. Upon lncRNA-EME1 being overexpressed in cervical cancer cells, the increase in repair factor recruitment and HR efficiency led to the cells developing radioresistance against cancer therapies. This highlights the need for tight regulation of DSB-related lncRNA expression in the cell, as excessive repair activity can promote therapeutic resistance rather than directly improving cellular outcomes.

Collectively, these studies demonstrate that lncRNAs scaffold and coordinate both NHEJ and HR repair complexes, highlighting their broad mechanistic diversity [9,20,21]. This highlights a unifying mechanistic principle in which structurally flexible lncRNA molecules serve as dynamic platforms for complex assembly that increase the local concentration and stability of repair factors. However, much of this evidence is derived from laser microirradiation systems, immortalised cell lines, and artificial overexpression models. While localisation and activity are supported in such systems, with necessity often being inferred from knockdowns, the sufficiency of these lncRNAs for initiating repair within physiological contexts in vitro remains less clearly established. Whether there is cross talk between these processes, or if they act independently, also remains unclear.

Overall, lncRNAs act primarily as structural scaffolds that organise and stabilise repair protein assemblies, functioning at the recruitment and early coordination stages of both NHEJ and HR to enhance repair

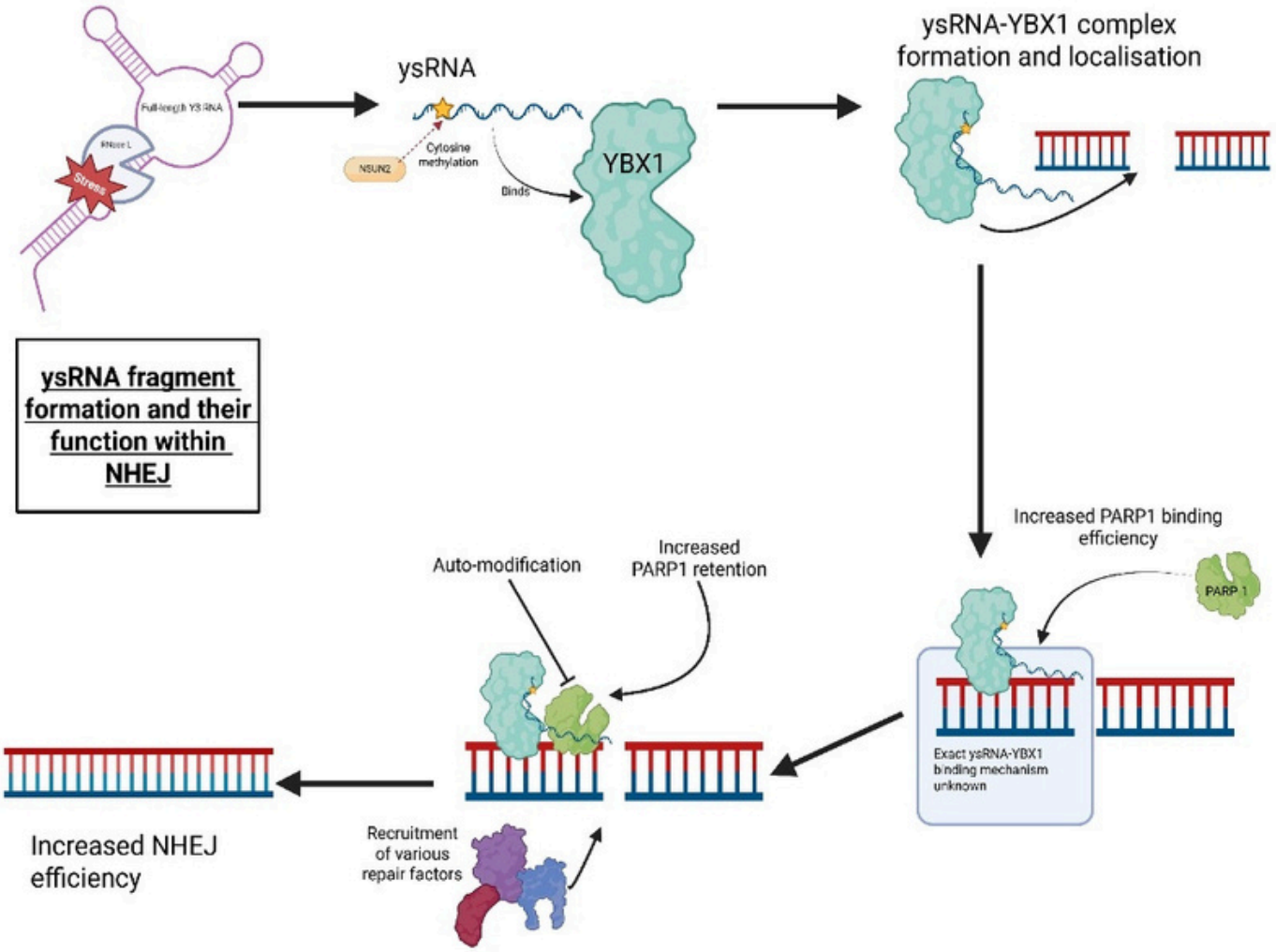


Figure 3: ***An overview of the role of Y3 RNA fragments (shown in blue) in NHEJ,*** *higlighting the stress-dependant activation and localisation of the Y RNA fragment to break sites, and the resulting retention of NHEJ factors at break sites.*

complex localisation and retention at DSB sites.

Small non-coding RNAs and RNA Fragments

Alongside lncRNAs, several classes of small non-coding RNAs (sncRNAs) participate in the DSB damage response. Defined as non-protein-coding transcripts under 200 nucleotides, sncRNAs hold both regulatory and structural functions by associating with RNA-binding proteins (RBPs) to regulate gene expression, chromatin dynamics, and DNA repair pathway activity [22]. With regards to the DSB repair response, sncRNAs are shown to function as targeting guides and modulators for larger protein complexes. Among these, fragments derived from Y RNAs have been shown to promote DSB repair [23].

Y RNAs are 80-120nt long sncRNAs originally identified through their association with the Ro60 antigen in autoimmune patients. They adopt a stem-loop secondary structure containing a double-stranded stem, a large internal loop and a single stranded tail, allowing them to specifically bind multiple RBPs [24,25]. Under stressful conditions, Y RNAs

are processed by the 3′-5′ exosome complex and cleaved at the loop region by stress-activated endonucleases such as RNase L [26]. This generates ~30 nucleotides long fragments known as ysRNAs.

DSBs were induced through ionising radiation, which led to an increase in cleavage of the Y3 isoform of Y RNA. This resulted in the accumulation of Y3-ysRNA in both the cytoplasm and the nucleus [23]. These fragments then underwent cytosine methylation by the RNA methyltransferase NSUN2, forming a 5-methylcytosine (m5C) residue. Following their formation, the ysRNAs were confirmed, through RIP-seq (a technique which immunoprecipitates RNA–protein complexes to identify RNAs bound to a specific protein by sequencing), to associate with the DDR-associated factor YBX1, which was shown by fluorescence microscopy to localise to γH2AX enriched sites. The cytosine modification was reported to be essential for YBX1 binding, with NSUN knockdowns reducing ysRNA-YBX1 binding affinity and diminishing ysRNA localisation to DSBs.

At the break site, the Y3-ysRNA-YBX1 complex enhanced PARP1 recruitment and activity on chromatin while reducing PARP1 auto-modification. This prolonged PARP1 chromatin association and correlated with enhanced downstream recruitment of repair factors, increasing repair efficiency. DSB repair efficiency was reduced when either Y3 RNA or YBX1 were knocked down. These findings, alongside PARP1's established role in NHEJ5, highlight the critical role of Y RNA fragments in DSB repair and suggest a mechanistic principle in which Y RNA fragments function through protein activity modulation rather than structural scaffolding, distinguishing them from lncRNA-mediated assembly processes [25] (Figure 3).

Another group of small RNAs which influence DSB repair are damage induced small RNAs (diRNAs). These are double-stranded small RNA fragments which are processed from dilncRNAs by various RNases (such as Drosha and Dicer) and often terminate at the end of a gene near the DSB site [27]. diRNAs are sequence-specific to the DNA flanking the break site and can therefore act as markers.

In the context of HR, the RNAi factor Argonaute 2 (Ago2) has been shown to localise at DSB sites in a diRNA dependent manner. While this has been directly observed in plant cells [27], the sequence specific recruitment of Ago2 to DSB sites in mammals is also supported by experimental evidence [28].

Ago2 interacts with Rad51 (the recombinase required for strand invasion) via its PAZ and MID RNA-binding domains, forming an Ago2-Rad51 complex. Following the formation of a DSB, the complex binds the diRNAs complementary to damaged DNA, forming a Ago2-Rad51-diRNA complex which assists in localising Rad51 to DSBs [28], although a direct interaction of this complex with the 3′ overhangs has yet to be demonstrated. This localisation promotes Rad51 loading and filament nucleation, which is required for strand invasion [7].

Knockdown of Dicer and Drosha reduced diRNA formation, which in turn reduced the association of the Ago2-Rad51 complex to the break site, although complex assembly was shown to be unaffected. The reduced association to the break site prevented Rad51 filament assembly, reducing repair efficiency. Upon reintroduction of diRNAs produced in non-knockout cells, it was recorded that HR activity was recovered [28], showing that diRNAs formed around DSBs are essential for the HR initiation, serving as guides to enable the correct placement of DSB repair factors.

Overall, sncRNAs function as sequence-dependent targeting and regulatory guides that act primarily at the initiation and recruitment stages of homologous recombination and end-joining pathways by directing repair factors such as Ago2 and Rad51 to DSB-proximal chromatin. This is supported by strong localisation evidence in both plant and mammalian models, as well as knockdown studies confirming the necessity of Dicer/Drosha activity. However, much of the mechanistic model is derived from laser-induced damage systems and immortalised cell lines, and while diRNAs appear sufficient to enhance recruitment in rescue experiments, their sufficiency for initiating physiological DSB repair in endogenous chromatin contexts remains less clearly established. In addition, direct biochemical and structural characterisation of these RNA-protein complexes remain limited, leaving uncertainty over the precise binding architecture underlying Ago2 and Rad51-mediated targeting. This contrasts with lncRNAs, which primarily act as structural scaffolds that organise and stabilise repair complex assembly.

R-loops: RNA-DNA Hybrids in DSB Repair

Some dilncRNAs, as well as being processed into diRNAs, can directly associate with complementary DNA flanking the break. This forms structures known as RNA-DNA hybrids [29], including *in cis* R-loops and *in trans* DR-loops. Functionally, these hybrids regulate repair by controlling repair-factor accessibility, stabilising repair intermediates, and influencing pathway choice between HR and end-joining mechanisms. These are, therefore, critical regulators of the DSB response, acting both as aids and obstacles to efficient repair.

It was demonstrated that accumulation of R-loops at DSB sites can be detrimental to repair efficiency, as their persistence after repair initiation impedes the recruitment of HR machinery [30]. The resolution of these R-loops was shown to be mediated by E3 ubiquitin-protein ligase (RNF8). Upon the detection of an R-loop, RNF8 ubiquitylates the exoribonuclease XRN2, which then localises to R-loop prone locations on the genome. XRN2 facilitates R-loop resolution, making way for repair factors to access the break site. In cancer cells expressing an inactive RNF8 mutant, XRN2 recruitment to R-loop sites was impaired, leading to an accumulation of R-loops around break sites, as observed by DRIP-qPCR. This resulted in defective HR and reduced genomic stability, highlighting the potentially detrimental role of RNA-DNA hybrids in DSB repair.

Conversely, DNA-RNA hybrid formation can promote DSB repair through DR-Loops, a

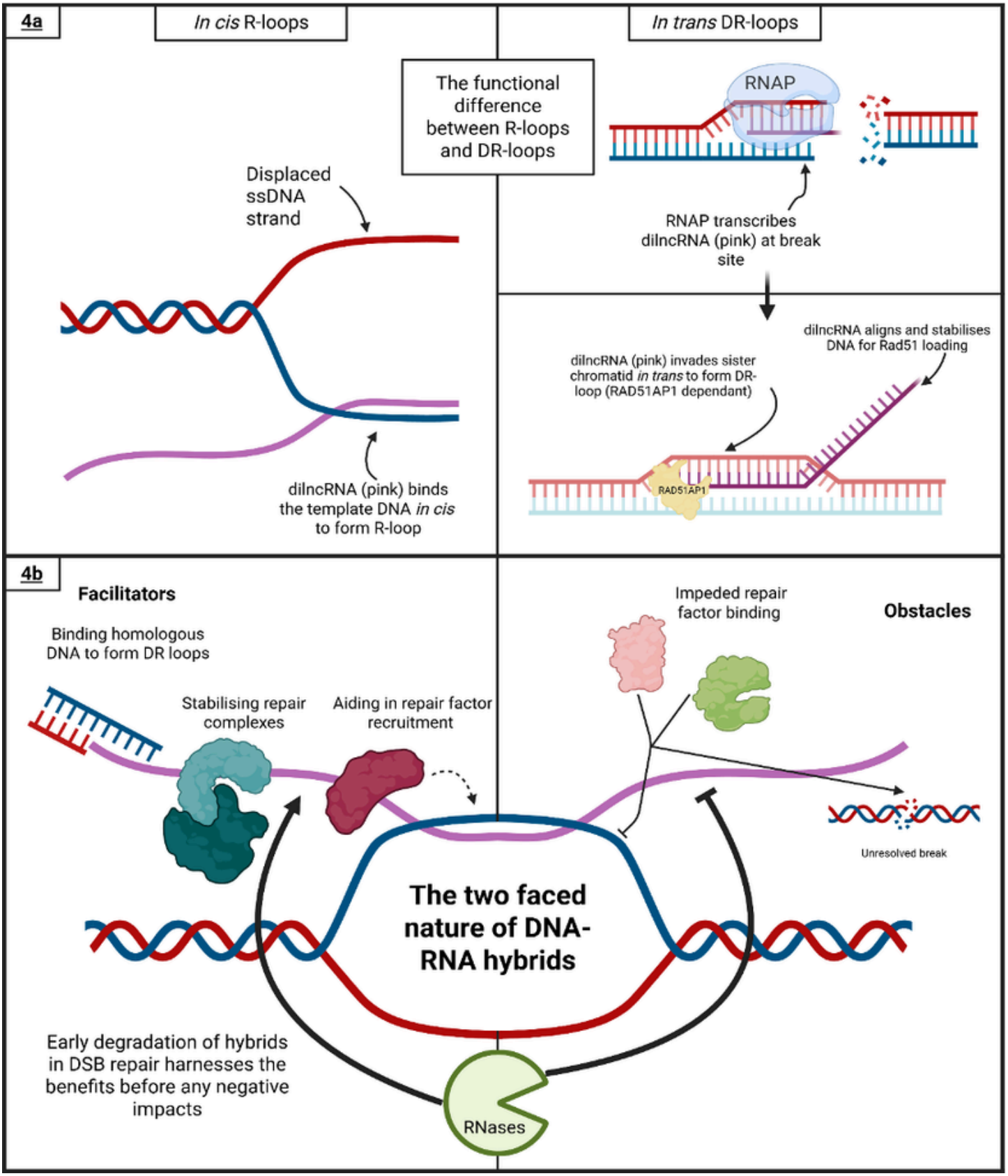


Figure 4: (a) A diagram showing the differences between R-loops and DR-loops. (b) Various examples demonstrating the two-faced role DNA-RNA hybrids play in DSB repair, with repair facilitators being shown on the left and obstacles on the right.

mechanistically separate class of DNA-RNA hybrids which form in trans through hybrid formation between RNA transcripts and homologous DNA sequences on a different molecule from which the RNA was transcribed (Figure 4a) [31]. When dilncRNAs associated with homologous sequences on the sister chromatid, their proximity and alignment stabilised the ssDNA at the break. This process depended on the protein RAD51AP1, which promoted the invasion of dilncRNA into the sister chromatid [31]. DR-loops acted as scaffolds to promote efficient Rad51 loading and nucleation, as confirmed by DRIP-seq and RNase-H sensitive assays. Degradation of DR-loops by RNase-H reduced RNA-DNA hybrid levels, suppressing the RNA-stimulated enhancement of HR observed in these experiments. When RNase-H was overexpressed prior to Rad51 recruitment, HR efficiency dropped. These results highlight the context-dependent roles of RNA-DNA hybrids, aptly described as "Janus-faced" by Marnef and Legube [10], as trans-acting hybrids such as DR-loops promote repair initiation, while persistent cis-acting R-loops impede successful repair (Figure 4b).

There is increasing evidence showing that RNA-DNA hybrids have a distinct role in repair pathway selection [11] (e.g. HR vs NHEJ/MMEJ). diRNAs generated at break sites can anneal with the opposite DNA termini, forming RNA-DNA hybrids that act as a molecular splint to align and stabilise the DNA ends. By stabilising the ends and recruiting end-joining proteins, RNA-DNA hybrids may bias repair towards NHEJ or MMEJ.

It was demonstrated that in yeast and human cells, the processing state of diRNAs influenced hybrid stability and repair pathway choice [11]. Using Cas9-induced DSBs within reporter constructs, spliced and non-spliced RNA transcripts capable of hybridising to sequences flanking the break were compared. Non-spliced transcripts showed a preference for NHEJ, with longer regions of homology providing stronger hybridisation to the DNA ends. In contrast, spliced transcripts, lacking the long homology regions present in the non-spliced variants, preferentially promoted MMEJ. The presence of RNA-DNA hybrids at these sites was confirmed by DRIP-qPCR and their sensitivity to RNase-H digestion, validating that the pathway bias was caused by the hybrids themselves. In both cases, HR was disfavoured, as hybrids likely impede end-resection and block Rad51 recruitment. This suggests that, at breaks where such hybrids form, end-joining mechanisms are favoured under conditions which would usually favour HR.

Together, these studies support a multifaceted role for DNA-RNA hybrids in DSB repair as context-dependent physical intermediates that influence repair pathway choice by modulating DNA end accessibility. Their functional outcome is determined by hybrid stability, genomic origin, and processing state. While biochemical and genetic experiments provide robust evidence for their functional involvement in repair outcome determination, much of the causal framework is derived from engineered reporter systems and defined break induction models, leaving their relative contribution and structural organisation at

in vivo chromatin breaks less clearly resolved. The ability to scaffold directly mirrors that of lncRNAs, however, the two-sided nature of these constructs highlights the necessity of strict regulation to prevent aberrant repair.

Overall, RNA-DNA hybrids act as context-dependent regulators of DSB repair. They function as dynamic intermediates that either facilitate repair factor recruitment and pathway progression or impede repair and destabilise genome integrity depending on their stability, localisation and processing state.

Discussion and Conclusions

The discovery that RNA molecules participate directly in the DSB response has expanded the scope of RNA function beyond just gene expression. Experimental evidence now supports the view that various distinct categories of RNA participate in DSB repair through structural, catalytic, and sequence-guiding roles, expanding upon the diverse role RNA plays in the cell. This review categorises RNA involvement in DSB repair into scaffolding, guiding, and hybrid-mediated regulatory mechanisms.

Mechanistically, the different RNAs take on distinct roles. lncRNAs, such as HOTAIRM1, act as scaffolds to aid in the structural assembly of repair complexes, while also preparing the site for repair by removing blockages [9]. sncRNAs function in a more direct way, whether it be diRNAs guiding repair factors such as Rad51 to the break sites, or Y RNA fragments enhancing NHEJ efficiency by modulating PARP1 activity [23,28]. Conversely, DNA-RNA hybrids formed early in the DDR are shown to facilitate repair factor recruitment and end-resection during HR, while persistent hybrid formation was shown to impede repair and induce genomic instability, demonstrating the two-faced role of DNA-RNA hybrids [29,30]. However, it remains unclear whether there is functional overlap between RNA classes in different repair contexts. Addressing these uncertainties is essential for a complete mechanistic framework of RNA-driven DSB repair and exposes an important conceptual gap in the current understanding.

Despite significant advances, several mechanistic gaps remain unresolved. The interactions that RNAs form are often inferred and not directly visualised, with direct biochemical evidence of binding being lacking. Determining the 3D structure and binding surfaces of these complexes and interactions is essential to understanding the nature of these mechanisms. Such information opens the door to determining the dynamics of these interactions and is necessary to define canonical RNA-mediated DSB repair pathways. Modern technologies, such as AlphaFold and 3D-NMR spectroscopy simplify this process, with AlphaFold3 being able to predict protein-substrate interactions [32], and 3D-NMR allowing RNA-Protein complexes to be modelled in solution [33], enabling conformational dynamics to be measured and quantified.

A large proportion of these mechanisms were characterised in vitro. While this allows for the characterisation of molecular interactions under controlled conditions, the use of immortalised

cell-free systems may not accurately represent the conditions of native cells [34]. Future studies should aim to visualise these mechanisms in vivo in model organisms, such as zebrafish, whose translucent epidermis facilitates fluorescent imaging [35], or Drosophila, due to high homology with the human genome and well characterised DSB repair pathways.

Looking ahead, determining the molecular interactions between RNA and classical DSB repair mechanisms may reveal potential drug targets. These studies showed that aberrant RNA processing, or overexpression of specific RNAs post-break, led to genomic instability, and in some cases radioresistant cancers [20,21]. Identifying the RNAs and repair complexes responsible allows for the development of next-generation targeted therapeutics aimed at selectively modulating the DSB response, whether it be knocking down hyperactive repair or by serving as a preventative measure in cells predisposed to oncogenesis.

Overall, emerging evidence clearly establishes RNA as a central regulator of DSB repair through scaffolding, guiding and modulation. Despite gaps in understanding precise molecular interactions *in vivo*, it is increasingly clear that RNA is not a passive bystander but actively shapes repair pathways and genome stability. Continued investigation of structural biology and *in vivo* characterisation is essential to fully understand the range of influence RNA has on genome integrity, and its potential exploitation in therapeutics and cancer research.

Interpreting and Assessing the Validity of Durkheim's Rules Under Conditions of Structural Injustice – A Standpoint Theory Approach

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Meet the Author

Sian Amekuedi is an MSc Social Science Research Methods student. Their undergraduate was in Mathematics and Philosophy, and since then, they have maintained a substantial interest in social epistemology, particularly standpoint theory. Sian especially enjoys the social epistemology of science and social science. Through writing this, they came to realise that they had judged Durkheim far too harshly. Recently however, they have been developing a growing curiosity about social network analysis. Outside of academics, Sian really enjoys DJing, boredom, and Doctor Who.

Keywords: Durkheim, Social Epistemology, Standpoint theory, Methodology, Structural Injustice

Abstract

This article interrogates the validity of Durkheim's Rules of Sociological Method under conditions of structural injustice. We argue that Durkheim's Rules can only be considered partially valid. Understanding Durkheim's rules as built upon an epistemic foundation that implicitly assumes universal observability, we suggest that this makes social research vulnerable to socially embedded ignorance. Drawing on standpoint theory and a contemporary case study, we highlight that such oversights can lead to epistemic and ethical harms. We then turn to standpoint theory for a potential solution, proposing an epistemic shift in line with contemporary medical research. Such an epistemological shift will bring with it methodological consequences. Hence, we speculate on a potential reformulation of Durkheim's fundamental rule that the sociologist must "treat social facts as things". Using Durkheim's own morphological/physiological distinction, we propose a reformulation of this rule, Rule 1*. The aim of Rule 1* is to encourage greater reflexivity and awareness of situated knowledge. We conclude that under conditions of structural injustice, Durkheim's Rules may be rendered conditionally valid.

Introduction

Durkheim's (2013) *Rules of Sociological Method* aimed to serve as a sociological manifesto. It offered an early framework for sociological research design. Durkheim's ultimate goal was for sociology to be a moral science. A scientific discipline capable of diagnosing social pathologies that undermine social solidarity (Lukes, 1985, pp. 193,226). Through diagnoses, sociology could prescribe directions for social reform and eventual social order (McLennan, 2011, p. 63).

We suggest that Durkheim's rules are only partially valid under conditions of structural injustice. However, they can be rendered conditionally valid when reformulated. Validity is assessed in relation to Durkheim's diagnostic aim. We suggest that structural injustice generates potential epistemic limitations. First, we establish the relationship between Durkheim's epistemology and methodology. Secondly, we introduce a case study and consider the validity of Durkheim's rules under conditions of structural injustice. Thirdly, standpoint theory is introduced as a comparison to signal Durkheim's epistemic limitations – suggesting only partial validity. Finally, we consider whether Durkheim could adopt a standpoint-based epistemology. A reformulation of his fundamental rule is suggested, potentially allowing conditional validity.

For clarification, our understanding of Durkheim focuses on the Rules of Sociological Method. Other works will only be used illustratively. Durkheim's primary rule will be the focus, with further rules not being discussed in depth. Throughout, we refer to and build upon standpoint theory.

Durkheim at a glance:

This section outlines Durkheim's

methodology and the epistemological assumptions upon which the validity of Durkheim's rules depends. Methodologically, Durkheim was a naturalist; he believed methods of the natural sciences should be replicated in sociology (Baert, 2005, p. 12). For Durkheim, scientific status required starting from observable and measurable data. Hence, the social fact:

Externality *- social facts exist outside of any individual consciousness.*

Constraint *– social facts impose rules and expectations, sometimes without our awareness.*

Generality *- social facts cannot be reduced to individual actions.*

Durkheim (2013, p. 7-9) asserts that sociologists must treat social facts as things. We will call this "Rule 1". By "things", Durkheim entails that social facts are impenetrable to the observer's introspection and thus require external methodological study. Three corollaries immediately followed. Firstly, preconceptions must be eliminated. Secondly, social facts must be defined by their external observable characteristics. Thirdly, social facts should be studied independently of individual manifestations. Together, these corollaries aimed to ensure research objectivity. Rule 1 is the basis from which the subsequent rules follow. The subsequent rules establish a quantitatively focused methodology aiming to produce sociological 'proof' grounded in claims of causality.

Durkheim's epistemology can be framed as a mixture of scientific rationalism and positivism (Lukes, 1985, pp. 72-76; Lukes, 2013, p. xii). Broadly, this position has been characterized by Bordo (1986) and Haraway (1988) as valuing rationality, objectivity, detachment, and a commitment to universal observability – a 'view from nowhere'. This is evident in Rule 1 and its corollaries. Durkheim aims to ground sociological arguments in systematic reasoning, explicitly aiming for an objective method. Through this, he seemingly assumes that the trained observer can bracket off these biases when examining social facts.

So, Rule 1 is the basis for Durkheim's methodology, and his methodology is seemingly built upon his epistemology. Together, these suggest that the validity of Durkheim's rules is dependent upon their underlying epistemological validity.

Turning to a contemporary example, this provides the intuitive grounds for the limitations of the seeming epistemological assumptions that ground his rules.

Chambers et al. as a case study

Chambers et al. (2021) look to understand how structural racism influences reproductive health. This case study aims to indicate the validity of alternative approaches in the context of racism. Specifically, how such approaches allow access to knowledge that risks being excluded under Durkheim's framework.

Chambers et al. (2021) note that racism/misogynoir operates at a societal level, imposes a constraining force, and

has generality across ethnically minoritised women. By Durkheim's own criteria, racism could be understood as a social fact. Similarly to Durkheim, Chambers et al.'s central aim is to prescribe policy changes to combat a social pathology. Methodologically, however, they deviate significantly from Durkheim's prescriptions. Their research design involves the following steps:

1. Defining the research problem.
2. Understanding their positionality.
3. Sampling participants based on lived experience.
4. Data collection via focus groups.
5. Data analysis.
6. Checking findings with an advisory board in the field.
7. Gathering feedback from participants to affirm the representativeness of findings.

Chambers et al. (2021) implement a small-N approach prioritising depth over generalisability. In contrast to Durkheim, they avoid the assumption that biases can be bracketed away through an objective method. Instead, they aim for reflexivity, seeking to mitigate and recognize the impact of external power dynamics. This is first evident in step 2 - by acknowledging their positionality, Chambers et al. encourage themselves to interrogate how this has come to shape the research process. In the case of step 3, this aims to ensure that research findings are derived from those with lived experience. Steps 7 and 6 moreover, look to integrate whether participants and the wider community have been adequately represented by researcher attitudes alongside assumptions.

As Bryman (2008, pp. 391-392) notes, however, such studies are also limited by their subjectivity and replicability. From a Durkheimian perspective, this small sample would provide an extremely limited basis for an inductive generalisation. Moreover, its reliance on lived experience undermines its objective basis. Durkheim (2013 p. 24) instead prioritises generalisability reflected in his use of official secondary data such as government statistics. By doing this, Durkheim sought detached, repeatable causal explanations.

Each of these approaches requires respective trade-offs and fundamentally different approaches to researcher bias. They each bring with them alternative epistemological assumptions. Chambers et al. sacrifice generalisability to access knowledge that purely quantitative methods can obscure. As suggested in the next section, Durkheim's epistemic commitments are vulnerable to blind spots. Unfortunately, these blind spots can have ethical consequences under conditions of structural injustice.

Standpoint Theory and Epistemic Blind Spots

This section examines standpoint theory to explain how Durkheim's epistemological assumptions may limit the applicability of his rules. Appealing to standpoint theory, this section critiques the assumption of universal observability under conditions of structural injustice.

Statements of positionality within

research (like those of Chambers et al.) are epistemic consequences of standpoint theory (Freeman, 2019). Standpoint theory has been characterized by Crasnow (2014) as maintaining three key theses. Firstly, knowledge is always tied to social positionality. Secondly, those in marginalized positions have access to knowledge invisible to dominant social groups. And thirdly, the achievement thesis – The standpoint is not automatic; it is cultivated through political and collective work. Methodologically, this can manifest as placing lived experience as the object of study, as done by Chambers et al. (2021).

Together, this immediately contrasts with Durkheim's seeming epistemological assumptions. In particular, his seeming assumption of universal observability. Haraway (1988, pp. 581-584) suggests that by assuming universal observability, the roles of societal power dynamics in shaping knowledge are ignored. Thus, the researcher's positionality can come to shape how they interpret data. Hence, steps 2, 6, and 7 in Chambers et al.'s (2021) research design.

Under conditions of structural injustice, a researcher assuming universal observability may unknowingly reproduce the social pathologies Durkheim is committed to diagnosing. Admittedly, Durkheim (2008) was not entirely blind to the existence of structural discrimination. In his account of antisemitism, he acknowledges that it meets each of his criteria of a social fact and is hence structural in nature. Nevertheless, Durkheim saw structural discrimination as resulting from factors such as social crises. What he seemingly didn't foresee was the possibility of social pathologies being embedded within the researcher's epistemology, shaping their ways of knowing.

Epistemic ignorance illustrates one way that social pathologies may persist undetected within an epistemology and act as a constraining force. It can be described as socially supported and strategic instances of not knowing that result in the reproduction of existing power structures. It includes bias, conceptual blind spots, denial, and systematic direction (Tuana, 2006; Mills, 2017). Crime statistics exemplify how the methodological consequences of ignorance may manifest in research.

Spohn (2015) argues that through the war on drugs, black men faced disproportionately harsher policing. This included harsher sentencing and racial profiling. Similar to Durkheim's suicide example, biases have shaped what data looks like. Naturally, this meant that subsequent crime rates would reflect patterns of enforcement over offending. Durkheim's suicide study indicates how blind spots may arise.

In Suicide, Durkheim partially overlooks biases that shape the production and reliability of data. Durkheim (2002, pp. xxxix-xl) opens with a warning of common prejudices of the public that distort researchers' understanding. However, Durkheim's own study relied heavily on the use of secondary statistics, assuming their neutrality. Though, as Montuschi

(2014, pp. 135-138) highlights, Durkheim underestimates the role of moral norms in shaping secondary data. For example, the societal stigma around suicide may have led to families lying.

The implication here is that Durkheim may lack the tools for identifying bias in secondary data, leading it to be taken as neutral evidence. Returning to the example of racialised policing, when a group is profiled and faces harsher sentencing, this is going to be reflected in official crime statistics. Accordingly, the use of biased data is likely to produce biased research. Hence, in the case of structural injustices, such oversights while claiming objectivity risk obscuring bias rather than challenging it. Unfortunately, the inability to identify biases presents a potential epistemic barrier to Durkheim's diagnostic aim.

Critically, Tuana (2006) argues that epistemic ignorance can be a systematic consequence of assuming universal observability. This can occur despite claims of objectivity. The Durkheimian researcher, then, may risk unknowingly reproducing structural bias within research from dominant social categories that shape data. For example, in the case of crime statistics. Therefore, Rule 1 and its corollaries may be insufficient on their own to adequately guard against systematically embedded epistemic ignorance. Such ignorance can contribute to ethical harm.

The British Sociological Association (2017, pp. 4-6) highlights the researcher's responsibility to safeguard those affected by their work. Likewise, the need to consider the effects and use of the research. Jensen (1969) for instance, attempted to correlate IQ with race. Through a different methodology, he drew upon 'objective' positivist methods – obscuring his own bias.

Responding to Jensen, Flynn (1980, pp. 15-20) asserts that such social research does not exist in an ethical vacuum. It can provide the racist with a 'rational justification' of their attitudes and/or racist policy. Hence, commitments to neutrality and objectivity can obscure embedded biases in research. Research can be mobilized to intentionally, or otherwise produce ethical harm. With epistemologically embedded ignorances, it follows that Rule 1's corollaries may be insufficient to remove researcher bias. Having understood Durkheim's epistemic limitations, the next section looks to the potential answer from standpoint theory.

Conversely, while standpoint epistemologies cannot guarantee the elimination of epistemic ignorance, they can offer a conceptual framework for uncovering it. Using the example of the women's health movement, (Tuana, 2006) alludes to ignorances being uncoverable by collective political work. This mirrors Collins's (2000, pp. 260-269) assertion that dialogue is a necessary ingredient for developing the standpoint. Methodologically, this is reflected in Chambers et al.'s (2021) implementation of focus groups.

Blakemore (2011) describes radical feminist consciousness-raising as women gathering to understand their experiences

of misogyny. Through this, they would develop a conceptual framework. Montell (1999) flags the consciousness-raising power of focus groups. This is through factors such as the creation of participant solidarity and dialogue that would not arise through the power dynamics of the interview. Through focus groups, Chambers et al. facilitate collective meaning-making, fostering political dialogue. Thus, Chambers et al present a potential methodological response to the problem of ignorance.

For Durkheim, however, Chambers et al's use of focus groups challenges his doubt of introspection. Montell (1999, p. 51) suggests that collective meaning-making can emerge through collective individual reflection. For (Chambers, et al., 2021), participants are collectively performing acts of introspection to foster dialogue. In doing so, participants are accessing situated knowledge of a social fact that Durkheim's quantitative methodology may not capture.

The challenges in this section suggest that Durkheim's epistemology is vulnerable to blind spots that may manifest as epistemic ignorance. The existence of this epistemic barrier implies that when put into practice, a Durkheimian could unknowingly reflect structural injustices. Hence, the epistemology that grounds Durkheim's rules potentially threatens their validity. Standpoint theory, however, provides epistemic tools for identifying these blind spots. The final section looks to assess whether Durkheim's rules may be rendered valid under epistemological revision.

(Re)Interpreting Durkheim

This section speculates on the consequences of Durkheim adopting a standpoint-based epistemology. In doing so, it suggests that Durkheim's rules can be rendered conditionally valid. Instead of rejecting Rule 1 outright, this section offers an alternative formulation. Developments within medical epistemology provide an illustrative comparative case.

Since the publication of The Rules of Sociological Method, health epistemology has experienced what Kuhn (1970) would call a paradigm shift. Specifically, it has seen a greater openness to standpoint epistemologies. Historical biases stemming from the universal observer have led to failures in understanding phenomena, causing individual harm. Grasswick (2017), for example, highlights a historical androcentric understanding of heart disease as a particular case of this phenomenon.

In response, at least two related subsequent developments can be seen within more contemporary research that reflect an epistemological shift. Firstly, health research is seeing increased scepticism towards the universal observer, as reflected in the example of McGregor's (2020) call for a greater diversity of practitioners. The implication here is that greater epistemic diversity can assist in combating shared reality bias. Anderson (2012, pp. 167-170) describes this as the convergence of perspectives and judgements amongst those who commonly interact. Anderson adds that when marginalised knowers are excluded from enquiry, the interpretive resources

can be insufficient for understanding the experiences of those marginalised knowers. Secondly, as we have seen with (Chambers, et al., 2021), some areas of medical research have adopted alternative methodologies more suited to capturing standpoint-dependent knowledge.

Durkheim's epistemology can be vulnerable to similar problems. However, Durkheim's methodology was the means through which his diagnostic goal could be achieved (Baert, 2005, pp. 10-13; Giddens, 1985, pp. 10-11) . Moreover, the intellectual climate contemporary to Durkheim was dominated by positivism, with the natural sciences seen as the most reliable routes to knowledge (Lukes, 1985, p. 67). From this perspective, Durkheim's epistemological commitments could be understood as historically situated. In line with contemporary medicine, this perhaps suggests making a similar epistemic shift. This could equip Durkheim with tools to identify epistemic ignorance, thereby encouraging the conditional validity of his rules. Adopting such a shift raises the question of whether his rules could be adapted.

Methodologically, this would not require Durkheim to abandon quantitative methods. As Neitz (2021, pp. 58,68) note, some standpoint theorists are overtly open to methodological plurality. Sprague & Zimmerman (1989) assertion puts this into perspective. They forward that qualitative methods can also reproduce dominant ideology through researcher privilege. Additionally, they suggest that quantitative methods can be a powerful tool for identifying patterns of structural inequality. Hence, while Durkheim would not need to abandon his methodology entirely, it would need to be used as one tool amongst many. It would, of course, need to be used reflexively, with a greater openness to how data categories can be socially produced. Under these conditions, his rules may maintain conditional validity while recognizing the potential for epistemic ignorance.

If the axioms of standpoint theory are to be accepted, Durkheim will need to reformulate rather than abandon Rule 1. As Rule 1 serves as the foundation of his methodology, abandoning it means abandoning the remaining rules. Therefore, any reformulation of Rule 1 must retain how social facts are treated. But it must account for how knowledge is accessed according to standpoint theory. On this basis, the following reformulation of Rule 1* is proposed as a speculative solution:

Rule 1*:

- Treat social facts as things (reflexively). If and only if morphological.
- Treat social facts as things (reflexively) with standpoint-dependent features only uncoverable via collective introspection under *specific conditions.* If and only if physiological.

Durkheim (2013, pp. 25-27) describes morphological social facts as material conditions that shape society. For example, population density or resource distribution. Physiological social facts are understood as dynamic aspects of life, such as norms, public ideology, or laws.

Racism and instances of structural injustice would therefore fall into this category. Physiological social facts can give rise to morphological social facts. For example, racism may impact child mortality rates (Chambers, et al., 2021). Moreover, physiological social facts can generate further physiological social facts, as evidenced through racism's role in the creation of segregationist laws. In each case, structural social facts can produce further social facts. Hence, distinguishing social facts through Rule 1* requires considering how structural social facts may have brought others into being and what investigation should look like.

In the case of morphological social facts, Rule 1* maintains that knowledge is not accessible via introspection. For example, if conducting a study of population density. Importantly, however, this does not always entail that quantitative methods are suitable for examining these social facts. Spatial arrangements, for example, may be studied both quantitatively and qualitatively. Moreover, the reflexivity clause is added to signal that these social facts remain epistemically non-neutral. Reflexivity becomes a necessary tool for interrogating how categories and measurements are produced.

Lammer (2024) provides an illustration of what quantitative reflexivity could look like. It begins with transparency about why a research topic was selected, and its relation to researcher positionality. Because positionality shapes enquiry, this entails that social research can benefit from greater diversity amongst researchers. At each subsequent stage of research, Lammer adds that researchers must transparently and critically examine how their standpoint, biases, and assumptions may shape the study. If we extend this to the context of secondary data, such as crime statistics, the reflexivity clause requires researchers to acknowledge structural forces that may have shaped the production of that data.
Nevertheless, while morphological social facts can indicate patterns of structural injustice, they cannot uncover how structural injustices are experienced by those subjected to them. This highlights the value of methodological pluralism, as different kinds of social fact may require different forms of investigation.

In the case of physiological social facts, introspection is restricted to when the standpoint may arise. These will be cases concerning the study of a structural social fact, such as racism. Here, collective introspection aims to provide the means for accessing standpoint-dependent knowledge. Importantly, this introspection must be combined with the dialogue needed for standpoint. By specifying "only uncoverable under certain conditions", we allow the three theses of standpoint theory outlined by Crasnow (2014) to be maintained.

In both physiological and morphological cases, Rule 1* aims to encourage criticality of the power dynamics that shape social facts of each kind; thus, the reflexivity clause. Reflexivity furthermore is a methodological commitment of standpoint theory. Through adopting standpoint theory, this necessitates the

adoption of alternative epistemic virtues. The switch from (weak) objectivity to strong objectivity is a clear example of this. Harding (1991, pp. 138-163; 1992) forwards that objectivity is not gained through neutrality, but reflexivity. Instead, it's gained through understanding how power dynamics shape knowledge. It further requires epistemic diversity and the rejection of universal observability.

The reformulation proposed in this section is intentionally limited to Rule 1. Rule 1 is the methodological foundation of Durkheim's rules. So, this suggests that the subsequent rules and corollaries could plausibly retain conditional validity under an epistemic shift. However, what these would look like and indeed whether it would be possible remains an open and speculative question. It may require further revisions to Durkheim's rules and this is outside the scope of this essay. It could, however, be an area for future investigation.

Conclusion

Durkheim's goal of establishing sociology as a diagnostic force remains a noble goal, still pursued in social research. In addition, his formulation of the social fact effectively produced an early conception of systemic injustice. This is a significant achievement for the time, one that deserves greater recognition. Despite this, when considering racism as a social fact, potential epistemic and ethical issues arise. Durkheim's epistemic commitment to detachment, objectivity, and universal observability potentially allows epistemic vices. Namely, epistemic ignorance. Epistemology shapes methodology, and together they can shape ethical outcomes. From this perspective, Durkheim's rules risk being an insufficient route to his aims because of Durkheim's epistemic foundations.

Standpoint theory, on the other hand, may offer a solution. Adopting it, Durkheim could gain an epistemology more suited to achieving his diagnostic aims. Of course, adopting a new epistemology brings baggage. In particular, a new set of epistemic virtues and vices as well as a need for methodological reformulation. In this essay, we have established what a reformulation of Rule 1 may look like – Rule 1*. If Durkheim accepts this reformulation, it could provide the validation of Rule 1 without discarding the entire methodology by virtue of pluralism. On this basis, we should consider Durkheim's rules conditionally valid, dependent upon epistemic revisions.

Political Hate Speech as an Electoral Strategy in India

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Meet the Author

Kongkita Konwar is a policy researcher, specialising in democratic governance, political communication, and qualitative research. Her academic work is grounded in three years of fieldwork experience across government, civil society, and electoral politics in India, where she developed a deep interest in how political narratives are constructed and weaponised to serve electoral ends. She believes that understanding hate speech not merely as a social harm but as a deliberate strategic instrument is essential to protecting democratic integrity in an era of accelerating disinformation. When not dissecting the mechanics of political manipulation, she restores her faith in humanity by reading, journalling, dancing, and watching trees and sunsets with a well-deserved glass of wine.

Keywords: political hate speech, electoral strategy, communal polarisation, India, ethnic democracy

Abstract

This paper uses the 2024-2025 election cycle as a case study to critically examine how political hate speech is used as a deliberate electoral strategy in India. The paper interprets the empirical data within broader theoretical debates of ethnic democracy, populist reason and dangerous speech. This paper identifies five hate speech patterns: conspiracy theories and dog whistles, construction of demographic threat, religious victimisation, punitive populism, and securitisation politics. It argues that hate speech in India is not a series of random incidents but is institutionally organised from the top down and deeply rooted within the majoritarian state project that Jaffrelot (2021) characterises as 'ethnic democracy.' Applying Mintrom's (2012) Implementation Analysis to three viable policy options: institutional and legal reform, civic peacebuilding, and curriculum development and community dialogue, the findings of the paper highlight that the central governance failure is one of political will rather than legislative absence. Furthermore, the paper contends that the analytical limitations of Implementation Analysis, particularly in its bureaucratic formalism and failure to account for power asymmetries, make it necessary to adopt structural and discourse-oriented perspectives. Finally, the paper concludes that any durable counter-hate speech framework must not only be grounded in institutional autonomy but also media accountability and long-term civic education, while continuing to survive the entrenched political interests that constrain each of these.

Introduction

India's 2024 general election, the largest exercise in history, was marked not only by its magnitude but also for the unusually high level of anti-minority language that dominated its campaign narrative. In March 2024, the United Nations Human Rights Council raised a formal warning of the ongoing attacks on religious and ethnic minorities and urged corrective action before polling began (UNHRC, 2024). Very unfortunately, that warning went unheeded. India Hate Lab (2025, p. 13) reports that in-person hate speech incidents against religious minority increased from 668 in 2023 to 1,165 in 2024 (74.4%) and reached 269 incidents in May during the campaign peak.

The analytical significance of this phenomenon lies not only in how widespread it is, but in its character. According to the argument presented, hate speech in contemporary India is not incidental to elections, but central to them, operating as a coordinated, top-down institutional strategy aimed at building majoritarian solidarity and mobilising votes by creating narratives of existential danger. The distinction between episodic bigotry and structured political violence is central to the (Benesch, 2012; 2016) literature on dangerous speech and to the comparative political sociology of Hindu nationalism (Jaffrelot, 2021; Chatterji, Hansen and Jaffrelot, 2019).

According to Andres and Slivko (2021),

hate speech refers to any communication that is hostile or derogatory towards individuals based on gender, race, religion or political identity. Pukallus and Arthur (2024, p. 467) defined it as 'communicative driver of oppression.' But, in the Indian context, this general definition needs to be refined because what is at stake is not simply offensive speech but what the UNESCO framework identifies as its institutionalisation, the chronic process by which hate speech 'shifts from unconventional exchange to policy discourse' in settings where ruling parties exert dominance over media and regulatory spaces (Gagliardone et al., 2015).

The paper proceeds in the following way. Section 2 explains the methodology used in the study, followed by Section 3 which examines five patterns of political hate speech used as an electoral strategy in India during the 2024-2025 elections. Section 4 looks at possible policy responses using Mintrom's (2012) Implementation Analysis, while Section 5 reflects on the limits of this framework. The paper concludes in Section 6.

Methodology

The sources of this paper are derived from databases of hate speech, legislative records, and Election Commission rules. It also analyses the recorded hate speeches made by politicians. Furthermore, a critical approach is being used here, and it is understood through a specific theoretical framework. This study also builds on existing research about populism, hate speech and how certain issues are constructed to present as security threats. Furthermore, reports from India Hate Lab, Human Rights Watch, Amnesty International, South Asia Justice Campaign, Citizens for Justice and Peace, and the Mahanirban Calcutta Research Group are being reviewed.

Mintrom's (2012, pp. 292-295) Implementation Analysis is applied to the policy options, a seven-step structured approach through which the institutional requirements and threats to successful implementation of policy is systematically interrogated. The selection of this particular framework is due to its emphasis on implementation gaps, which is particularly apt for the Indian context, where the Supreme Court of India itself has noted that 'the root of the problem is not the absence of laws but a lack of effective execution' (PUCL, 2024). The paper, however, also critically interrogates the limitations of this framework, drawing on theory of populist reason by Laclau (2005) and dangerous speech framework by Benesch (2012; 2016) to supplement its bureaucratic logic with structural and discursive analysis.

A note on scope and positionality:
Hate speech events recorded from January 2024 and early 2025, with special focus on the Lok Sabha elections is being covered in this analysis. The report doesn't provide an all-inclusive record of events; however, it does provide evidence that has been verified against external sources, and based upon the evidence provided, it highlights some larger structural trends. Furthermore, it acknowledges the contested nature of 'hate speech' as a category and addresses the tension

between free expression and minority protection that runs through all regulatory debates in this domain.

Key Findings

According to research data collected in 2024, it appears that instances of hate speech were shaped by a clear logical framework rather than a random arrangement. The India Hate Lab (2025) further verified this by reporting that the BJP directly facilitated 340 gatherings, 29.2% of all documented hate speech events. This represents a 580% increase from 2023, when the party organised only 50 such events. Evidently, while the state-level politicians were determined as the primary instigators in 2023, the year 2024 saw national figures such as Prime Minister Narendra Modi, Home Minister Amit Shah, and Uttar Pradesh Chief Minister Yogi Adityanath stand out as the central actors of communal rhetoric (India Hate Lab, 2025). The analysis of this top-down structural shift is significant because it signals not only intensification of societal bigotry but also deliberate mainstreaming of hate speech as an official mode of campaign communication. As the Citizens for Justice and Peace (CJP, 2025) concluded in their structural analysis, the absence of enforcement does not fail to stop speech but actively incentivises it.

In this architecture of electoral hate speech as a political campaign strategy, five recurring patterns can be identified, each linked to a distinct theoretical framework.

Conspiracy Theories and Dog Whistles

The deployment of politically constructed conspiracy theories has served as the ideological bedrock of the BJP's electoral campaigns. The concept of 'love jihad,' a specious claim that Muslim men systematically ensnare Hindu women in marriage to convert them to Islam, functions as what Laclau (2005) would term an 'empty signifier': a term flexible enough to encapsulate a diverse chain of anxieties (demographic decline, sexual vulnerability, cultural contamination) into a single, mobilising narrative. Froystad (2021) similarly identifies these narratives as 'sound bites' whose rhetorical power lies in their ability to trigger affective responses without the burden of evidential support.

By 2024, the entire ecosystem had proliferated into an extensive repertoire: 'land jihad,' 'vote jihad,' 'halal jihad,' and others (India Hate Lab, 2025, pp. 9-10). Critically, in 2024, out of 1,165,
581 hate speech events were documented, in which almost exactly half incorporated such conspiracy theories (India Hate Lab, 2025). In a paradigmatic instance, the Uttar Pradesh Chief Minister, Yogi Adityanath, during his campaign in Jharkhand, alleged that mass Bangladeshi and Rohingya infiltration have led to 'love jihad' and 'land jihad,' posing a threat to women, jobs, and land (Hindutva Watch, 2024). What is notable here is not the content alone but the mechanism: the 'jihad' framing operates through Benesch's (2012) concept of 'accusation in a mirror,' which frames the targeted group as the aggressor in order to rationalise defensive violence against them.

Demographic Threat

The securitisation of cross-border migration, where a social and administrative issue is framed as a security threat, conforms to the theoretical logic advanced by Buzan, Weaver and Wilde (1998, p. 26), who argue that any issue can be transformed into a security matter through 'speech acts' of authorised 'security actors.' In the Indian context, this mechanism has been systematically instrumentalised against Muslim migrants and Indian Muslims, who are constructed as proxies for Pakistani or Bangladeshi infiltrators.

Mahanirban Calcutta Research Group (2024, pp. 12-13) demonstrates how this securitisation has resulted in discriminatory and exclusionary practices in border regions. For instance, the Chief Minister of Assam, Himanta Biswa Sarma, at a campaign event in Jharkhand, stated that the election was about 'driving out infiltrators from Jharkhand and saving Hindus' and he warned that if infiltration went unchecked, the Hindu population in Jharkhand would fall to 50% within twenty years (The Indian Express, 2024). Jaffrelot (2021) argues that such demographic threats serve a dual function, which is that they construct a unified Hindu 'people' by invoking a logic of equivalence, in which all Hindus are framed as subject to a common threat, while concurrently dehumanising Muslims as existential others. Benesch (2016) identifies this exact structure as dangerous speech, in which the target group is portrayed as a moral threat, making violence appear not merely legitimate but necessary.

Religious Victimisation

Construction of Hindu victimhood, reframing majority as a narrative of cultural persecution is one of the most strategically refined elements of BJP's rhetorical repertoire. Prime Minister Narendra Modi's speech at Jaipur on 23rd April 2024, demonstrates this further pattern. He alleged that Congress had protected those who 'pelted stones' at Ram Navami processions and framed the electoral victory of BJP as the guarantee of unrestricted Hindu religious practice. This speech was one of the seventeen by senior BJP officials that appeared to breach Section 123(3) of the Representation of the People Act, 1951, which prohibits seeking votes on religious grounds (MG, 2024). The South Asia Justice Campaign (2024) documented that between the official notification of the election (16 March) and the end of campaigning (29 May), they recorded 380 instances of hate speech by senior political and religious figures, of which 72 (19%) met the UN's threshold for 'top-level' hate speech. Despite multiple complaints, the Election Commission of India (ECI) failed to take meaningful action, and the Election Commission Chief later admitted that the body had 'deliberately decided' not to take action against the top leaders of either party, which reflects a statement amounting to institutional abdication (The Wire, 2024).

Punitive Populism

Punitive populism, as defined by Wood (2014), refers to the mobilisation of popular support for coercive state action against targeted groups, framed as a means of restoring social order. In the Indian context,

this has taken its most visceral form, commonly known as 'bulldozer justice,' which involves the extrajudicial demolition of Muslim properties, residences and places of worship by state authorities, often within the immediate aftermath of communal incidents.

Amnesty International (2024) documented 128 targeted demolitions across Assam, Delhi, Gujarat, Madhya Pradesh, and Uttar Pradesh. A paradigmatic example occurred on 15 June 2024 in Mandla, Madhya Pradesh, where homes of eleven Muslim families were demolished by authorities on the basis of alleged presence of beef in their refrigerators, while sixteen other allegedly illegal structures in the same neighbourhood, where no beef was found, were left intact (Anand Mohan J, 2024). While the Supreme Court subsequently issued guidelines to restrain discriminatory demolitions (Karpuram, 2024), the political symbolism of bulldozer had already been established as a campaign signifier, which in Laclau's (2005) terms, is a floating signifier, which is capable of condensing anxieties about crime, sovereignty, and Muslim presence into a single image of majoritarian authority. As the CJP (2025) observed, the bulldozer has become a 'structured and institutionalised feature of Indian political life,' not an aberration.

Securitisation politics

The final and most dangerous pattern is expressed through the explicit dehumanisation of minority communities in the language of national security, a move that Benesch (2012) identifies as a key indicator of dangerous speech, associated historically with the escalation of mass violence. For instance, the Chief Minister of UP, Yogi Adityanath, stated that the opposition would 'distribute the wealth of the citizens to infiltrators, i.e., Bangladeshi, Pakistani or any other Muslim infiltrators' (India Hate Lab, 2025, p. 28). This is precisely what Benesch (2016) describes as a 'accusation in a mirror' logic, which represents the targeted group as the aggressor. This represents the advancement of what Jaffrelot (2021, p. 82) terms as 'civilisational majoritarianism', a systemic project aimed toward Hinduisation of India through exclusion and subordination of minority populations.

Evaluating Policy Options

Mintrom's (2012) Implementation Analysis is used to evaluate three policy options. The framework is used not as a technocratic exercise but as a structured lens for identifying where the gap between policy aspiration and political reality is more pronounced and why.

Strengthening Institutional Mechanisms and Legal Procedures

India's existing legal framework is not devoid of resources. Sections 153A and 295A of the Indian Penal Code criminalise communal incitement. Section 123(3) of the Representation of the People Act, 1951, prohibits religious appeals in electoral campaigns, and the ECI's Model Code of Conduct (MCC) sets out an explicit prohibition of communal campaigning. As the Supreme Court itself has acknowledged, the central failure is not the absence of law but paralysis in

enforcement (PUCL, 2024). The ECI, once regarded as a global reference model for electoral administration, has, in The Wire (2024) assessment, become 'increasingly susceptible to executive influence,' with its Chief Commissioner publicly defending the non-imposition of sanctions on the Prime Minister for speech violations through the logic of institutional symmetry.

Howlett (2018) argues that effective policy instruments must 'fit' their operational context. In this context, the ECI cannot be an impartial enforcer when its independence is itself contested. The 2024 election saw thousands of citizen complaints lodged with the ECI regarding Modi's speeches with no meaningful impact (HRW, 2024). According to a PUCL (2024) cited survey, 58% of respondents expressed distrust in the ECI by 2024, compared with 78% who reported trust following the 2019 elections.

At a minimum, institutional reform would need to establish constitutionally guaranteed independence for ECI appointments, removing any executive discretion. A multilingual monitoring unit for hate speech, with algorithmic support akin to Indonesia's NLP-based BAWASLU system (Wijnarko et al., 2024); time-bound, mandatory investigation protocols for Model Code of Conduct (MCC) violations; and alongside graduated sanctions that apply even to 'star campaigners.' The Implementation Analysis highlights that the main threat to this option is political, as enforcement institutions are controlled by those whose behaviour the policy seeks to curb.

Civic Engagement for Peacebuilding

The structural problem with India's media landscape, which Gagliardone et al. (2015) identify as the capture of media houses by ruling parties, is exacerbated by ownership patterns, particularly the fact that several leading Hindi-language television networks are owned by individuals closely tied to the BJP government. As Pukallus and Arthur (2024) proposed, this concentration of media power means that the 'mixed advisory and regulatory space' faces a fundamental challenge, which suggests that any government-involved body is vulnerable to co-optation, while civil society options lack the power to enforce decisions.

Nonetheless, civil society initiatives represent a crucial second line of resistance. A glaring example could be derived from the Hate Speech Beda initiative in Karnataka (Boruah, 2024), which tracks, documents, and legally challenges hate speech, demonstrating that civil society can create accountability pressure even without institutional enforcement powers. Through training young leaders to map digital identity dynamics (Pukallus and Arthur, 2024, p. 478), the DMAPS programme presents a scalable model for strengthening media literacy and counter-narrative capacity. The Strong Cities Network (2024) provides an international framework for local government engagement.

Curriculum Development and Community Dialogue

According to UNESCO (2023), critical thinking, socio-emotional skills, and intercultural dialogue should be integrated

into education systems as foundational commitments rather than supplementary initiatives. Education as a long-term resilience is the most compelling case, as Gagliardone et al. (2015) argues that societies with stronger traditions of civic education and historical literacy have shown greater resistance to the escalation of hate speech. However, in the context of Indian curriculum, a specific political challenge since 2014 is that the BJP government, through systematic textbook revisions, has reduced the prominence of content perceived as incompatible with Hindutva ideology, such as India's pluralist constitutional traditions, the cultural contribution of Muslim rulers, and historical accounts of communal violence (Chatterji, Hansen and Jaffrelot, 2019).

Community-based programmes which are delivered through non-governmental organisations, self-help groups, and civil society organisations at the grassroots level face fewer risks of top-down curricular intervention than state-controlled education systems and are better positioned to address India's rural-urban education divide. A particularly relevant example here is The Strong Cities Network (2024) model, which equips local authorities with context-specific interventions against hate speech and polarisation. The Implementation Analysis highlights that implementation may be hindered by institutional inertia in state education boards, many of which are under BJP-aligned administrators.

Critical Reflection on Implementation Analysis:

Mintrom's (2012) Implementation Analysis is a useful diagnostic tool precisely because it rejects the idea that well-designed policies lead to well implementation of its own.

However, the limitations of the framework are significant and must be acknowledged. In the Indian context, where the ECI, the judiciary, and the police operate under varying degrees of political pressure from the executive, a framework that assumes these institutions as neutral agents of implementation misconstrues the political economy of implementation. As Fowler (2021) contends that ambiguity and uncertainty in implementation are not merely administrative problems but are often politically produced and politically functional, allowing powerful actors to selectively apply or ignore rules fitted to their interests. Second, the framework's emphasis on quantifiable outcomes (a 30% reduction in hate speech events or faster response times) risks reducing a multifaceted social and political phenomenon to a set of administrative metrics. Hate speech is not simply an outcome to be measured and reduced; it reflects deeper structures of ethnic majoritarianism whose roots demand structural political remedies, not just administrative. Laclau's (2005) theory of populist reason is illuminating here: BJP's construction of 'the Hindu people' against the Muslim 'other' is not a policy error to be corrected but a hegemonic project that has reshaped Indian political identity. No implementation plan addresses this at the level of its political logic. Third, the framework pays insufficient attention to multilingual complexity. India's hate speech landscape is overwhelmingly

conducted in Hindi and regional languages; the most dangerous speeches are often precisely those that do not translate into English and therefore escape the notice of national and international monitoring systems. This requires not merely regional language moderators but a fundamentally multilingual approach to monitoring, adjudication, and counter-speech.

Conclusion

Since 2014, under the BJP, political hate speech in India has undergone a qualitative transformation. What was once episodic has become normalized in a more structured, top-down strategy through the manufacture of Muslim threat. The 74.4% surge in verified hate speech events between 2023 and 2024, and the 580% increase in BJP-organised hate speech gatherings. From normalisation of hate speech to the institutionalisation of what Jaffrelot (2021) calls ethnic democracy and what Benesch (2012) identifies as the preconditions for dangerous speech to produce real-world violence.

The Implementation Analysis of three policy responses - institutional reform, civic peacebuilding, and education - reveals that none is sufficient in isolation, as all confront a common structural obstacle in which the political actors driving hate speech also control its regulators. ECI reform without genuine constitutional independence will remain cosmetic; media regulation without structural change in ownership patterns will be captured; curriculum reform without political independence will be reversed. As mentioned above, the Supreme Court's own observation that the problem is not the absence of law, but the failure of execution implies that implementation is not a technical problem but a political one.

The paper has its own limitations. The reliance on English-language sources and documented incidents inevitably undercounts the true volume of hate speech in regional language contexts, where the most virulent and locally effective rhetoric circulates. Implementation Analysis, as argued above, underestimates the structural and discursive dimensions of the problem. Future research should engage more deeply with the lived experiences of affected minority communities' perspectives that are systematically absent from policy-oriented frameworks, and with the comparative literature on hate speech regulation in analogous contexts of democratic backsliding, including Hungary, Brazil, and the United States. What India's trajectory makes clear, however, is that the defence of pluralist democracy against majoritarian populism is not primarily a legal or institutional problem, but a political and moral one, and that the international community's capacity to address it is constrained by the very norms of sovereignty and non-interference that authoritarian populists routinely exploit.

Serum Hydrogen Sulphide as a Biochemical Correlate of Major Depressive Disorder: A Cross-Sectional Case-Control Study from a Tertiary Care Centre in Sub-Himalayan India

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Atrim is an MSc Molecular Neuroscience student, with a background in Biotechnology. Atrim's academic interests lie at the intersection of neuroscience, biotechnology, artificial intelligence, and translational medicine. Through research experiences, Atrim has explored areas ranging from psychiatric biomarkers and clinical biochemistry to gene therapy, molecular cloning, and AI-driven healthcare tools. Atrim is particularly interested in how molecular and computational approaches can help us understand brain disorders and develop more accessible, precise, and patient-centred solutions, and hopes to contribute to research that bridges laboratory science, clinical insight, and real-world impact.

Keywords: hydrogen sulphide; major depressive disorder; gasotransmitter; biomarker; neuroinflammation

Abstract

Hydrogen sulphide (H_2S) acts as a vital signalling molecule in the brain, regulating neuroinflammation and long-term potentiation (LTP), the sustained strengthening of synaptic connections necessary for memory. Since these neural pathways are often disrupted in Major Depressive Disorder (MDD), this study investigated whether measurable serum H_2S concentrations differ between depressed patients and healthy individuals. This cross-sectional study evaluated 50 drug-naive MDD patients and 50 healthy controls. Depression severity was assessed using the Hamilton Depression Rating Scale (HAM-D-17), and serum H_2S was quantified via a spectrophotometric methylene blue method. Mean serum H_2S was lower in the MDD group (0.068 ± 0.044 µmol/L) than in healthy controls (0.541 ± 0.272 µmol/L), representing an approximately 8-fold difference. HAM-D-17 scores confirmed very severe depression in the MDD cohort (28.94 ± 12.78 versus 3.96 ± 2.31). No significant correlation between serum H_2S and symptom severity was found within the MDD group. These findings suggest that substantial H_2S depletion is a core biochemical feature of untreated MDD, supporting the neuroprotective role of H_2S and highlighting the CBS/CSE–H_2S axis as a candidate biomarker and therapeutic target warranting further investigation.

1. Introduction

Major Depressive Disorder (MDD) is a globally disabling condition affecting approximately 280 million people, ranking as a leading cause of years lived with disability[1]. The World Health Organisation defines MDD as a persistent depressed mood or loss of pleasure lasting at least two weeks, paired with physical and cognitive disruptions that severely interfere with daily life.1 Despite decades of research, the exact mechanisms remain partially obscured, and approximately one-third of patients do not respond to standard first-line medications [2]. For over sixty years, the monoamine hypothesis has dominated antidepressant research, attributing depression to deficits in serotonin, norepinephrine, and dopamine. While influential, this model struggles to explain the delayed onset of antidepressant action, high rates of treatment resistance, and the heterogeneity of depressive symptoms.3 Scientific consensus is shifting toward a broader framework encompassing disrupted synaptic plasticity, dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, neuroinflammation, and oxidative stress [4,5]. Hydrogen sulphide (H_2S) represents a promising candidate in this search. Known alongside nitric oxide and carbon monoxide as the third member of the gasotransmitter family, H_2S was once viewed strictly as an environmental toxin [6] but is now recognised as a naturally occurring signalling molecule. In the brain, it is primarily synthesised by cystathionine-beta-synthase (CBS), with cystathionine-gamma-lyase (CSE) operating in peripheral tissues and

3-mercaptopyruvate sulphur transferase (3-MST) functioning within neurons [7]. CBS is highly concentrated in the hippocampus and cerebellum, predominantly in astrocytes, and is activated by calcium and calmodulin. At physiological concentrations, H_2S directly supports hippocampal long-term potentiation (LTP) by enhancing NMDA receptor activity, a process essential for memory and learning [8]. Subsequent research has expanded our understanding of H_2S, demonstrating that it suppresses neuroinflammation by modulating microglial and astrocytic activity, mitigates oxidative stress by restoring superoxide dismutase activity, modulates the HPA axis by suppressing corticotropin-releasing hormone, promotes neurogenesis via BDNF-related pathways, and activates the mTORC1 signalling pathway similarly to rapid-acting antidepressants [9,10,11]. In animal models subjected to chronic unpredictable stress, hippocampal H_2S is consistently depleted, and administering an H_2S donor reliably reverses depressive behaviours.11 Despite robust preclinical findings, direct measurements of circulating H_2S in human MDD patients remain scarce. Yang et al. (2021) reported significantly lower plasma H_2S in depressed patients, with a negative correlation with symptom severity scores [12]. Dutta et al. (2024) found reduced serum H_2S in an MDD cohort from North Bengal, identifying a potential diagnostic threshold of less than 78.5 µmol/L with 96% sensitivity and 88% specificity [13]. A primary limitation of earlier studies is the inclusion of patients already receiving pharmacological treatment, which introduces confounding due to the antioxidant effects of antidepressants. India faces a severe burden of untreated depression. The National Mental Health Survey (2015–2016) indicated that one in twenty Indians experiences depression, yet over 75% remain untreated [14]. Developing accessible and cost-effective biological markers could improve early detection in resource-limited settings. This study was therefore designed to compare serum H_2S concentrations between strictly drug-naive MDD patients and healthy controls at a tertiary care centre in the sub-Himalayan foothills of West Bengal, using the Hamilton Depression Rating Scale (HAM-D-17) for clinical assessment and the spectrophotometric methylene blue method for biochemical quantification.

2. Methods

2.1 Study Design and Setting

A hospital-based, cross-sectional case-control study was conducted over twelve months (January to December 2024) at North Bengal Medical College and Hospital (NBMCH), Sushruta Nagar, Darjeeling, a 1,000-bed tertiary referral centre serving the sub-Himalayan region. The research protocol received formal ethical approval from the NBMCH Institutional Ethics Committee, and all participants provided written informed consent in their preferred language.

2.2 Participants

Fifty consecutive patients attending the Psychiatry outpatient department who met DSM-5 criteria for MDD were enrolled. All were classified as drug-naive, defined as having received no psychotropic medications for at least six weeks before

enrolment. A control group of fifty healthy volunteers with no personal or close family history of psychiatric illness was recruited. All participants were aged 18 to 65 years and capable of providing informed consent. Individuals with chronic physical illness, co-occurring psychiatric conditions, substance use disorders, or bipolar depression were excluded. Sample size was calculated based on previously published standard deviations for serum H_2S in depressed and healthy populations, yielding a minimum of 47 participants per group (rounded to 50).

2.3 Clinical Assessment

A standardised questionnaire collected demographic data, including age, sex, residence, language, religion, marital status, education, occupation, and family structure. Socioeconomic status was assessed using the Modified B.G. Prasad Scale. Depression severity was quantified by trained psychiatrists using the Hamilton Depression Rating Scale (HAM-D-17), a validated 17-item structured interview scored 0–52, with established thresholds: no depression (0–7), mild (8–12), moderate (13–17), severe (18–24), and very severe (≥25) [15].

2.4 Biochemical Analysis

A 5 mL venous blood sample was collected from each participant into a standard red-cap tube and processed immediately. Serum H_2S concentration was quantified using the validated spectrophotometric methylene blue method. Briefly, serum was mixed with phosphate-buffered saline and trichloroacetic acid, centrifuged at 3,000 rpm for 30 minutes, and the supernatant was treated with zinc acetate to precipitate sulphide. The precipitate was dissolved in N, N-dimethyl-p-phenylenediamine sulphate and ferric chloride, followed by sodium hydroxide. After 10 minutes of incubation, absorbance was measured at 650 nm using a double-beam UV/Visible spectrophotometer. Concentrations were calculated against a sodium hydrosulphide standard curve (R^2 = 0.99) and expressed in µmol/L.

2.5 Statistical Analysis

Data were organised in Microsoft Excel and analysed using IBM SPSS version 20. Continuous variables are presented as mean ± standard deviation, and categorical variables as counts and percentages. Group differences in continuous variables (serum H_2S, optical density, HAM-D-17 score) were assessed using independent-samples t-tests after confirming approximate normality by histogram and Q-Q plot inspection. Associations between categorical sociodemographic variables and serum H_2S were evaluated using chi-square tests. The relationship between serum H_2S and HAM-D-17 score was examined by linear regression within each group and across the combined sample. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Sociodemographic Characteristics

The study enrolled 100 participants (50 MDD, 50 controls). Both groups shared highly similar demographic profiles with no statistically significant differences across any variable (all $p > 0.05$, chi-square test; Table 1). Most participants in both cohorts were young adults aged

Variable	Category	MDD (n=50) n (%)	Controls (n=50) n (%)	p-value
Age (years)	<20	7 (14.0)	2 (4.0)	
	21–30	23 (46.0)	22 (44.0)	
	31–40	12 (24.0)	15 (30.0)	
	41–50	4 (8.0)	3 (6.0)	
	51–60	4 (8.0)	8 (16.0)	>0.05
Sex	Female	37 (74.0)	36 (72.0)	
	Male	13 (26.0)	14 (28.0)	>0.05
Residence	Rural	39 (78.0)	35 (70.0)	
	Urban	11 (22.0)	15 (30.0)	>0.05
Marital status	Single	31 (62.0)	23 (46.0)	
	Married	10 (20.0)	22 (44.0)	
	Widowed	9 (18.0)	5 (10.0)	>0.05
Education	Illiterate	25 (50.0)	16 (32.0)	
	Primary	10 (20.0)	23 (46.0)	
	High School	5 (10.0)	7 (14.0)	
	Graduate+	10 (20.0)	4 (8.0)	>0.05
Family type	Nuclear	34 (68.0)	31 (62.0)	
	Joint	16 (32.0)	19 (38.0)	>0.05
SES (Prasad)	Class I–II	13 (26.0)	15 (30.0)	
	Class III	5 (10.0)	4 (8.0)	
	Class IV–V	32 (64.0)	31 (62.0)	>0.05
Duration of illness (months)	Mean ± SD	19.26 ± 10.63	18.32 ± 10.32	>0.05

Table 1: Sociodemographic profile of MDD cases and healthy controls (n = 100) SES = socioeconomic status; Prasad = Modified B.G. Prasad Scale 2024. All p-values from the chi-square test.

Parameter	MDD Group (n=50) Mean ± SD	Control Group (n=50) Mean ± SD	Difference	p-value
Serum H_2S (µmol/L)	0.068 ± 0.044	0.541 ± 0.272	~8-fold lower in MDD	< 0.0001
Optical Density (650 nm)	1.44 ± 1.18	81.32 ± 57.85	~56-fold lower in MDD	< 0.0001
HAM-D-17 Score	28.94 ± 12.78	3.96 ± 2.31	Very severe vs Normal	< 0.0001
Duration of illness (months)	19.26 ± 10.63	18.32 ± 10.32	Comparable	> 0.05

Table 2: Key biochemical and clinical parameters in MDD cases and healthy controls (n = 100). H_2S = hydrogen sulphide; HAM-D = Hamilton Depression Rating Scale; SD = standard deviation. All group comparisons by an independent-samples t-test after confirming approximate normality by histogram and Q-Q plot inspection.

21–30 years. Females accounted for 74% of the MDD group and 72% of controls, consistent with the well-documented higher prevalence of depression in women. The majority of participants across both groups were from rural areas, spoke Bengali, and fell into lower socioeconomic tiers (Classes IV and V, Modified B.G. Prasad Scale). Patients with MDD were more likely to be single (62% vs 46%) and had higher rates of illiteracy (50% vs 32%). Mean illness duration in the MDD cohort was 19.26 ± 10.63 months.

3.2 Biochemical and Clinical Parameters

Mean serum H_2S was lower in the MDD group (0.068 ± 0.044 µmol/L) than in healthy controls (0.541 ± 0.272 µmol/L) [Table 2], representing an approximately 8-fold difference ($p < 0.0001$, independent-samples t-test; Figure 1A). This difference was directly reflected in the raw optical density readings at 650 nm, which averaged 1.44 ± 1.18 in the MDD group versus 81.32 ± 57.85 in controls ($p < 0.0001$; Figure 1C). HAM-D-17 scores confirmed very severe depression in the MDD cohort (28.94 ± 12.78) compared with the normal range in controls (3.96 ± 2.31; $p < 0.0001$; Figure 1B).

3.3 Correlation Between H_2S and Depression Severity

Within the MDD group (Figure 2A), no significant correlation was observed (R^2 = 0.061, p = n.s.), with data points widely scattered without a clear trajectory. This indicates that while H_2S is substantially lower in clinical depression, the degree of depletion does not act as a linear predictor of symptom severity once the disorder is established. This pattern is consistent with a threshold-like relationship, in which H_2S may need to fall below a critical level for MDD to manifest, with secondary factors such as genetics and psychosocial stressors then determining symptom severity, though this interpretation remains speculative and requires prospective testing. In contrast,

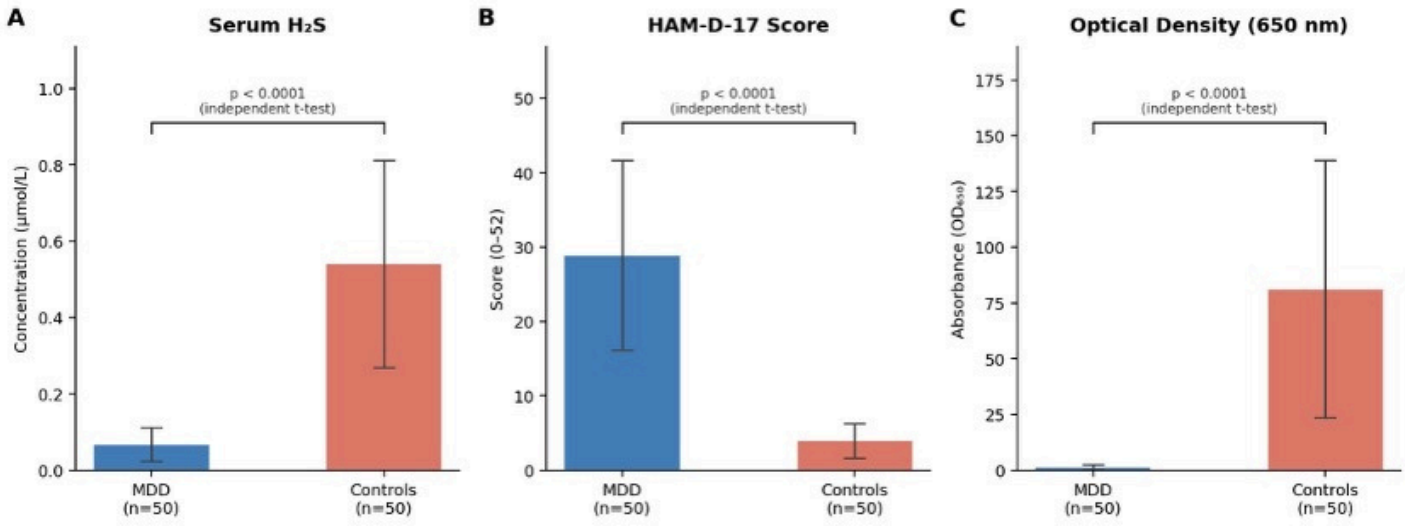


Figure 1: Group comparison of key biochemical and clinical parameters (n = 100). Bars = group means; error bars = ±1 SD. All comparisons by independent-samples t-test, $p < 0.0001$. (A) Serum H_2S is approximately 8-fold lower in MDD. (B) HAM-D-17 scores confirm very severe depression in MDD vs. the normal range in controls. (C) Optical density at 650 nm mirrors the H_2S difference.

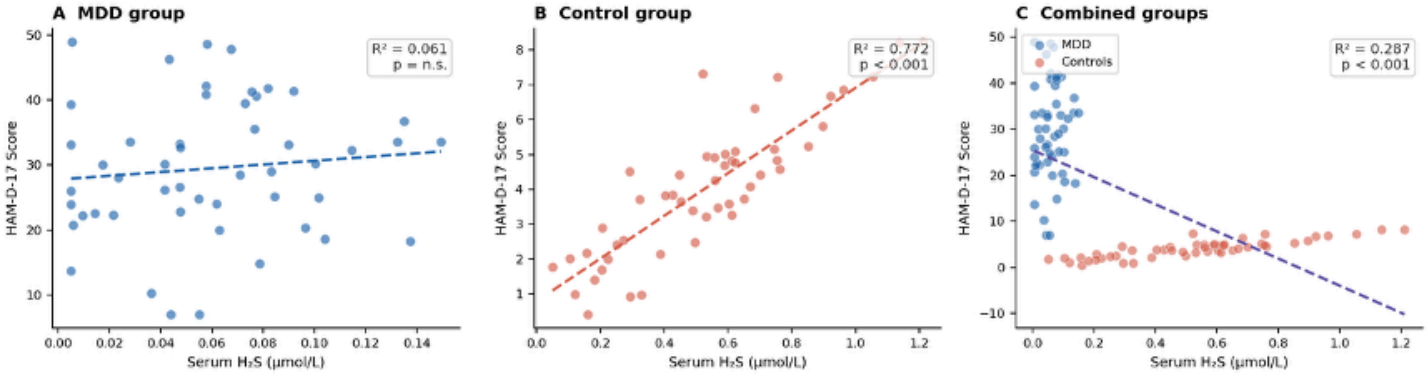


Figure 2: Linear regression analysis: serum H_2S vs HAM-D-17 score. (A) MDD group: no significant correlation ($R^2 = 0.061$, p = n.s.), consistent with a threshold-like relationship. (B) Control group: strong positive correlation ($R^2 = 0.772$, $p < 0.001$). (C) Combined groups: moderate negative correlation ($R^2 = 0.287$)

the control group displayed a strong positive correlation ($R^2 = 0.772$, $p < 0.001$; Figure 2B), reflecting the natural co-variation of H_2S and mood-related measures in the absence of psychiatric illness. The combined dataset showed a moderate negative correlation ($R^2 = 0.287$; Figure 2C), confirming that higher H_2S concentrations generally correspond to lower depressive symptom burden across the full sample.

3.4 Associations with Sociodemographic Variables

Chi-square analyses in both the MDD and control groups found no statistically significant associations between serum H_2S concentration and any sociodemographic variable, including age, sex, residence, language, religion, marital

status, education, occupation, socioeconomic status, family type, or duration of illness (all $p > 0.05$). This suggests that the observed H_2S depletion in MDD reflects a disease-specific biochemical phenomenon rather than demographic or social determinants.

4. Discussion

This study provides direct clinical evidence from a drug-naive South Asian population that serum H_2S is substantially lower in untreated MDD than in healthy controls. An approximately 8-fold lower mean serum H_2S in MDD patients compared with healthy controls is consistent with the hypothesis that H_2S deficiency is a biological correlate of MDD, though the cross-sectional design precludes causal inference. By focusing on a drug-naive cohort, this research offers a clearer view of the disease's baseline biochemistry, unconfounded by antidepressant effects. The near-complete suppression of circulating H_2S (mean 0.068 µmol/L) aligns with earlier findings by Yang et al. (2021) [12] and Dutta et al. (2024) [13]. Biologically, this is coherent: H_2S is essential for activating NMDA receptors to stimulate LTP, the process of strengthening neural connections critical for emotional regulation and memory [8,4]. Its depletion removes several layers of neurological defence. Normally, H_2S acts as a potent antioxidant and animal models show that replenishing it restores chemical balance and reverses depressive behaviours.11,5 It also modulates the brain's immune response, lowering inflammatory markers frequently elevated in depressed patients [16], buffers against stress hormones [17], encourages neurogenesis [11], and activates rapid-acting neural pathways similar to ketamine [9]. The absence of a significant within-MDD correlation between serum H_2S and HAM-D-17 score ($R^2 = 0.061$, p = n.s.) suggests that serum H_2S distinguished MDD patients from controls more clearly than it tracked symptom severity within the MDD group. This pattern is compatible with a threshold-like relationship, in which H_2S may fall below a critical range in MDD, while other factors such as genetics, inflammation, sleep disruption, illness duration, and psychosocial stress shape clinical severity. However, this interpretation remains speculative and requires prospective testing. The control-group association should also be interpreted cautiously because HAM-D-17 scores in healthy participants occupy a narrow non-clinical range. Similarly, the combined-cohort correlation likely reflects case-control separation rather than a continuous severity relationship within MDD. The strong positive correlation in healthy individuals ($R^2 = 0.772$) confirms that H_2S naturally fluctuates alongside healthy mood regulation. Crucially, social factors like whether a patient was rural, single, or facing educational barriers had no significant impact on H_2S concentrations, confirming that the H_2S deficit is a distinct biochemical feature of the disease rather than a reflection of sociodemographic disadvantage. These results carry implications for psychiatric care. The spectrophotometric methylene blue method is low-cost and requires only standard laboratory equipment, making H_2S measurement feasible in resource-limited settings. Beyond diagnostics, this study points toward therapeutic

innovation: drugs designed to safely release H_2S have already shown efficacy in reversing depression-like symptoms in animal models, offering a potential new avenue for antidepressant development [18]. This study has several limitations. The cross-sectional design captures a single time point and cannot establish whether H_2S depletion precedes, accompanies, or follows the onset of MDD. The sample was drawn from a single tertiary centre, which may limit generalisability. Formal normality testing was not performed before parametric analysis, and future studies should consider non-parametric alternatives given the near-floor distribution of H_2S in the MDD group. Multivariate adjustment for potential confounders such as diet, physical activity, and gut microbiome composition was not performed. Future directions include longitudinal tracking to determine whether H_2S drops before a depressive episode, cerebrospinal fluid analysis to map H_2S directly within the central nervous system, and exploration of dietary and genetic influences on this protective gasotransmitter.

5. Conclusion

This study demonstrates that serum H_2S is substantially and specifically lower in drug-naive MDD patients compared with healthy controls, independent of sociodemographic factors. The finding is biologically coherent with H_2S's established roles in hippocampal synaptic plasticity, neuroinflammation, oxidative stress defence, HPA axis regulation, and neurogenesis, all pathways disrupted in MDD. The CBS/CSE–H_2S axis warrants further investigation as a candidate biomarker and therapeutic target in major depressive disorder.

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