CORDAP R&D TECHNOLOGY ROADMAP FOR

UNDERSTANDING NATURAL ADAPTATION AND ASSISTED EVOLUTION OF CORALS TO CLIMATE CHANGE

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SECTION 1

Executive Summary

Coral reefs cover less than 1% of the ocean floor yet support up to 30% of all marine life (Plaisance *et al.*, 2011) and generate US$6.8 billion in annual net profits from global fisheries (Burke *et al.*, 2011).

Reefs also help reduce coastal floods, saving US$1 billion per year in the US alone (Reguero *et al.*, 2021), and attract US$36 billion in global tourism annually (Spalding *et al.*, 2017).

Since the 1950s, however, coral cover has declined due to overfishing, coastal development, and systemic increases in pollution and ocean temperature.

By 2050, and without considering adaptation, 70% to 90% of coral reefs are likely to disappear, given a warming scenario of only 1.5 °C; with warming of 2 °C, 99% of all coral reefs will be lost in less than 30 years (IPCC, 2022).

A new '30x30' COP15 initiative aims to protect 30% of the world's land, freshwater, and sea areas by the year 2030 (CBD, 2022). This effort strives to safeguard ecosystems that are biologically essential due to the goods and services they provide, in addition to maintaining a diverse range of habitat types.

Coral reefs provide a myriad of ecological benefits to ocean and coastline communities, including human populations, and are noted as a critical ecosystem to prioritise (ICRI, 2022). Protection in this context is multi-faceted and entails:

- Mitigating impacts on global (e.g., reduction of greenhouse gas emissions) and local (e.g., management of water quality and invasive species) scales (Mumby & Steneck, 2008).
- Increasing the success of programmes to restore degraded reefs (Knowlton *et al.*, 2021; National Academies of Sciences & Medicine, 2019).
- Identifying coral populations that are resistant or that might recover rapidly after heat stress events (ICRI, 2022).
- Understanding how to assist corals with adaptation to ocean warming (Baums *et al.*, 2019; Goergen *et al.*, 2020; Shaver *et al.*, 2022).

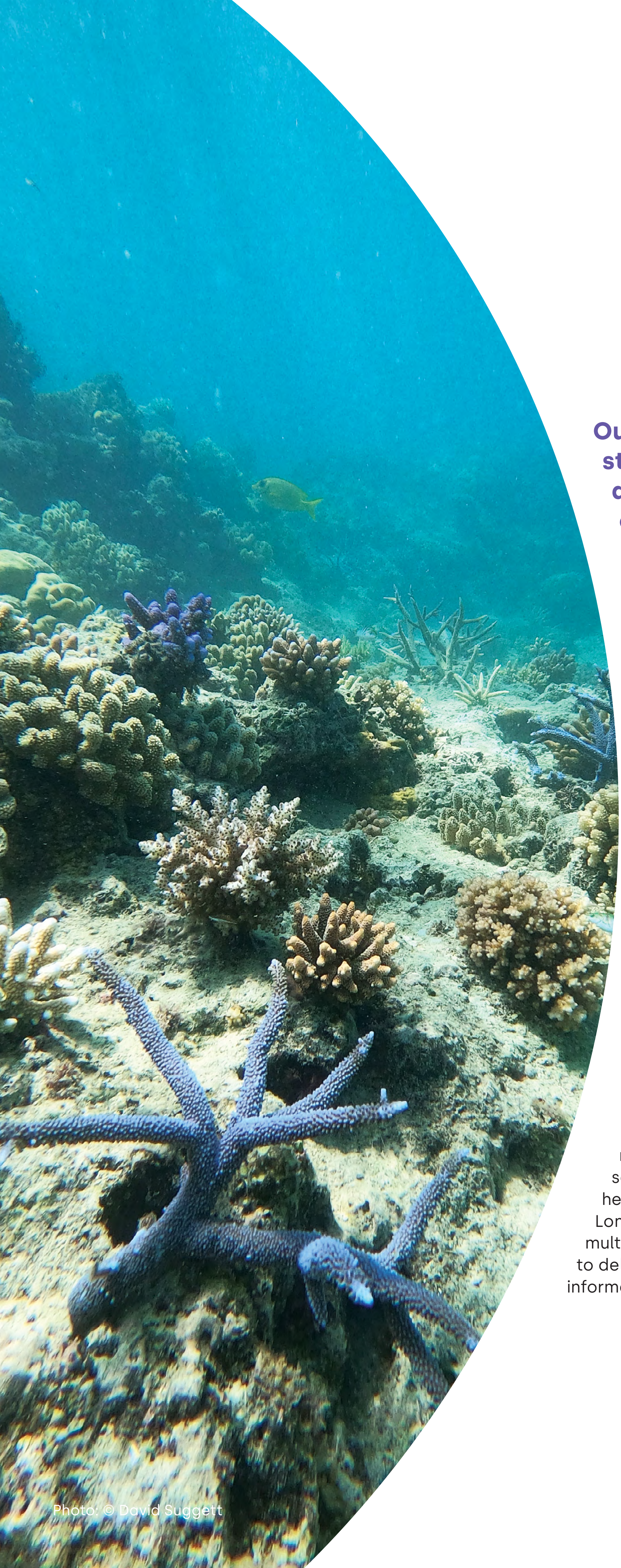
Substantial investment, such as through the Reef Restoration and Adaptation Program (RRAP), the Defense Advanced Research Projects Agency (DARPA), and the Coral Research & Development Accelerator Platform (CORDAP) is needed to support Research & Development (R&D) for novel natural resource management tools that enhance the capacity of corals to adapt to climate change (i.e., assisted evolution; van Oppen *et al.* 2015).

Assisted evolution refers to human actions seeking to accelerate the rate of evolutionary processes within populations or species to help them adapt to a changing environment.

Over recent years the number of studies on the assisted evolution of corals has increased dramatically, with most able to demonstrate enhanced tolerance of the coral holobiont (that is, the cnidarian host, the photosynthetic symbionts and other microbes), (Drury *et al.*, 2022 and more). Yet significant knowledge gaps exist in our fundamental understanding of how coral heat tolerance evolves and the efficacy or effect size of interventions that aim to improve this process.

Further, the technologies or strategies to implement these approaches at scale are largely absent in both restoration and adaptation contexts. As a result, resources including funding and time cannot be applied effectively and strategically. To address these issues, we evaluated gaps in knowledge about coral thermal adaptation and the interventions used to enhance their tolerance and adaptation to global heating. Specifically, we:

1. Systematically reviewed studies published between 1975 and 2022 on the responses of corals, their microalgal symbionts (hereafter called symbionts), or bacterial microbiota (hereafter referred to as microbes) to heat stress either under laboratory conditions or during marine heatwaves. We compiled studies into a database, which we used to objectively examine what is known about the potential for natural adaptation of corals to increased temperatures and the effect sizes of assisted evolution efforts that have been documented to date.
2. Obtained the expert opinions of 22 coral reef scientists currently leading research and development on assisted evolution and six evolutionary biologists via a 3-day workshop at King Abdullah University of Science and Technology (KAUST) in Saudi Arabia (24 - 26 January 2023), as well as via pre- and post-workshop questionnaires. In doing so, we identified gaps in fundamental knowledge of coral evolution and established priorities for knowledge acquisition relevant to using management strategies that aim to increase adaptive resilience.
3. Prepared a report and synthesised findings into a Roadmap using the systematic review of the literature, questionnaires, and workshop discussions. The recommendations for funding prioritisation that we provide here were informed by the participants of the workshop, a review of the literature, and weekly discussions amongst the ten participants comprising the core group.



Our was to develop a visionary strategy for research into the adaptation of corals to climate change via assisted evolution.

We make tangible recommendations for future investment that will fill current critical knowledge gaps to assist coral reefs in a warming world and provide recommendations for R&D on natural adaptation and assisted evolution, especially related to heat tolerance.

The workshop discussed the challenges of working with corals and their associated symbionts and microbes, as well as future climate predictions and the potential impact of climate change on coral cover. The logistic constraints, known and unknown effects, and effect sizes of assisted evolution methods were discussed.

The group unanimously agreed that assisted evolution methods cannot be understood and evaluated without a solid foundational understanding of natural adaptation.

Many gaps remain in the knowledge of corals' natural adaptation to temperature stress, and filling these gaps is important to ensure the success of assisted evolution methods.

- We argue that R&D to understand natural adaptation and assisted evolution must be fast-tracked, and propose that standardising methods, experimental designs, species selection guidelines and terminologies will help achieve this understanding more rapidly Long-term funding is critical to facilitate multigenerational studies, which are needed to deliver essential, but largely missing information about coral evolution (Figure 1).

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- We developed the R&D Roadmap for Understanding the Natural Adaptation and Assisted Evolution of Corals to Climate Change based on four foundational elements:

  Theme 1: Lead global coordination and synthesis.

  Theme 2: Optimise generation and use of knowledge.

  Theme 3: Fill critical knowledge gaps in multigenerational coral data in the laboratory and field.

  Theme 4: Support the advance of existing and new technologies.

  The Roadmap identifies the funding structures and research priorities that are most likely to yield the knowledge needed to ensure that assisted evolution methods can be implemented effectively.

  Ultimately, conserving and restoring coral reefs in warming climates will require an inclusive infrastructure involving many partners at local, national and international scales (Table 1).

- Assisted evolution methods can be applied to corals and their symbionts and include the physiological and genetic mechanisms associated with acclimatisation and adaptation. Using the database and register of ongoing projects that we constructed as part of this project (database available here), we identified eight methods that are currently under evaluation globally, as well as several others that have been described but are not under current investigation.

  Whilst substantial variation in the terminology still exists, the methods are hereafter referred to in this Roadmap as: assisted migration[1], transgressive segregation[2], intraspecific hybridisation or assisted gene flow[3], hybridisation between species, conditioning, algal symbiont and microbiome manipulation, and gene editing (Figure 3).

  While the number of studies on assisted evolution is rapidly growing, only a fraction permit the calculation of effect sizes (i.e., quantifiable increases in thermal tolerance) across life stages and generations - information that is essential to understanding the long term benefits of these methods (Table 4).

  At present, current knowledge is insufficient to critically assess the benefits and risks of assisted evolution methods, especially across coral generations.

- Finally, we recommend investment into a mixed portfolio of R&D, ranging from technologies with lower perceived risks to those with higher risks and longer R&D horizons. This strategy is advised because of uncertainty around future heating trajectories and thus requirements for enhancement of tolerance.

1. Intraspecific breeding outside current range.
2. Intraspecific breeding between extreme environments.
3. Intraspecific breeding within and between reefs within the current range.

Photo: © CORDAP

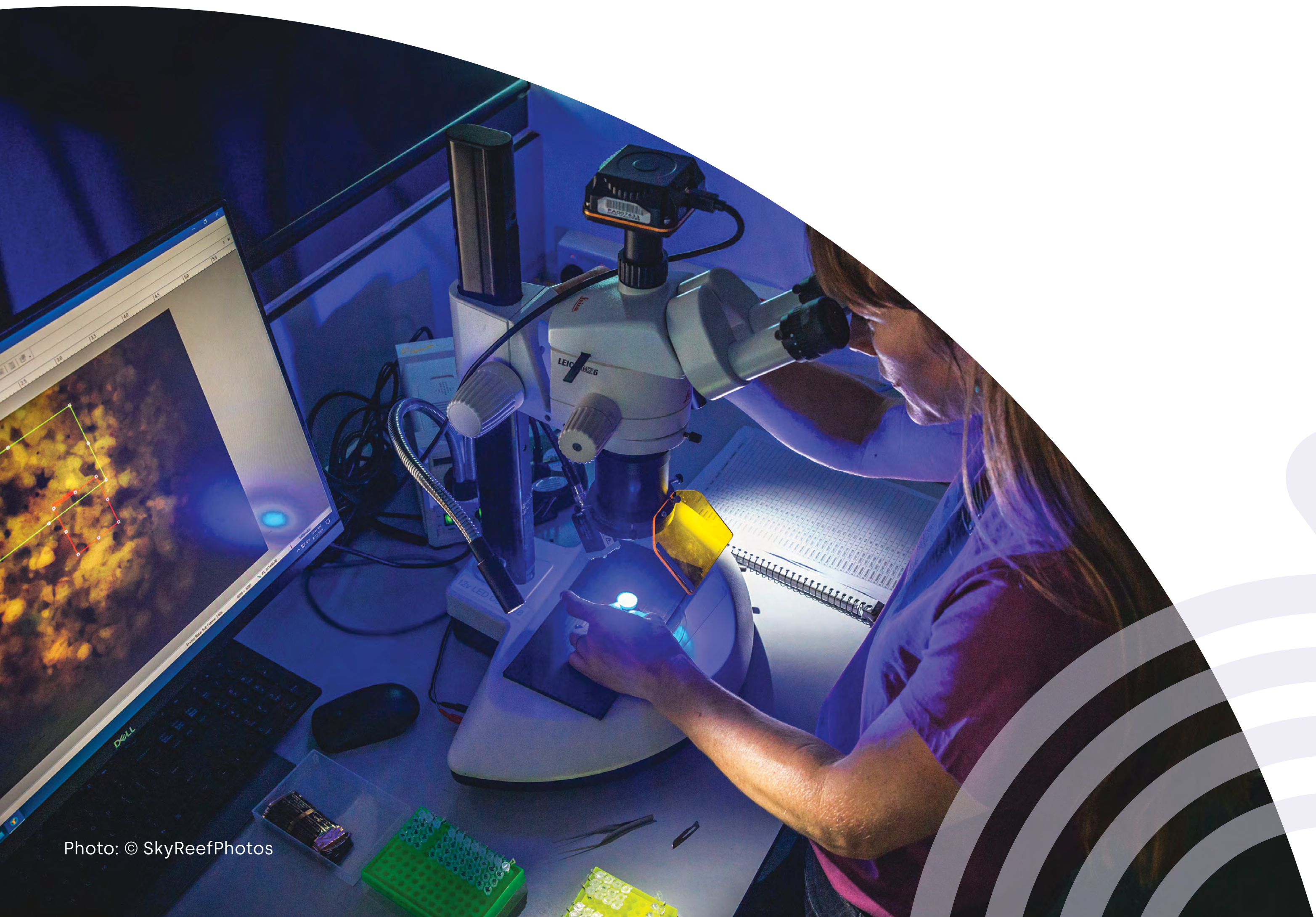
A research and development pathway towards assisted evolution methods backed by science.

To ensure the persistence of coral reefs, the highest priority must be reducing global greenhouse gas emissions. However, even if swift and substantial reductions in emissions are realised, corals will continue to face increasing temperatures for the foreseeable future, which will result in extensive coral mortality and local extinction of some coral species.

Workshop participants concluded that corals must adapt to survive, and assisted evolution methods exist that could facilitate and accelerate coral adaptation.

While the fundamental principles for assisted evolution methods have been developed, and methods are being explored by a number of research groups, critical knowledge gaps remain that hinder the application of these methods at scale.

Here we identify gaps that can be closed with relatively minimal effort, and others that will require substantial investment of time and resources (Table 1). Because corals are under imminent and severe threat due to climate change, new knowledge must be generated quickly.



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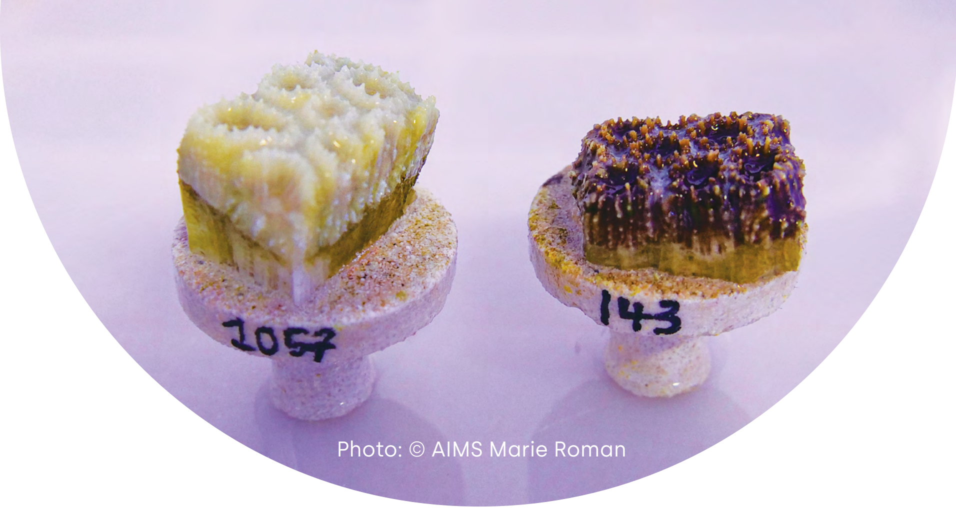

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Table 1: Justification and proposed delivery methods of recommendations.

Area & funding type	Justification	Proposed delivery method
Theme 1: Lead global coordination and synthesis	Strategic leadership is needed to optimise data utility, value-for- money, collaboration and alignment to reach common goals quickly (see Figure 3 for further detail).	To commission workshops/groups on topics including: • Methods and protocols including response to natural bleaching. • Annual synthesis and revised funding calls. • Scoping studies for the formation of research hubs and a coordinated network
Theme 2: Optimise generation and use of knowledge	Some essential knowledge gaps in basic coral evolution can be filled with existing methods within the scope of existing funding opportunities. Results will enable progress towards longer term and sequential goals.	• To develop project briefs based on Roadmap recommendations for open calls. • To solicit an additional statistical and methodological review by an external committee of experts to ensure optimal alignment among shortlisted projects.
Theme 3: Fill critical knowledge gaps in multigenerational coral data in the laboratory and field	Key data to estimate cost and benefits of interventions is needed but can only be obtained with substantial investment of resources and time.	• To develop project briefs based on Roadmap recommendations for open calls. • To award longer grants with appropriate mechanisms to track progress.
Theme 4: Support the advance of existing and new technologies	Longer horizon R&D to explore methods with potentially greater effects and risk profile.	• To develop project briefs based on Roadmap recommendations for open calls. • To award some funding to high-risk but potentially high reward assisted evolution solutions.

To address these issues, we recommend:

1. Committing to global coordination, collaboration, and synthesis

We suggest that building global infrastructure to support research would dramatically accelerate the generation of knowledge around the natural and assisted evolution of corals.

Infrastructure could include compiling and committing to a set of standards and methods that will allow more studies to be used in predictive models, as well as establishing a global resource-sharing network and database to facilitate meta-analysis and synthesis.

We recommend further funding for workshops to synthesise existing data and to advance specialist topics.

We also recommend that the feasibility of research hubs within a network structure be explored as a cost-effective way to facilitate and build collaboration capacity in diverse locations - this idea should be further scoped by a diverse and inclusive specialist working group.

Hubs could focus research on overlapping sets of species to further benefit data coordination and synthesis (Madin *et al.*, 2023 provide approaches for how species could be selected).

2. Optimising the generation and use of knowledge

Workshop participants identified research targets that could close important knowledge gaps quickly and with relatively little effort. These included characterising the relationship between different metrics of thermal stress and coral fitness across experimental and natural settings and putting greater emphasis on understanding how selection for heat tolerance might come at the cost of trade-offs in other traits.

Because reefs are formed by many coral species, this knowledge base should encompass traits and mechanisms for thermal resistance across divergent coral taxa.

Other studies could take advantage of natural bleaching events, which are highly undesirable from the standpoint of coral health but may offer unique insights into the thermal tolerance of wild coral populations 'in action'.

Because bleaching events are difficult to plan for, stand-by funding should be made available to ensure that researchers can investigate coral heat tolerance during unforeseen bleaching events, especially if their studies facilitate comparisons with experiments at the colony level.

3. Filling critical knowledge gaps in multigenerational coral data

Adaptation studies in corals are challenging. The long generation times of coral hosts mean that experiments must run for many years to generate the data needed to understand adaptive responses of corals to heat stress.

Many questions remain about how hosts acquire and transmit symbionts and microbes, including across host generations. Although evolution occurs at the time scale of generations, most funding models (e.g., 3 to 5 years) preclude multigenerational studies in corals. New funding models that enable longer studies and support interdisciplinary teams are therefore key to filling knowledge gaps and optimising assisted evolution methods.

Longer-term funding would be a game-changer by allowing research groups to gain the evolutionary knowledge needed to estimate the multi-generational benefits and risks of implementing assisted evolution methods in the wild.

Furthermore, we suggest that multiple methods - based on the same species and using standardised approaches and experiments - should be tested concurrently within focal hubs or regions; the provision of shared resources and infrastructure to these hubs may produce economies of scale. Consolidation of efforts would also support place-based genetic management and monitoring schemes.

Because many reefs are located in remote areas of coral reef (i.e. small islands, atolls, and archipelagos), it may be important to implement approaches that do not rely on advanced or permanent infrastructure (i.e. molecular laboratory facilities).

4. Advancing existing and new technologies for long term success

Currently, numerous assisted evolution methods have the potential to yield positive effects but need to mature from proof-of-principle to implementation.

We recommend continuing their development and testing *ex situ* and, in the field, as appropriate. For current AE methods this ranges from open field test ready, limited field trials or laboratory trials to optimise positive effects and minimise risks. Participants acknowledged that, due to the gaps in existing knowledge, it is not currently possible to accurately assess effect sizes for any of the assisted evolution methods.

There was consensus, however, that the riskiest methods were also the ones that could potentially yield a larger effect (e.g., gene editing, hybridisation between species, and assisted migration) and would need considerable R&D.

Presently, methods deemed to pose a high ecological risk or that have long R&D horizons are not being explored.

We recommend that development of these technologies be immediately but cautiously pursued in laboratory or quarantine conditions, alongside low risk, more mature technologies. Investment today in both suites of interventions (e.g., high risk, and low risk) may increase the toolkit that managers have available for coral conservation and restoration in the mid to near future.

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SECTION 2

CORDAP R&D Technology Roadmap for

Understanding Natural Adaptation & Assisted Evolution of Corals to Climate Change

The CORDAP R&D Technology Roadmap for Understanding Natural Adaptation and Assisted Evolution of Corals to Climate Change is based on an analysis of the major barriers and catalysts for understanding the adaptive potential of corals to meet 2030 targets (i.e., protection of 30% of the world's land, freshwater, and sea area by the year 2030; CBD, 2022) and future reef needs.

The following recommendations were informed by expert opinions (workshop, questionnaires, feedback), a systematic review of the literature (database), and weekly discussions amongst a core group (summary to be provided shortly).

Today, climate change is impacting ecosystems at an unprecedented rate, and we still have much to learn about how corals will respond to these changes or how we might harness the natural mechanisms of adaptation and acclimatisation to restore coral reefs more effectively.

Clearly - given predicted greenhouse gas emission scenarios - R&D around natural adaptation and assisted evolution must be prioritised to deliver solutions for coral reefs into the future.

Our recommendations fall under four major themes (Figure 1) that represent different project scopes and include a shift in funding duration and collaborative philosophy to fuel rapid discovery. The core working group rationalised and categorised the specific recommendations under

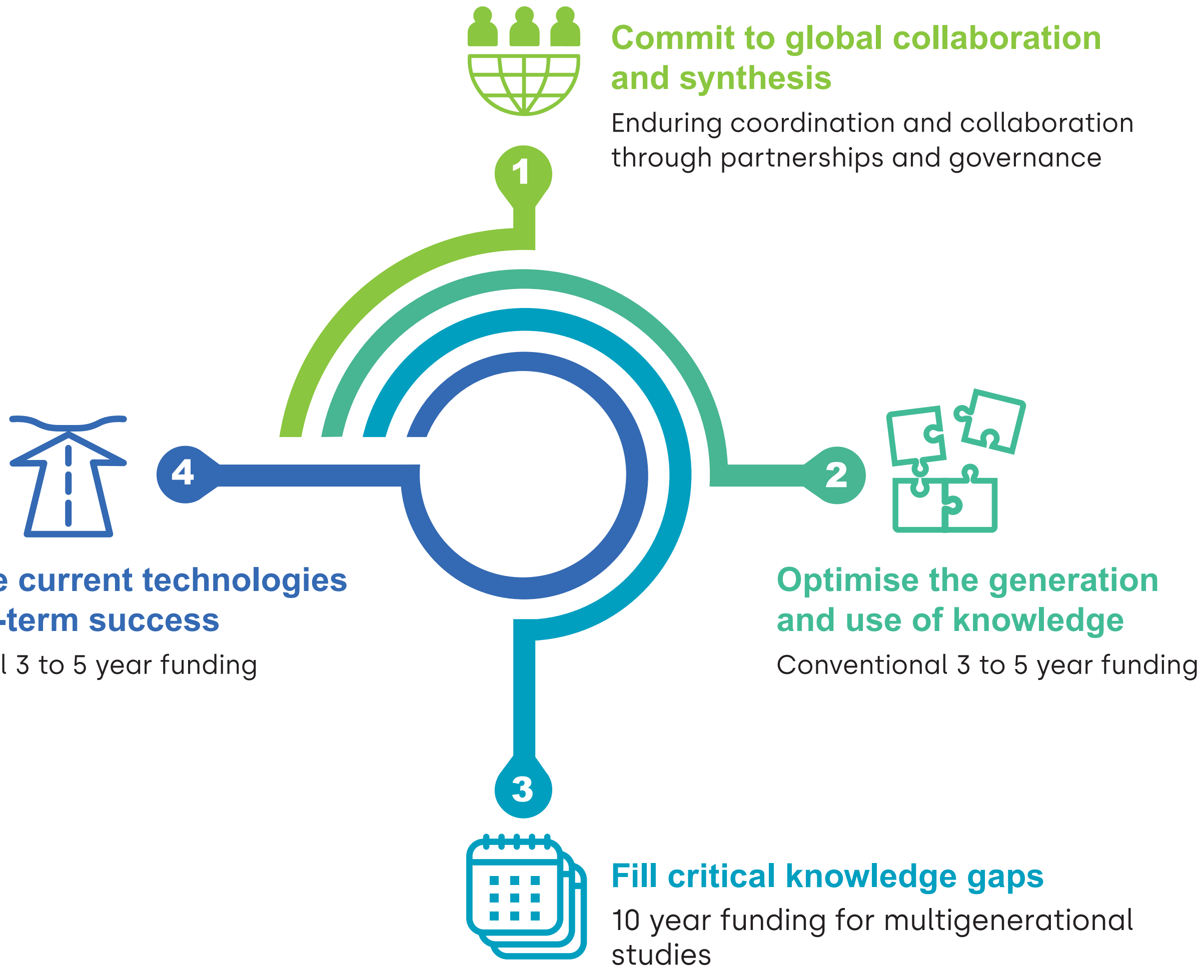

Figure 1: A four-themed investment portfolio with shorter and longer time horizons. Nineteen specific recommendations are made within these four themes and listed in Table 2.

Table 2: Summary of recommendations informed by the participants of the workshop, a review of the literature, and weekly discussions amongst the core group. AE = assisted evolution.

Theme	Recommendation	Recommendation outline	Impact
1. Lead global coordination and synthesis	1.1	Exert strategic leadership in research of natural adaptation and assisted evolution of corals by convening specialist workshops and working groups.	• Accelerate knowledge generation on assisted evolution (AE) methods and deployments to enable simultaneous comparison of AE methods and effect size. • Build local capacity to ensure the ability to deploy the ideal interventions in the appropriate locations at the required scale.
	1.2	Develop 'best-practice' protocols and metadata standards.	• Optimise efforts in generating quality data. • Improve cost-efficiency. • Ensure FAIR principles are upheld so that open access data can be used optimally (Jacobsen et al., 2020; Wilkinson et al., 2016).
	1.3	Establish a mechanism to provide statistical and methodological review to align funded projects.	• Funded studies follow best practice for data collection. • Ensure results can be consolidated to answer global questions. • This goes beyond the normal review process and cuts across all shortlisted projects.
	1.4	Build and maintain a global resource-sharing network and database (align with existing).	• Democratise access to coral heat tolerance knowledge.
	1.5	Build context-specific research capacity in developing economies and local communities.	• Support AE methods with lower perceived risks as appropriate in developing economies, home to the majority of coral reefs.
2. Optimise generation and use of knowledge	2.1	Link common heat tolerance metrics with coral fitness.	• Maximise our ability to predict future coral persistence and optimise the effectiveness of AE methods under different deployment and climate change scenarios.
	2.2	Estimate correlations among key heat tolerance metrics and between simulated and field heat stress events.	• Ensure interpretability of results within a natural context. • Ensure that phenotypes are correctly selected to withstand natural thermal stress events.
	2.3	Characterise trade-offs between coral heat tolerance metrics and other traits that impact fitness	• Reduce the ecological risks associated with assisted evolution methods.
	2.4	Increase research to elucidate the genetic correlations between key traits.	• Improve predictions of the limits of natural adaptation and AE under climate warming. • Support the discovery of individuals with beneficial phenotypes. • Allow risks to be more precisely estimated.
	2.5	Characterise geographical variation in phenotypic traits related to heat tolerance and other adaptations.	• Determine the geographic distance over which different evolutionary solutions have been reached and identify geographic and environmental locations with high thermal tolerance.
	2.6	Provide stand-by funding to undertake surveys and sample corals during natural bleaching events.	• Investigate mechanisms and factors that contribute to coral heat tolerance.
	2.7	Establish a common terminology for natural adaptation, heat tolerance and assisted evolution methods.	• Improve communication around AE methods.

Theme	Recommendation	Recommendation outline	Impact
3. Fill critical knowledge gaps on field performance of enhanced corals	3.1	Provide funding over longer time periods to evaluate multiple AE methods across multiple generations of corals in the field.	• Examine several AE methods including selective breeding within and between reefs and some symbiont manipulations (Figure 2). Combine methods where possible. • Build predictive models with biologically realistic evolutionary parameters based on actual data on the success of AE methods in the field.
	3.2	Elucidate the diversity, persistence, and specificity of microalgal symbionts and bacteria across host life stages, generations, and species.	• Estimate the effect size conferred by microbial manipulation methods. • Support fundamental research that investigates evolutionary and selective dynamics of symbionts and microbes and their interactions with cnidarian hosts. • Estimate "environmental leakage" of enhanced microbes under field conditions.
	3.3	Determine the effect size of AE methods across generations.	• Identify AE methods able to provide highest and most persistent impacts. • Improve our understanding of risks associated with AE methods.
	3.4	Estimate correlations of heat tolerance across life stages.	• Speed up R&D and reduce costs with predictive capacity of early life performance.
4. Support the advance of existing and new technologies	4.1	Create enhanced tolerance via breeding among extreme phenotypes, habitats, or regions to create novel genetic combinations.	• Potentially yield large effect sizes that may persist or increase over generations. • Better understand associated risks.
	4.2	Improve gene editing and splicing technologies to assess gene function and genomically label enhanced corals for genetic monitoring purposes.	• Provide foundational knowledge by identifying genetic basis of key traits. • Enable genome tagging (short-term) and enhancement (long-term) of deployed corals.
	4.3	Support innovations that enable the implementation of assisted evolution and acclimatisation tools.	• Accelerate delivery of recommendations.

these themes (Table 2), and the complete group of experts edited and commented.

We recommend that additional workshops or expert groups are solicted to further develop and consider the proposed recommendations. This includes the development of common methods, tools, and standards, and setting in place a statistical and methodological review and consultation of funded projects to ensure maximum alignment of results (Theme 1).

Our specific recommendations can be achieved under existing funding models (Theme 2) but also include multigenerational studies which, for most corals, would transcend current grant durations (Theme 3).

We recommend supporting the development of higher-risk methods that have the potential for high-reward outcomes but will require longer periods of funding to be safely developed (Theme 4).

A diverse portfolio of approaches, as recommended here, will be required to evaluate both the benefits and risks of proposed assisted evolution methods. Specialist examinations delivered from workshops or expert group outputs can also inform risk assessments that are underway in several partnerships (e.g., RRAP, CORDAP and others).

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RECOMMENDATION 1:

Commit to global coordination, collaboration and synthesis

Enduring coordination and collaboration through partnerships and governance

A major challenge for proposed restoration and adaptation initiatives is the rate at which R&D must occur for methods to be available when needed. While knowledge of coral adaptation has advanced (see recent review in Howells *et al.*, 2022), our analysis of the current literature clearly demonstrates a disconnect in methods and terminologies across research groups and locations that impedes progress (Figure 2).

In addition, adjacent fields such as animal or crop breeding or metaorganism and microbiome research outside corals may provide key insights as to how to enhance desirable traits in wild populations.

There is an urgent need to transition to a coordinated global effort to maximise and catalyse knowledge generation through a collaborative approach.

While we consider it too early to eliminate or prioritise specific assisted evolution methods, this may change in the future if funded studies begin to collect data using standardised methods.

Standardisation will also allow results to be included in population and ecosystems models to predict the impacts of assisted evolution under future climate scenarios.

Ultimately, a global collaborative program will produce synergistic benefits across studies and accelerate the generation of knowledge.

Here we provide high-level recommendations that we consider critical to achieve the goal of global alignment, which should be immediately considered for implementation.

Van Oppen *et al.* 2015	Assisted gene flow	Selective breeding	Hybridisation within and between species	Hybridisation within and between species	Preconditioning	Experimental evolution	Probiotics	
NAASEM Palumbi *et al.* 2019		Supportive breeding (within populations)	Outcrossing between populations	Hybridisation among species	Pre-exposure	Algal symbiont manipulations	Microbione manipulation	Genetic manipulation
RRAP Bay *et al.* 2019	Assisted migration	Marker assisted selective breeding	Assisted gene flow	Hybridisation among species	Conditioning	Algal symbiont manipulations	Microbione manipulation	Genetic manipulation
CORDAP 2023	Assisted migration	Intraspecific breeding within reefs	Intraspecific breeding between reefs	Hybridisation among species	Conditioning	Algal symbiont manipulations	Microbione manipulation	Genetic editing
# of studies per AE intervention	**0**	**4**	**5**	**2**	**38**	**17**	**3**	**1**

Figure 2: Terminology used to describe eight assisted evolution methods in the literature and in this Roadmap. The number of published studies per method (indicated on the horizontal axis) was determined using the database.

1.1

Establish mechanisms for strategic leadership in research of natural adaptation and assisted evolution of corals

To speed up the generation of knowledge, we endorse a growing call recommending mission-driven research, in which research efforts are aligned toward achieving a common goal, supported by governance and structure (Hein *et al.*, 2022).

Mission-driven research requires strategic guidance through the funding cycle with an overarching vision to ensure that program level goals are achieved through the combined outputs of independent projects.

- **To improve global alignment, we recommend funding meetings by specialist working groups, committees, and expert workshops to advance the existing roadmap into a strategic plan, evaluate progress and synthesise outputs on key topics (Figure 3).**

Impact: Global analysis and synthesis is essential to ensure that R&D for the assisted evolution of corals can keep up with moderate and extreme emission scenarios (McWhorter *et al.*, 2022).

We list a number of potential topics for meetings and working groups (Figure 3) but acknowledge that this list will change over time and should be guided by annual analyses and syntheses. Multi-generational studies are critical to understanding the long-term benefits of assisted evolution methods (see Theme 3 for full justification). Therefore, longer-term research funding (beyond the usual grant length of 3 to 5 years) is needed to account for the time required to produce the second-generation stage of corals.

While details were not discussed in the workshop, longer term projects typically have built-in stages at which to gauge performance and could be designed with the flexibility to adapt to results as they are analysed and synthesised. If a longer-term commitment to a research project is secured in one location, then infrastructure and capacity can be built, enabling teams to test other questions or assisted evolution methods through leverage and co-investment with matched research partners.

A hub structure consisting of researchers, practitioners, Traditional Custodians, community stakeholders, and citizen scientists who co-plan research and share resources would be ideal to examine the performance of multiple generations of corals on the reef. Research hubs are typically well positioned to produce higher-impact outcomes for a broader range of stakeholders (Bikard & Marx, 2020) and could be hosted under the network umbrella (Recommendation 1.4).

While there was enthusiasm around this fundamental shift in R&D strategy at the workshop, significant further discussion is required before it can be operationalised.

- **We recommend further examination of the best ways to set up hubs to catalyse R&D across coral generations.**

Impact: Accelerate the production of knowledge on assisted evolution methods and deployments in wild populations. This knowledge will allow the simultaneous evaluation of assisted evolution methods and the comparison of effect sizes in adaptive resilience-based management plans.

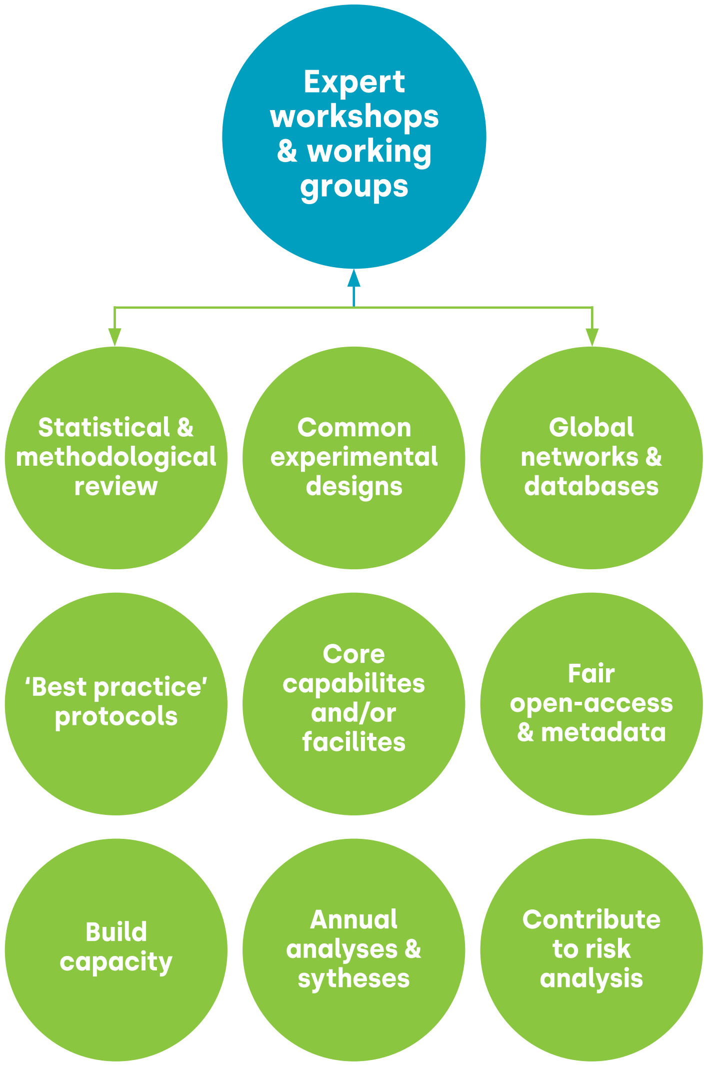

Figure 3: Recommended mechanisms to accelerate acquisition and use of knowledge on the natural adaptation and assisted evolution of corals. Working groups, committees and workshops with contributions from people and groups with diverse perspectives can provide detailed advice around the topics presented in dark blue.

1.2

Develop 'best-practice' protocols and metadata standards to enhance data utility and value-for-money

Our database analysis, along with other recent studies (e.g., McLachlan *et al.*, 2020), demonstrates that the methods used to characterise and understand the mechanisms underlying coral heat tolerance are disparate, making it difficult to translate results across sites or systems.

To overcome this issue, standard metrics are required that quantify the amount of stress that corals have been subjected to in experimental settings or during marine heatwaves. Several existing metrics, such as bleaching thresholds (Fitt *et al.*, 2001) or degree heating weeks (DHW; Heron *et al.*, 2016), can be used for this, each with its own implications and limitations.

DHW, for example, is routinely used to estimate the intensity and duration of heating conditions to which corals are exposed during marine heatwaves. However it cannot be used to contrast variation in annual and extreme thermal profiles (Heron *et al.*, 2016; Leggat *et al.*, 2022), and does not take into account the impact of short term anomalies that can also incite coral stress responses (Ainsworth *et al.*, 2016).

Although data sufficient for DHW calculation were provided in 80.4% of studies in the database, only 8.4% actually reported this metric, hindering easy comparisons between studies.

To facilitate comparison, studies should at least report the data required to calculate the amount of heat stress to which corals have been subjected (i.e., coordinates of coral collection sites, date of collection, temperature during the temperature stress event or experiment recorded hourly for each site or tank replicate, and duration of the stress event).

Currently, research methods, protocols and standard operating procedures (SOPs) for studying corals and their symbionts and microbes vary across projects, which limits the ability to compare or extrapolate results to other systems.

We therefore recommend that protocols and SOPs are standardised - in line with similar calls (e.g., Grottoli *et al.*, 2021; Thurber *et al.*, 2022). This could be achieved through a series of expert workshops and facilitated by the statistical and methodological reviewers (the SEP; Recommendation 1.3). Workshop topics could include the development of common heat stress experimental designs and best practice for monitoring natural bleaching events, where many approaches can be replicated with limited resources and budgets (Evensen *et al.*, 2023).

Research on a variety of reef-building coral species is particularly important because they may require very different experimental approaches and field protocols while, at the same time, selecting a few "model" species can greatly accelerate insights.

Workshop structure and selection of participants must also ensure diversity from across the coral research and restoration community, as well as local stakeholders, including Traditional Custodians. We strongly recommend that non-coral experts are included in these workshops for the valuable perspectives that they offer.

- **We recommend investment in the development of standardised 'best-practice' protocols for conducting experiments and reporting associated metadata, ranked by complexity, scalability, and cost.**

Impact: Unifying and harmonising protocols (i.e., McLachlan *et al.*, 2020; Thurber *et al.*, 2022; Voolstra *et al.*, 2021; Evensen *et al.*, 2023) will help researchers and managers make the most of efforts to produce quality data for the collective good and save money in the long run.

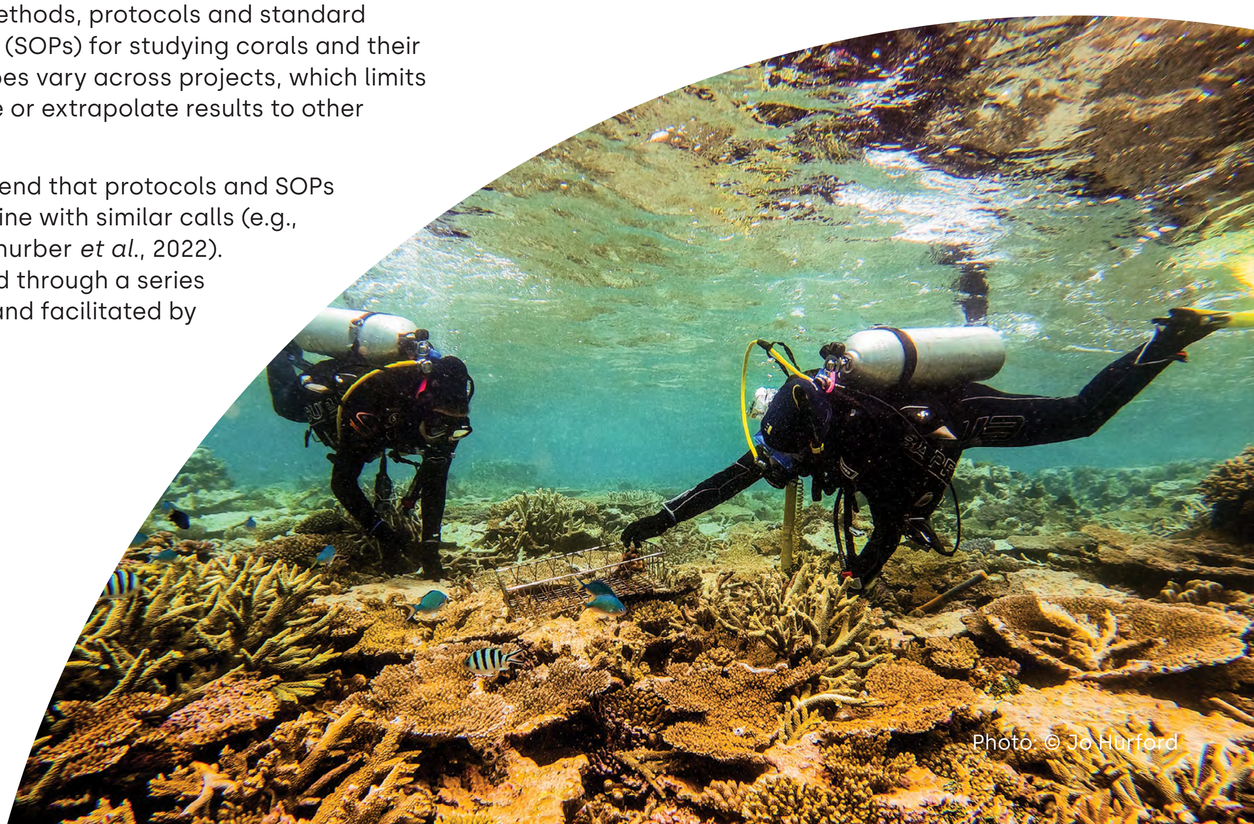

Photo: © Jo Hurford

1.3

Establish a mechanism to provide statistical and methodological review of selected projects for funding

We recommend to coral R&D funders that all relevant projects adhere to standardised protocols and procedures and that compliance is evaluated as part of the existing review process. If projects are too complex, or until the time that standards are fully in place, funders could request that a statistical expert panel (SEP) work with successful applicants to ensure projects are maximally aligned with complimentary global efforts and best contribute to key collective knowledge needs. Ultimately the Network will promote compliance across projects.

- **We recommend the creation of a statistical expert panel (SEP), in coral R&D funding programs to provide recommendations on the experimental design of selected projects to ensure that data collection follows standard protocols and experimental designs with statistical power to detect differences among treatments.**

Impact: Ensure that funded studies follow best practice for data collection and analysis, and that results can be consolidated to answer global questions.

1.4

Build and maintain a global resource-sharing network and database to improve collaboration, research alignment, dissemination of successes, and data use

An active and well-organised global network will keep track of standards and procedures, significantly improve communication among diverse teams, strengthen collaborative potential, and help researchers and managers better align their efforts.

We base this recommendation on the success of similar initiatives like like SPI Birds, the Nutrient Network, Coral Restoration Consortium, Kelp Forest Alliance, Green Gravel and others.

The network should help guide data collection for all projects and can support the collation of much-needed population genetic and environmental data for focal areas. Resource-sharing on this scale will require long-term web hosting, data maintenance and curation, and system upgrades.

Coral R&D funders have an opportunity to demonstrate global leadership by developing and managing this network, significantly increasing research productivity and improving outcomes for coral reefs globally.

A Research Network can support and catalyse local focal points for R&D, which we envisage as research hubs (Further scoping recommended in 1.1). We suggest that hub developments occur in a staged manner, are adaptive, and are leveraged with additional R&D partners where possible. Many published methods and outcomes are behind paywalls and thus are not accessible to all practitioners. To address this issue,

- **We recommend investment in the development and maintenance of a global network and database for sharing methods, papers, data and other resources. This network should include researchers and managers working on all reef types, including temperate and deep reefs.**

Impact: Democratising access to knowledge of coral heat tolerance, expanding the possibilities for conserving and restoring reefs in developing economies.

1.5

Build research capacity in developing economies and local communities

Coral reefs are in peril world-wide, but some regions have lost more coral cover than others. Reefs in the Caribbean, for example, are in a particularly bad state (Jackson et al., 2014), due to a combination of high temperature events (Eakin et al., 2010), hurricanes (Gardner et al., 2005), eutrophication, herbivory declines (Cramer et al., 2020) and infectious disease outbreaks (Aronson & Precht, 2001) that have reduced coral cover significantly.

As a result, major Caribbean reef-builders are now listed as threatened or endangered (Souter et al., 2021), and important reef ecosystem services reliant on the structural complexity provided by corals have been lost (Alvarez-Filip et al., 2009; Perry et al., 2013).

Globally, coral loss is exacerbated by differences in the capacity of countries and communities to protect, conserve and restore reefs due to variability in the availability of trained personnel and monetary resources to maintain laboratories and computing infrastructure.

Many of the assisted evolution methods described in this report are resource- or equipment-intensive, limiting their use in developing economies, which govern the majority of the world's coral reefs.

We suggest that implementation of R&D and restoration efforts be calibrated within local contexts (Gibbs et al., 2021) to make the best use of available equipment, local researchers, students, and conservation leaders, online mentoring, machine-assisted data compilation and other methods to create robust data sets in remote settings - all of which will improve the success of interventions.

- **We recommend that studies are funded to develop methods that can be deployed in resource-limited environments, accompanied by human capacity-building efforts and supported by global networks.**

Impact: Enhanced development, adoption and success of assisted evolution initiatives in developing economies, which are home to the majority of coral reefs.

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RECOMMENDATION 2:

Optimise the generation and use of knowledge

Conventional 3 to 5 year funding

In the last 30 years, more than 2,000 research papers have been published on the impact of temperature stress on coral reef organisms (database available here) - yet the genetic basis of heat tolerance remains poorly understood.

Many questions remain unanswered about the mechanisms, ecology and evolution of coral heat tolerance, due in part to the length and complexity of the studies that would be required. However, progress is also limited by a lack of standardisation in metrics and confusing and inconsistent terminologies.

Of particular concern is the lack of understanding of how commonly used metrics of thermal stress[4] (e.g., symbiont photochemical efficiency, pigment concentration, symbiont density) relate to colony fitness[5]; how responses to experimentally induced heat stress events compare to those induced by marine heatwaves; and how heat tolerance traits or responses trade off with vital rates (e.g., growth).

Furthermore, heat tolerance is a complex trait involving the physiology and genetics of the host, symbionts, and associated microbes - all of which have different potential metrics of heat stress.

We propose a series of short-term studies characterising the relationship between thermal stress metrics and vital rates in a variety of species, comparing these standardised metrics across experimental approaches to better correlate them to heat stress events that occur in the reef, and expanding the exploration of fitness trade-offs into the more robust space of genetic covariance between traits.

Such experiments would be appropriate for conventional (3 to 5 year) funding models, such as the one proposed by CORDAP or used by many national scientific funding bodies (e.g., the National Science Foundation, the Australian Research Council, Horizon Europe, Natural Environmental Research Council (NERC), UK).

2.1

Link common heat tolerance metrics with long term coral fitness to unlock the evolutionary significance of existing data

Using our database, we identified 220 studies that evaluated the physiological or demographic performance of corals under heat stress conditions - altogether using 76 different metrics to do so. Because coral performance traits are measured using so many different methods, taxa, and experimental conditions, their relationship to fitness is not well understood. As a result, studies that only report heat tolerance metrics cannot be leveraged to estimate the adaptive potential of corals to warming temperatures. Furthermore, due to the limited number of comparable data points per taxon, it is not currently possible to characterise relationships among these metrics using a meta-analysis of existing data.

For the five stress response metrics most frequently appearing in our database, the number of comparable studies for the same taxa varies between 2 and 10, with most having fewer than 5 studies (Table 3). After accounting for the experimental conditions in each of these studies, we are left with only a small number of data points for a very narrow range of thermal stress levels, encompassing only four coral genera. To address this issue,

- **We recommend a series of integrated experiments that consistently measure multiple stress response metrics together with fitness proxies (survival, fecundity or growth) on the same colonies under a wide range of genotypes and thermal stress levels. Experiments like this should ideally be performed in many locations, testing a large number of colonies and be designed using consistent experimental design principles in order to allow the integration of data and generation of general relationships.**

Impact: Elucidating the relationships between common heat stress metrics and coral fitness will advance our understanding of coral heat tolerance, dramatically improving our ability to predict coral persistence under different climate change scenarios when implementing assisted evolution methods in wild populations.

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4. Heat tolerance metrics hereafter refer to metrics measured during heat stress exposure.
5. Coral fitness refers to the quantitative representation of individual reproductive success that is affected by any factor that alters growth, fecundity and survivorship (vital rates) of the coral colony.

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Table 3: Number of studies per species that measured at least one of the five most common stress response metrics (symbiont photosynthetic efficiency (*Fv/Fm*), symbiont density and physiology, colour, photosynthesis and respiration) alongside proxies of fitness (survivorship, growth or fecundity). In addition to the aforementioned criteria, studies also had to provide the information needed to estimate the amount of heat stress exposure (i.e., to estimate DHW the collection sites of corals must be provided and thermal stress events must last longer than seven days), and metrics had to be measured on the same coral species and life stages.

Species	Vital rate	Metric	Number of studies
Acropora hyacinthus	Fecundity	Colour	3
Acropora millepora	Growth	Symbiont density	6
	Mortality	Symbiont physiology	6
Montipora capitata	Growth	Colour	7
		Photosynthesis and respiration	4
	Mortality	Colour	10
		Photosynthesis and respiration	2
Pocillopora damicornis	Fecundity	Symbiont photosynthetic efficiency	3
		Photosynthesis and respiration	3
	Growth	Symbiont photosynthetic efficiency	4
		Symbiont physiology	6
	Mortality	Symbiont photosynthetic efficiency	7
		Symbiont density	7
		Symbiont physiology	6
		Photosynthesis and respiration	2
Porites astreoides	Fecundity	Symbiont density	3
		Symbiont physiology	2
Porites compressa	Growth	Photosynthesis and respiration	4
	Mortality	Photosynthesis and respiration	2

2.2

Estimate phenotypic correlations among key heat tolerance metrics and experimental approaches (natural vs short- and long-term experiments)

The database demonstrates that most coral heat stress experiments have been conducted under controlled laboratory conditions over short time periods (i.e., hours to days). Only four studies (Chase et al., 2018; Shore-Maggio et al., 2018; Noonan & Fabricius, 2015; Anton et al., 2020) have attempted to link the experimental responses of corals under laboratory conditions to wild population responses during marine heatwaves - each using only one coral species and not using the same colonies.

Six other studies (each with one species) attempted to compare responses to short-term (<7 days) and moderate- or long-term (>30 days) heat stress in the laboratory. None of these studies, however, linked their results to holobiont fitness (where holobiont refers to the cnidarian animal and associated symbionts and microbes). In many cases, the effects of assisted evolution on coral heat tolerance can only be determined using large sample sizes that are logistically difficult to manage in long-term experiments.

Therefore, it is critical to understand how short-term laboratory research correlates with the longer-term natural exposures faced by corals during marine heatwaves.

Therefore,

- **We recommend conducting matched experiments in the laboratory (short- and long-term) and observations during marine heatwaves of individual (tagged or geo-referenced) colonies to correlate common responses under short- and long-term heat stress exposures. It is critical that these correlations are contextualised with colony-level growth, fecundity or survivorship to evaluate the effect of implementing adaptive resilience-based management strategies.**

Impact: Understanding these relationships will enable us to calibrate metrics across short- and long-term laboratory experiments to ensure that results can be interpreted within natural contexts. Critically, this will ensure that short- or long-term heat stress experiments can accurately identify individual colonies that are most likely to have high heat tolerance when exposed to natural thermal stress events. This knowledge will greatly aid in the implementation of many assisted evolution initiatives.

2.3

Characterise trade-offs between coral heat tolerance metrics and other traits that impact fitness

Heat tolerance is a complex trait that is determined by a number of holobiont attributes (i.e., several genes of small effect, the associated symbionts and microbes, etc.) and environmental factors.

Because traits are frequently genetically correlated (Walsh & Blows, 2009), coral heat tolerance traits may evolve concurrently and at a cost to other traits (trade-offs).

We found a total of 23 studies that have examined trade-offs related to heat tolerance in 15 coral species. Some of these studies reported the existence of trade-offs (Cunning *et al.*, 2015; Muller *et al.*, 2018, 2021; Shore-Maggio *et al.*, 2018), while others did not (i.e., Quigley *et al.*, 2020; Wright *et al.*, 2019), emphasising the need for more research in this area before general conclusions can be made.

Moreover, there is a lack of studies focused on estimating trade-offs across multiple life stages, compromising our ability to predict the effects and risks of assisted evolution methods to enhance heat tolerance. To address these knowledge gaps,

- **We recommend investing in studies with experimental designs suitable to estimate trade-offs on heat tolerance. These studies must include a control for the enhancement and be conducted across multiple life stages.**

Impact: Understanding the costs - if any - that may be associated with increased coral heat tolerance enhancement reduces the risk that assisted evolution methods might inadvertently select for 'maladapted corals', that are, for example, more susceptible to disease, grow more slowly, or reproduce less.

2.4

Increase research on genetic covariance of traits with fitness

Although population variation in heat tolerance has been reported in numerous studies, the causes of this variation can be many, and the degree to which coral offspring inherit this trait is unclear. Additive genetic variation, usually estimated as narrow sense heritability, is an indicator of how a single trait is passed on to the next generation and a population's ability to shift its phenotype in response to selection.

Only nine published studies report (host) additive genetic variation, and only three report values for adult stages when exposed to either short or moderate-term temperature stress conditions. As a result, our current understanding of coral heat tolerance is based on a few studies of single traits that are more likely to have been assessed in early life history stages rather than adults.

Moreover, few of the studies attempted to characterise the additive genetic variance of fitness, namely survival and reproductive success, the attributes that are most relevant to evolutionary adaptation (relevant studies: six for survivorship, one for growth, none for fecundity). To date, genomic and breeding studies suggest that heat tolerance is a complex trait governed by many different genes, many of which have a low effect (e.g., Dixon *et al.*, 2015; Fuller *et al.*, 2020).

These findings are consistent with other species that have been more thoroughly studied, such as fruit flies and crops, indicating that quantitative genetic approaches are appropriate for studying adaptation in corals. Our findings are consistent with those of recent reviews by Howells *et al.* (2022) and Bairos-Novak *et al.* (2021), as well as the opinions of workshop participants.

An emphasis to date on studies of single traits overlooks the fact that genetic covariance among different traits is a primary determinant of evolutionary trade-offs. In other words, genetic covariance determines how selection on one trait, such as heat tolerance, affects other traits, such as holobiont fitness.

Estimating genetic covariances is critical in understanding the response to selection in multi-trait phenotypes (discussed further in Richards *et al.*, 2023). Estimating genetic covariances yields information on the additive genetic variation of the individual traits. These covariances can be different for different species, or even populations, and would need to be evaluated broadly.

No study in the database was identified as having estimated genetic covariances between traits. Furthermore, not knowing correlations between traits means that if we select strongly for heat tolerance, we risk losing important genetic diversity in other traits or can shift other traits in unwanted directions (Baums *et al.*, 2019). As a result, estimates of evolutionary potential may be overestimated if there are undesirable trade-offs between traits.

- **We recommend investing in studies with experimental designs that are sufficiently powered to investigate genetic covariances among traits and to prioritise traits that directly affect fitness (e.g. survival and reproductive success). Using experimental designs based on the "animal model" (Gienapp et al., 2017; Wilson et al., 2010) - used successfully by plant and animal breeders to infer relatedness from genetic similarity - may be especially useful.**

Coral habitat quality is rapidly deteriorating. Therefore - despite the risk of losing genetic diversity during selective breeding - we argue that development must continue on assisted evolution and selective breeding methods for increased temperature tolerance at the colony and population levels, even as the genetic architecture of traits is investigated.

This research can be fast-tracked if maps or other inventories of heat tolerant corals for restoration or breeding exists, because it allows breeding designs based on many heat-tolerant parents, and genomic prediction methods developed for agricultural species (which can also avoid breeding experiments).

- **We recommend that selective breeding of corals for increased temperature tolerance is further developed by utilising classical genetics (BLUP - Genomic best linear unbiased prediction) and or genomic predictions that do not rely on a priori knowledge of the genetic architecture of target traits.**

Impact: Better understanding of genetic correlations for diverse species will improve modelled predictions of adaptive potential, support the discovery of individuals with beneficial phenotypes, and allow risks to be more precisely estimated. Genetic prediction by BLUP will improve accuracy of selection when the pedigree structure is known (e.g. in captive breeding designs). Genomic enabled approaches such as application of 'pedigree-free' animal models (i.e., utilising genomic relatedness matrices in pace of pedigree-derived estimates) and genomic prediction would allow wild corals to be assessed in situ and bypass multigeneration breeding and phenotyping experiments.

2.5

Characterise the geography of phenotypic traits related to heat tolerance and other key elements of fitness

The genetic basis of variation in heat tolerance is highly relevant to coral assisted evolution. To date, research into the genetic architecture of coral heat tolerance traits suggests that it is species specific and has a polygenic basis, involving many genes, each with (mostly) small effects on the phenotype (see 2.5).

A polygenic architecture for thermal tolerance or fitness indicates that alterations to single host genes are unlikely to cause much shift in phenotype. On the other hand, a polygenic basis often implies that standing genetic variation for heat tolerance will not easily be exhausted after a few generations of selection, and that many different avenues of heat tolerance evolution exist.

Following issues identified in 2.5, the capacity of populations to adapt may differ based on their geographic, historical and environmental context (e.g., Schoepf *et al.*, 2023; Ziegler *et al.*, 2021). For example, if there is substantial genetic divergence (reflecting some combination of adaptive differences and/or low gene flow), the key alleles contributing to relevant phenotypes are likely to differ in frequency across populations or be absent in some locations.

For polygenic traits, different species or even populations of the same species from different regions may have similar phenotypes and use different combinations of loci to achieve these similar phenotypes.

At present, we do not know over what spatial scale this occurs for corals, especially as surveys of genome-wide genetic differentiation may be confounded with cryptic species (Sheets *et al.*, 2018) and spurious geographic patterns driven by limited gene flow.

The same genotypes can yield different phenotypes in different environments through a process termed genotype-by-environment interactions as found for inshore and offshore populations of *Porites astreoides* (Kenkel & Matz, 2016; Kenkel & Wright, 2022).

It is important to note that some methods for studying the geographic distribution of genetic variation underlying heat tolerance may require more time than the current three-year timeframe suggested in this section.

As a result, it may be necessary to prioritise and carefully plan research efforts in order to make the best use of available resources to increase our understanding of the genetic basis of heat tolerance and its geographic variability.

Symbiont genetics is also a key feature of coral holobiont heat tolerance and can vary among species and over small spatial scales. The genetic basis of the contribution of symbionts to heat tolerance is a growing field, propelled by recent experiments on artificial selection for heat tolerant symbionts (Buerger *et al.* 2020). The intersection of coral genetic variation and symbiont genetic variation and its role in determining holobiont traits is poorly known, and would benefit from collaborative study at the physiological, genetic and ecological levels.

- **We recommend investment in studies that evaluate genetic architectures of heat tolerance in terms of reef geography.**

This would include studies that:

(a) Robustly measure heat tolerant phenotypes in multiple ways (see Recommendation 2.2 above).

(b) Measure genome-wide genetic variation of these same corals, as well as their symbionts and microbes.

(c) Survey the geographic distribution of alleles for loci contributing to key traits across various spatial scales (small-scale/within reef to large-scale/among reef).

(d) Document genotype-by-environment interactions.

Impact: Determining the geographic distance over which different evolutionary solutions have been reached - such as locations that have adapted to warm temperatures by using different sets of loci - would support breeding experiments (e.g., selected breeding, intraspecific hybridisation) to create phenotypically variable progeny from warm-by-warm crosses.

Photo © Jo Hurford

Genotype environment association (GEA) studies (Lasky *et al.*, 2023) could be used as a fast method to complement quantitative genetic investigations to yield insights into the geography of adaptive potential.

GEA studies bypass time-consuming phenotyping and therefore are more scalable across populations and species. For example. common garden experiments, similar to those used regularly in plant biology, use heat tolerant corals from different reef locations and show which ones retain their heat tolerance when grown together (Morikawa & Palumbi, 2019).

- **We recommend that population genomic surveys be undertaken for species with diverse life histories. These data can be used to infer the important environmental axes describing within species genetic differentiation using genotype environment association methods.**

Impact: Increased geographic knowledge of adaptive potential will help guide selection of corals for interventions such as assisted migration, assisted gene flow, and selective breeding, including suitable sources for translocation or breeding and suitable reefs for settlement or outplanting.

The approaches discussed above rely on molecular genomic tools, with investigators being able to genotype large numbers of coral colonies. Not only are genomic methods expensive and infeasible for many research programs, but the extreme species richness of corals suggests that low-cost and high throughput approaches may be practical (even if they are less informative on fundamental understandings).

Enabling widespread baseline studies of heat tolerance and its variation (using standardised protocols - section 1.3) across local reef habitats and in many different reef locations - particularly in remote areas far from human stressors - could yield a "map" of heat phenotypes.

Streamlined heat testing, followed by common garden experiments, could quickly pinpoint colonies with durable heat resistance, setting them up as the raw material needed for local restoration, heat tolerance breeding, and potential agents of assisted evolution.

This relatively low-tech approach has the advantage of being implementable immediately across a wide range of locations. It can lead to identification of colonies or regions with unexpectedly high heat tolerance for further study using the approaches above, especially if global networks help connect research groups to collaborate.

- **We recommend that studies mapping thermal tolerance across diverse habitats, geographies, and species be supported, contingent upon studies following established protocols (1.3) and making data accessible (Recommendation 1.4).**

Impact: Key benefits of this approach are that it is immediately open to research on hundreds of coral species on thousands of reefs, can be implemented by investigators from all countries, and will enable incremental progress to be made during the long development of some of the methods proposed above.

2.6

Stand-by funding to undertake surveys and sample corals during natural bleaching events

Natural bleaching events offer researchers the opportunity to collect large sample sizes across species, stress and geographical gradients, but they cannot be scheduled and are thus impractical to incorporate into regular grant applications. Samples and high throughput observations from natural bleaching and mortality events are indispensable to estimate the strength of selection and detect shifts in allele frequencies in wild populations.

In particular, the distribution of extreme phenotypes that remain unbleached is a valuable resource for continued research and, possibly, selective breeding and restoration. Such surveys gain even more value if colonies can be tagged, repeatedly surveyed, and experimentally phenotyped. Therefore,

- **We recommend that an emergency fund be established to support rapid response bleaching investigations. Such studies should follow a standard process and use recommended methods (Recommendation 1.2). Studies that further leverage knowledge through tagging, repeated surveys, photogrammetry, or other methods are preferred.**
- **We also recommend that standing protocols, including priority measurements with assembly of instruments and reagents (if required) be developed as part of Recommendation 1.1 as soon as possible. This should also include one or several sampling sets to tag colonies and collect samples for genetic analysis that can be shipped at short notice where they are needed.**

Impact: The findings of these studies will allow us to identify heat tolerant colonies and investigate the mechanisms and factors that contribute to their tolerance. Furthermore, phenotyping colonies in an experimental setting (field or laboratory) will allow comparison of their responses under controlled conditions to those observed in the wild during natural marine heat stress events (in accordance with Recommendation 2.3).

2.7

Establish a common terminology for natural adaptation, heat tolerance and assisted evolution methods

Our workshop discussions and literature review revealed that a confusing variety of terms are used to define coral heat tolerance and assisted evolution methods (Figure 2).

According to the database, only eight studies formally define coral heat tolerance, and 98 different metrics are used as proxies for the resistance of corals to temperature stress.

It is therefore essential to define coral heat tolerance, revisit the terms used across restoration activities and publications in the broader field of conservation genetics, and craft an updated, unified lexicon to aid communication among scientists, practitioners, and the general public.

- **We recommend that the relevant coral organisations, such as CORDAP and the CRC, establish a working group to create common lexicons for:**

1) heat tolerance, and,
2) assisted evolution methods that can be used in the contexts of natural and social science engagement and aligned with terminologies used in terrestrial restoration. We recommend that working groups include key coral experts in the disciplines along with general experts from natural and social science, Traditional Custodians, stakeholders, and communication experts.

Impact: Consistent usage of simple terms that are acceptable across sectors will aid communication and build trust in science.

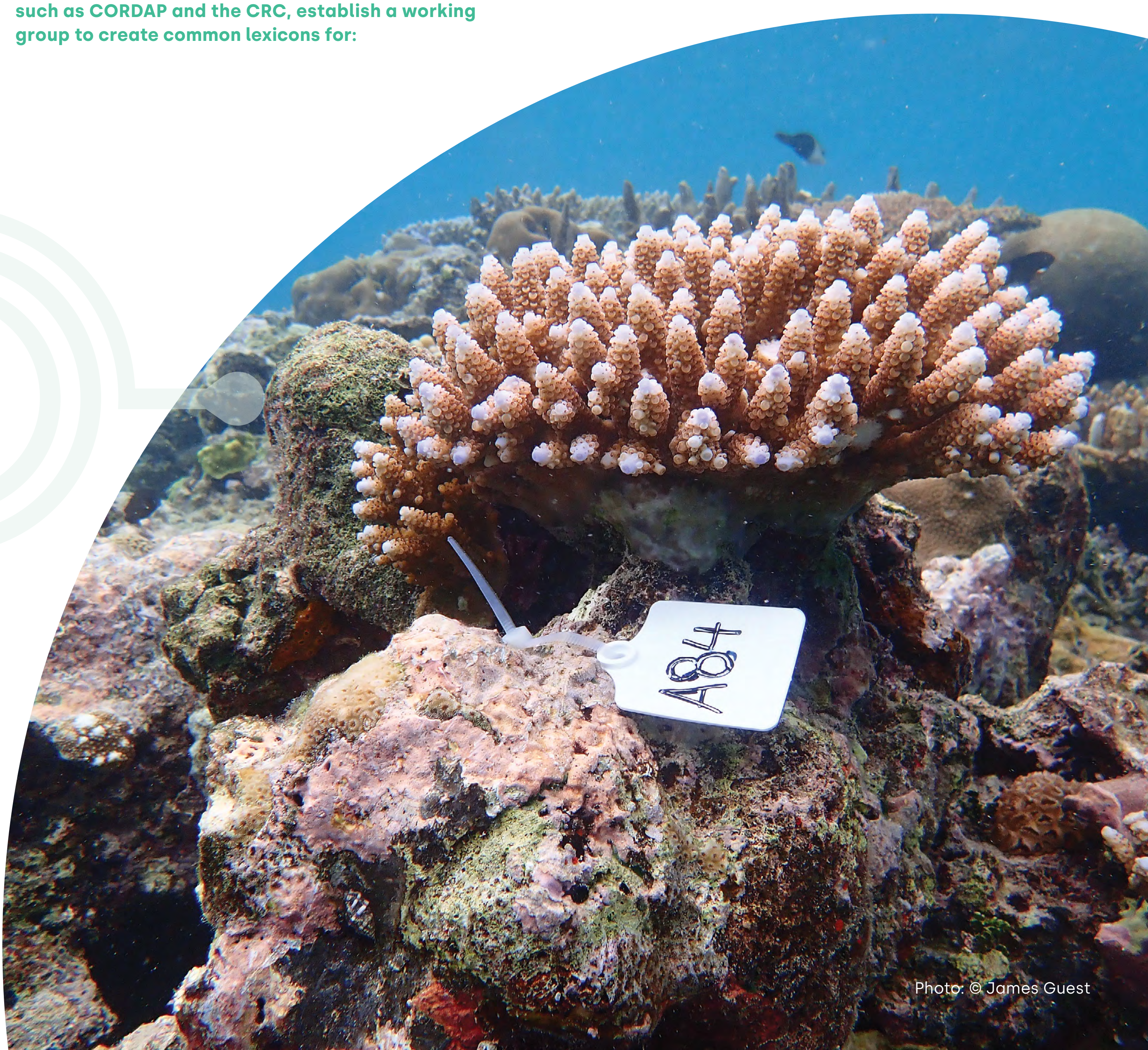

Photo: © James Guest


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RECOMMENDATION 3:

Fill critical multigenerational knowledge gaps

10 year funding for multigenerational studies

Adaptation with or without human assistance is predicated on changes to the genetic composition of the coral holobionts. If changes do not carry forward to the next generation, then the ultimate impact of any intervention will be short lived and unable to spread from the point of deployment.

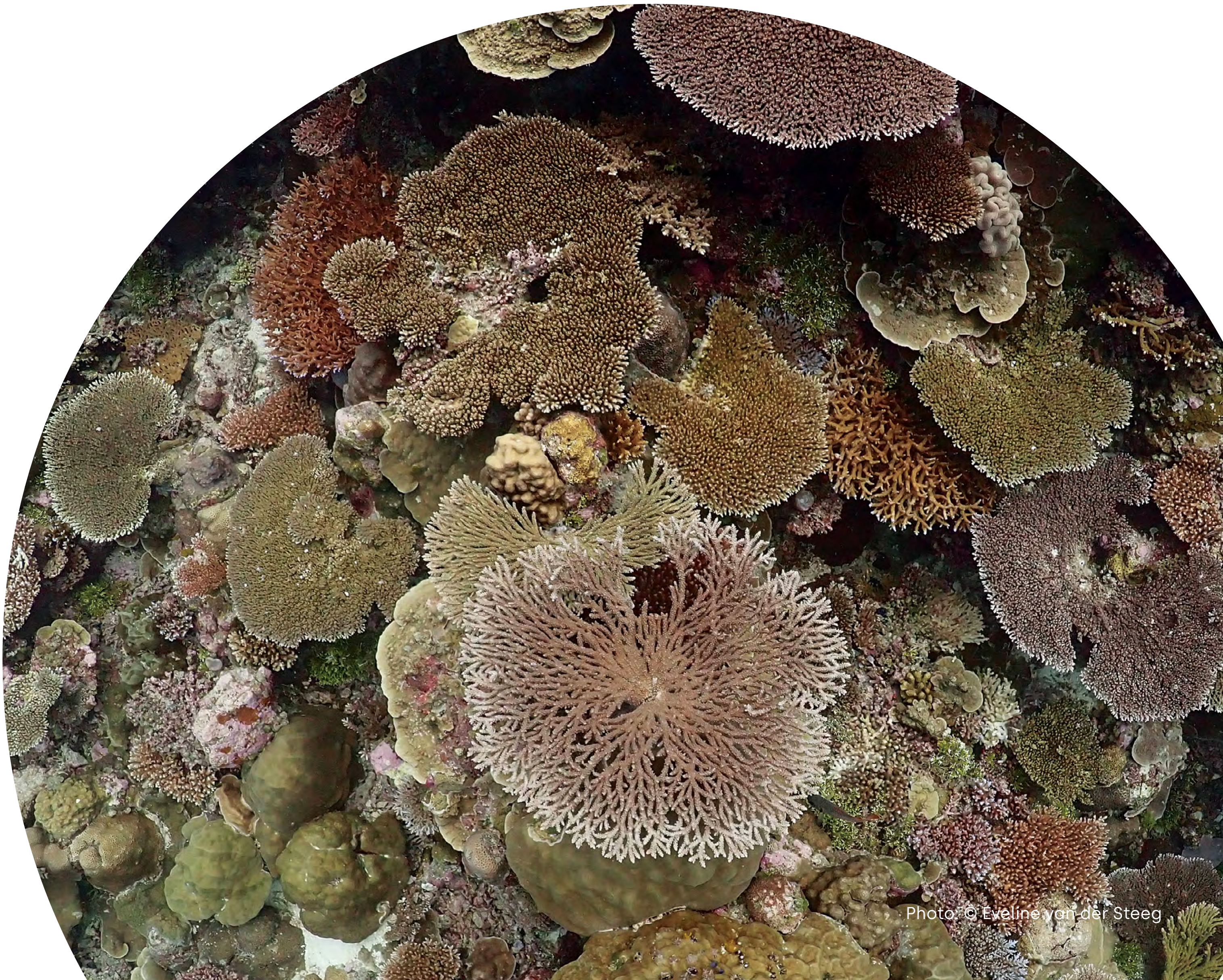
Even worse, some interventions are likely to have their greatest effects beyond the second generation (e.g. assisted migration, interspecific hybridisation) and thus may fail to deliver on promise.

An improved ability to predict the ecosystem benefits of assisted evolution requires studies that span more than the time needed for corals to become reproductively active (between 4 - 34 years, Hall & Hughes (1996)).

We identified numerous knowledge gaps through the database analysis, workshop discussions and knowledge shared that, if filled, would dramatically improve conservation and restoration outcomes for corals globally.

The studies we recommend either require longer-term investigations to capture information across generations or necessitate more complex designs than those that can reasonably be delivered in conventional funding scopes.

Understanding of both the effect of enhancement for increased heat tolerance, as well as the maintenance and spread of tolerance across generations, is critical for cost-benefit analyses of assisted evolution methods.



3.1

Provide funding over longer time periods to enable the assessment of assisted evolution methods across multiple generations of corals

Conventional funding models, where projects are constrained to 5-year (or more commonly 3 year) durations, are incompatible with studying multiple generations of corals. This constraint prevents researchers from designing critical experiments and investigations necessary to advance our understanding of evolutionary processes.

- **We recommend that funding be provided over longer time periods (i.e., longer than the usual three to five years) to enable the multigenerational and multidimensional studies that are required to advance fundamental knowledge of and confidence in effect sizes, durations, and spread of interventions.**

Impact: Creating a reliable and consistent stream of long-term funding will transform coral research and eventually lead to predictive models based on biologically realistic evolutionary parameters.

The alignment of long-term projects will be achieved through collaborative Networks that uphold and refresh standards and procedures (building off Recommendation 1.4).

Secured funding projects over longer time frames will build local capacity and capability including research infrastructure (Recommendation 1.5).

A longer-term commitment to projects also supports leverage from collaboration and can lead to the establishment of hubs for local R&D activity (building off Recommendation 1.1).


Photo: © David Suggett

3.2

Elucidate the diversity, persistence and specificity of symbionts and microbes across host life stages, generations, and species

The susceptibility of corals to temperature stress is strongly dependent on the heat tolerance of their symbiont and microbe communities (van Oppen & Nitschke, 2022). Manipulating the algal symbiont and microbe communities of corals (Peixoto et al., 2017, 2021) can be key methods to increase the heat tolerances of coral holobionts and could be delivered in addition to host-focussed breeding methods.

Preliminary studies have demonstrated that symbiont manipulation (Buerger *et al.*, 2020; Quigley & van Oppen, 2022) and microbe probiotics (Rosado et al., 2019; Santoro et al., 2021) can reduce the bleaching susceptibility of coral larvae and adults over one to three weeks of heat stress exposure.

Given their inherently short generation times, symbionts and microbes are potentially more amenable to experimental evolution than their animal hosts. Using experimental evolution, the heat tolerance of symbionts can be enhanced *in vitro,* and subsequent reintroduction into corals can boost host fitness under temperature stress (Buerger *et al.*, 2020; Chakravarti *et al.*, 2017).

However, critical questions about the persistence of such symbiotic relationships and their implications for coral fitness remain. For example, it is unknown how long corals inoculated with algal symbionts retain them, although prokaryotic probiotics can trigger beneficial effects independently of colonisation or retention, through epigenetic and immune responses (Lebber et al., 2018; Barno et al., 2021).

Exposure to exogenous sources of algal symbionts (e.g., surrounding coral colonies), the coexistence of inoculated and native symbionts within the same coral, and exposure to repeated and/or multiple stressors are all factors that may contribute to the elimination or replacement of introduced algal symbionts.

Moreover, there is limited information about the transgenerational persistence of algal symbionts. While transgenerational persistence of symbionts is feasible in corals that transmit algal symbionts to their offspring via gametes (mainly brooders), broadcast spawners primarily rely on environmental uptake of symbionts, which depends on the persistence of algal symbionts in environmental reservoirs and their transmissibility between host organisms.

Understanding the persistence, transmissibility, and fidelity of algal symbionts is critical for determining how long benefits conferred by their manipulation might last. Elucidating host-symbiont specificity would provide greater confidence in the transferability of algal symbiont manipulation methods between coral taxonomic groups or ocean basins, over long-term applications.

Probiotics might be applied to corals as medicine, to retain natural genetic diversity and coral cover during heatwaves and disease outbreaks. Yet, the natural diversity and functions of symbionts and microbes remain poorly characterised (reviewed by Shah *et al.*, 2022).

Expanding culture collections of host-associated microbes is key to enable more extensive testing of phenotypes, both in culture and in association with a multitude of coral hosts. Given the significant phenotypic diversity existing between symbionts at the intra-genus level, it is also important that studies report, at a minimum, species-level genotypes.

This will enable functional and taxonomic diversity to be accurately linked and help unlock the phylogenetic diversity of symbionts, which will provide opportunities to incorporate strains or species that have not yet been studied/discovered into algal symbiont manipulation efforts.

- **We recommend investment in studies that:**
  - **(a) Investigate the fidelity of algal symbionts after uptake into a host;**
  - **(b) Investigate algal symbiont specificity to coral animal hosts;**
  - **(c) Characterise the taxonomic and functional diversity of algal and prokaryotic symbionts, especially from populations predicted to have high heat tolerance;**
  - **(d) Expand collections of algal symbionts to cover more species and regions, and so enable development of additional experimentally evolved strains;**
  - **(e) Investigate short and long-term effects of prokaryotic probiotics and alternative probiotic microbial therapies (such as postbiotics); and**
  - **(f) Evaluate the combined use of prokaryotic microbial therapies with other interventions**

Impact: Increasing knowledge in these fundamental areas will enable studies to estimate effect sizes for assisted evolution methods based on microbial manipulations. Additionally, growing our knowledge of symbiont and microbial diversity and their associations with hosts may open up development of new strains suitable for use in interventions and targeting additional coral hosts.

3.3

Determine the expected increase in heat tolerance that is achievable through different assisted evolution methods across generations

One of the main challenges for assisted evolution initiatives is determining whether achievable rates of adaptive change, if successful, match predicted increases in extreme ocean temperatures - namely, whether or not they can provide measurable ecosystem benefits at desired temporal and spatial scales.

Multiple hurdles need to be overcome, including the methodological challenges of enhancing corals and the logistical hurdles in deploying them at the necessary scale to ensure that their alleles spread across the population; this process can take a long time, at least for the coral genome - symbionts and the microbiome may be able to achieve this faster.

Accordingly, understanding the effect size is critical for the planning, development, and successful implementation of assisted evolution methods. To be able to calculate the predicted effect size of an intervention, information is required on additive genetic variance and covariance, as well as changes in mortality and reproductive success in enhanced corals as a function of cumulative heat stress.

Almost no data exist currently that quantify such effect sizes beyond rough estimates of mean tolerance, variation in tolerance, and broad sense heritability.

We examined the database to determine how many studies provided the necessary information required to estimate and compare heat stress exposures (in DHWs), and to calculate effect size of interventions. Our analysis shows that only a very small number of studies (between 1 and 9 studies, Table 4) provide the required data. Furthermore, when considering the degree of cumulative stress and taxa that are targeted in each of these studies, only one or two data points from fewer than four taxa are available.

Table 4: Number of studies in the database providing data that can be used to calculate enhancement effect size.

Assisted evolution method	Number of studies
Conditioning	9
Assisted migration	0
Intraspecific breeding between reefs	3
Intraspecific breeding within reefs	1
Hybridization among species	2
Algal symbiont manipulation	8
Microbiome manipulation	0
Gene editing	0

It is of paramount importance to develop studies that can be used to evaluate the impact of assisted evolution methods by providing the required empirical information to properly calculate expected effect sizes. Therefore,

- **We recommend funding a variety of assisted evolution methods that target the coral host and its symbionts. The proposals should be evaluated based on their ability to meet stated cost-benefit parameters. They should adhere to best practices developed in Recommendation 1 and contribute to the mission of filling critical knowledge gaps (Recommendation 2)**
- **We also recommend that generating information to estimate potential effect size of assisted evolution methods should be required as an early milestone of these studies.**

Impact: The generation of this knowledge will enable comparisons across assisted evolution methods, allowing us to identify those methods that can provide higher impacts in terms of coral heat tolerance enhancement and improve our understanding of their associated risks.

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3.4

Estimate correlations of heat tolerance across life stages

Although some empirical evidence suggests that performance during temperature stress events varies between different coral life stages (Humanes *et al.*, 2016), these differences have not been thoroughly documented.

Developing assisted evolution methods to identify heat tolerant corals or to increase their resistance while they are still in their early stages (larvae and juvenile corals) provides significant logistical benefits that could be critical for spatially scaling up these interventions.

However, our database revealed that 86.6% of published heat stress studies focused solely on adult stages, while only 3% evaluated both early and adult stages, with a maximum of three studies for the same taxa.

Further analysis is necessary to determine if early and later life stage responses are correlated. The majority of studies that investigated the effect of assisted evolutionary methods on heat tolerance (N = 66) focused either on adult stages (N = 44) or larvae (N = 18), with none investigating the effects of enhancing early-stage tolerance on adult (reproductive) colony resistance to heat stress. Therefore,

- **We recommend investing in research that estimates the persistence of heat tolerance across coral life stages. This entails characterising heat stress responses of larvae, juveniles and adult reproductive colonies originated from the same cohort.**

Impact: The findings of these studies will provide critical information in determining whether heat tolerance at early stages is maintained throughout the lifespan of the colony, maximising the success of existing and new interventions.

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RECOMMENDATION 4:

Advance existing and new technologies for long-term success

Sequential 3 to 5 year funding

According to current knowledge and expert opinions, the obtained effect size of most assisted evolution methods is relatively small in comparison to possible or even likely warming scenarios; therefore, R&D is warranted for other methods that may be able to achieve larger effect sizes.

These methods may include gene editing technologies to increase expression of traits or interbreeding species from outside their current range or from extreme environments to enhance local performance. These methods are considered to be of higher risk ecologically and in delivery and have generally not been prioritised to date (Bay *et al.*, 2019; National Academies of Sciences, Engineering & Medicine, 2019).

R&D on methods with higher risks and longer development horizons must be commenced soon so that their development has the time needed to follow all safeguards and protocols and still be available when needed.

The workshop group and the scientific community at large are aware of both positive and negative effects of assisted evolution methods and strongly recommend that risk assessments and safety precautions are taken when higher risk methods are examined.

4.1

Use transgressive segregation to create extreme phenotypes

In response to environmental challenges like high(er) temperatures, isolated populations may evolve similar phenotypic adaptations that are underpinned by different genetic mechanisms. By combining different genetic solutions for the same trait from historically separated populations or species, plant and animal breeders have generated desirable transgressive (extreme) phenotypes such as higher growth rates, drought resistance and disease resistance (Mackay *et al.*, 2021).

It is reasonable to expect that such results can be achieved in corals as well, and that effect sizes will be larger than those observed for assisted evolution methods currently under investigation. Coral populations and species may maintain the ability to interbreed over geographic space (Hagedorn *et al.*, 2021) and, for some genera, across species boundaries (Chan *et al.*, 2019).

However, later generation hybrids (beyond the F1) are required to create transgressive phenotypes and demonstrate multigenerational effects, and these studies are very rare. While transgressive segregation should work for any crosses between historically isolated groups, the longer the isolation time between parental groups, the more extreme the expected spread of phenotypes (Rieseberg *et al.*, 1999).

Therefore, interspecific hybridization would likely yield larger effects than intraspecific hybridization. As such, we recommend investment in,

- **Laboratory based crossing trials of corals that extend across multiple generations and that track phenotypes and genotypes to gauge effect sizes. These crossing trials should be conducted between species (interspecific hybridisation) and between geographically separated populations (intraspecific hybridisation).**
- **Studies that can find wild hybrid corals to be used as parents in laboratory-based trials, thereby bypassing one or more generations.**
- **Methods to induce sexual reproduction of symbionts, as transgressive segregation relies on recombination that accompanies sexual reproduction.**

Impact: Methods that rely on transgressive segregation (inter and intraspecific hybridisation) have the potential to yield large effect sizes that will persist and even increase over generations. Concurrently, there is the risk of inadvertently creating highly competitive "invasive" species, so laboratory-based studies should minimise risks before these methods are considered for any field-based trials.

4.2

Improve gene editing and splicing technologies to accelerate adaptation of natural populations

CRISPR-Cas9 is a recently discovered molecular tool that can be used to make precise changes to an organism's genome (Doudna & Charpentier, 2014). Gene editing can be used to determine the roles of specific genes in the regulation of ecologically important traits (e.g., heat tolerance, biomineralisation, and bleaching) and to modulate (up or downregulate) these genes to enhance traits (van Oppen & Oakeshott, 2020). A functional understanding of key coral traits is central to identifying resilient individuals and populations and developing genetic tags or markers of heat tolerance (Cleves, 2022).

To date CRISPR/Cas9 has been used to understand the function of coral genes involved in ecologically relevant traits such as heat tolerance (Cleves *et al.*, 2020) and calcification (Tinoco *et al.*, 2023). The number of genomic studies in corals has been rapidly growing over the past several decades, generating many hypotheses about the genetic basis of coral resilience and other traits. However, the lack of functional testing of these hypotheses has led to a significant knowledge gap that prevents researchers from using solid genetic information in conservation and assisted evolution. Using existing and expanding current CRISPR/Cas9 methods (i.e targeted knock in and allele replacement) offers an opportunity to fill these critical knowledge gaps to unify genetics with conservation (Cleves, 2022).

Currently, CRISPR/Cas9 can be delivered using microinjection for several coral species (e.g., Cleves *et al.*, 2018, 2020; Tinoco *et al.*, 2023), but some species are more difficult to inject than others. Upscaled methods for CRISPR/CAs9 delivery (such as chemical transfection) must be developed to realise the full potential of this method. Improving gene editing and knock-on technologies will yield important information on coral genetic architecture and ways to enhance heat tolerance traits to support methods with larger effect sizes. Therefore, we recommend investment in:

- **Improved gene editing and splicing methods to understand the genetic architecture of key tolerance traits and ways to enhance them in both coral hosts and symbionts.**
- **Investment in studies that develop tools and methods to upscale gene editing.**
- **Investment in the application of CRISPR-Cas9 in diverse coral species.**

Impact: Gene editing tools provide a test of the genetic basis of key traits and the possibility to manipulate and/or tag coral genomes - providing essential foundational knowledge with immediate (tags) and longer term (enhancement of key traits) benefits for seeding corals onto reefs.

Photo: © James Guest

4.3

Support technologies that enable the development and deployment of assisted evolution

The workshop identified a number of supporting technologies that are important or essential for various recommendations to understand natural and assisted adaptation.

For example, coral propagation is an essential enabler to deliver AE corals in higher numbers onto reefs. Most of the methods require successful sexual reproduction of the coral host and, in some instances, the symbionts.

This is particularly true for selective breeding, hybridisation, generation of extreme phenotypes via transgressive segregation and gene editing. In some places, research is needed to understand natural coral reproductive cycles whereas in others the time may be right to manipulate the timing of reproduction and length of the life cycle to fast-track research and propagation.

Cryopreservation is a supporting technology that - in addition to preserving modern genotypes - could enable corals from different locations and different breeding seasons to be crossed.

Focusing on gametes could also reduce risks of parasite spreading (Baums *et al.*, 2019). Methods to better understand pathogens in coral AE methods and tools to support coral cell culture were also flagged as important by the workshop group.

To advance work on assisted evolutionary methods, particularly assisted migration and transgressive segregation, we recommend investment into studies that:

- **Upscale coral aquaculture processes.**
- **Accelerate the reproductive cycle of corals.**
- **Solve the problem of cryopreserving coral host gametes/ larvae and symbionts, especially those that are resistant to culturing.**

We recognise this list is incomplete and propose it is further scoped and regularly. Thus, we recommend to:

- **Continue to review and update the list of priority supporting technologies.**

Impact: Continued development of supporting technologies such as coral breeding and cryopreservation underpin the ability to deliver fast results across the recommendations in this roadmap.

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