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Census of heat tolerance among Florida's threatened staghorn corals finds resilient individuals throughout existing nursery populations

Ross Cunning, Katherine E. Parker, Kelsey Johnson-Sapp, Richard F. Karp, Alexandra D. Wen, Olivia M. Williamson, Erich Bartels, Martine D'Alessandro, David S. Gilliam, Grace Hanson, Jessica Levy, Diego Lirman, Kerry Maxwell, Wyatt C. Million, Alison L. Moulding, Amelia Moura, Erinn M. Muller, Ken Nedimyer, Brian Reckenbeil, Ruben van Hooidonk, Craig Dahlgren, Carly Kenkel, John E. Parkinson and Andrew C. Baker

Article citation details
Proc. R. Soc. B **288**: 20211613.
http://dx.doi.org/10.1098/rspb.2021.1613

Review timeline

Original submission:	16 July 2021
Revised submission:	24 September 2021
Final acceptance:	27 September 2021

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2021-1613.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
Cunning et al. present the results of acute heat stress assays for thermal tolerance in Acropora cervicornis held in nurseries across the Florida Reef Tract. Overall they find that variation in thermal tolerance is higher within than among nurseries and that tolerance individuals can be found in all source populations. Despite its obvious importance in future ecosystem stability, historically, high-throughput measurement of thermal tolerance in corals has been limited by the feasibility holding many individuals for long-term aquarium experiments. Cunning et al. show not only that the emerging CBASS systems can be used to measure relative thermal tolerance, but that the results are repeatable. This opens the door for many future eco-evolutionary studies aimed at understanding and predicting climate response in corals. The paper is very clearly written, the analysis is thorough, and the results are of broad interest. I have just a few minor comments:

I found myself a little confused about when the authors used ED50 vs. ED50adj. For example, in lines 270-272 states: "Mean ED50 was highest at RR, intermediate at CRF, NSU, MML, and UM, and lowest 271 at FWC (Fig. 2B), but these differences were not related to the maximum monthly mean (MMM) 272 temperature." However, Figure 3 shows lack of relationship between ED50adj and MMM. It's unclear to me whether both analyses were done. Was ED50 used for all downstream analysis?

Related to the above, I worry that removing the nursery effects may eliminate more than just environmental effects. For example, based on the figure in the supplementary figures there is bias in where colonies were originally sourced from, with colonies more likely to be kept in nurseries nearby. In this case, if there is genetic variation for thermal tolerance across latitude, this signal would be reduced in the ED50adj statistic. I wonder if the conclusions about the distribution of the most tolerant individuals also hold for the raw ED50 statistic?

I think there is a mistake in the figure citation on line 276: "Variation in Symbiodiniaceae had no effect, since all colonies analyzed (n = 182) hosted exclusively Symbiodinium, except for a single individual from RR that was dominated by Durusdinium (see Fig. 2B)." This figure does not reference Symbiodiniaceae.

I am having trouble reconciling what the authors term a small effect of nursery with the fact that there is little correlation across genets reared in multiple nurseries. The first would suggest that plasticity is not large while the second would suggest plasticity plays a large role in thermal tolerance variation. I wonder if the authors have any possible explanations for this?

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Excellent

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

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Is it accessible?
Yes

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
See attached PDF. (See Appendix A)

Decision letter (RSPB-2021-1613.R0)

17-Sep-2021

Dear Dr Cunning

I am pleased to inform you that your Review manuscript RSPB-2021-1613 entitled "Census of heat tolerance among Florida's threatened staghorn corals finds resilient individuals throughout existing nursery populations" has been accepted for publication in Proceedings B.

The referee(s) do not recommend any further changes. Therefore, please proof-read your manuscript carefully and upload your final files for publication. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let me know immediately.

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2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and the file name should contain the author's name and journal name, e.g authorname_procb_ESM_figures.pdf
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please see: https://royalsociety.org/journals/authors/author-guidelines/

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It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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5) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your final version. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Dr Daniel Costa
mailto:proceedingsb@royalsociety.org

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s)
Cunning et al. present the results of acute heat stress assays for thermal tolerance in Acropora cervicornis held in nurseries across the Florida Reef Tract. Overall they find that variation in thermal tolerance is higher within than among nurseries and that tolerance individuals can be found in all source populations. Despite its obvious importance in future ecosystem stability, historically, high-throughput measurement of thermal tolerance in corals has been limited by the feasibility holding many individuals for long-term aquarium experiments. Cunning et al. show not only that the emerging CBASS systems can be used to measure relative thermal tolerance, but that the results are repeatable. This opens the door for many future eco-evolutionary studies aimed at understanding and predicting climate response in corals. The paper is very clearly written, the analysis is thorough, and the results are of broad interest. I have just a few minor comments:

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Referee: 2
Comments to the Author(s)
See attached PDF

Decision letter (RSPB-2021-1613.R1)

27-Sep-2021

Dear Dr Cunning

I am pleased to inform you that your manuscript entitled "Census of heat tolerance among Florida's threatened staghorn corals finds resilient individuals throughout existing nursery populations" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

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Electronic supplementary material:
All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

This paper uses a system (CBASS) developed for use in the Red Sea to measure photosynthetic responses to short-term (7 hr) elevated temperatures by symbiotic zooxanthellae in the once dominant, now severely threatened coral *Acropora cervicornis* transplanted to six nurseries spanning much of the Florida Reef Tract. Photochemical efficiency (Fv/Fm) was measured for cloned coral fragments exposed simultaneously at eight temperatures, and expressed as dose response curves; the ED50 provides a quantitative measure of physiological heat stress/tolerance. Contributions include: modifying the laboratory CBASS for shipboard use and conducting surveys at six nurseries; using CBASS for rapid identification of heat-tolerant genotypes, and demonstrating that heat-tolerant genotypes exist naturally throughout the region; and providing evidence that much of the phenotypic variation in heat tolerance is genetically determined.

This paper should interest global audiences from a wide range of backgrounds, since it has many potential applications going far beyond *A. cervicornis* in Florida, including: other corals; other photosynthetic organisms (e.g., algae, foraminifera…); other regions, habitats and ecosystems; and other stressors that affect photosynthesis. Despite the breadth of potential applications, the paper seems directed at a very narrow readership – some included details, and the absence of other details, seem to presume all readers are very familiar with minutae of Florida corals and reefs, and of photosynthetic measurements. A broader readership could be attracted by adding brief sentences about potential uses to the Abstract, Introduction and Discussion; by minor editing, from the perspective of non-specialists and workers in unrelated systems; and possibly by modifying the title.

GENERAL COMMENTS

Abstract: This has relatively low information content (see Minor Points for some suggestions). While most corals tested (170+?) were unique genotypes, 31 genotypes were at 2 or 3 nurseries – allowing separation of genotypic and environmental effects. The June measurements and June-October comparisons should be mentioned.

Introduction and **Discussion:** Both are generally easy to read and understand, at least partly because the text is less terse than that of many papers today. While unnecessary words and phrases could be edited out, I only have a few general comments and minor stylistic suggestions that may enhance these sections. There could be more discussion of two major assumptions:

1. The relevance of short stress tests to bleaching - i.e. what is the evidence that a 7 hr stress test is a good predictor of resistance to prolonged stress causing bleaching (usually attributed to 4-6 degree-weeks above normal maximum at that site) – citing a paper with no indication of its content seems inadequate?
2. What is the evidence that persistent differences between cloned corals in different nurseries are due to genetic variation in the coral rather than the symbiont?

Materials and Methods: This contains many relevant details but would benefit from some reorganization, more consistent terminology, and a few exchanges of information with the Supplement. The Methods should contain, very briefly, some information about:

Nurseries – especially criteria for inclusion of original source colonies in nurseries. My understanding is that most (if not all) were added because they were survivors of major bleaching events – i.e., already known known or suspected to be heat tolerant.

How long were corals at nursery before CBASS trials (i.e., possible roles of long-term acclimatization)?

Are ambient temperatures and field variation (diurnal, tidal, lunar) the same or different among nurseries (i.e., possible roles of short-term acclimation)?

What criteria were used to select the colonies used for this study at each nursery (by genotype, known source, random…).

June measurements and the Jun-Oct comparisons are not mentioned in the Abstract or Introduction, and are alluded to without being named or described at the end of the statistical Methods, even though they support an important Fig. 5 demonstrating consistency of relative ED50 over time, and of constant absolute changes in ED50 values between seasons at three nurseries. Relegating the entire description of the June measurements to the very end of the Supplement is insufficient – essential points, especially differences from shipboard procedures, should be included in the main Methods.

Results: The text seems surprisingly brief, considering the scope and complexity of the study. Full comprehension requires a reader to look closely at figures and tables in both the main text and the supplement. Figure legends tend to be confusing due to inadequate descriptions, unnecessary details, missing information, or inconsistent terminology, and the figures themselves tend to diverge from common conventions for presenting scientific data.

Fig. 1A: Contains very little real information. First, it is not a map – it appears to be (or be based on) satellite imagery [low resolution Google Earth?] – but no source is cited. Most land details are irrelevant, the marine color scheme is not explained, and the colors over the reef tract obscure rather than illustrate anything about the reefs. It should also be extended westward to include all source reefs (i.e., the Far Tortugas are mentioned in the text and are the source of 2 corals). A clearer, more informative figure would be a black & white map outlining the land and reefs, a few useful isobaths (e.g., 0 m? 10 m? 50 m? 100 m?), some place names to orient readers (e.g., Far Tortugas, Key West, Miami …), and the names of locations of each nursery. These suggest a larger figure would be helpful. [The mixture of Counties and Keys regions given as broad "locations" in Table S1 is confusing – I believe all Keys are in Monroe County, which is not even mentioned]. Any and all place names mentioned in the text (including Broward County, Key regions and Far Tortugas) should be indicated and labelled in Fig. 1A.

A reader's understanding of the design and of the results (especially genotypic aspects and analyses), could be enhanced by extending Fig 1A to its right as a complementary Table containing much of the information now in Table S1. Particularly relevant would be dates of measurement (perhaps 3 columns for Jun, Aug, Oct?) and a breakdown of total colony numbers into multiple columns including: number of local source corals (genotypes?); number imported (clones) from other nurseries; local corals shared with other nurseries (identified by codes); and numbers used in the June 2020 experiment. If nursery names and codes are the first column(s) in such a Table, they can be deleted from the legend.

Fig. 1B: These are more than "target" temperatures, they are time courses of each temperature treatment. The colors add no information and adjacent intermediate shades are not readily told

apart. Black lines would be clearer (and, if desired, each could be labelled above the plateau with its "intended" maximum).

Fig. 1C: Appears to be an example of one dose-response curve for one coral, and should be labelled as such (not plural "curves"). Colors are hard to distinguish, merge duplicate points (at lower temperatures), and provide no new information. Black symbols would enable better separation of duplicates. Legend should explain the 2 points per temperature; and legend or plot could include nursery/coral ID and date of measurement. Stress that the x-axis is the maximum "achieved" or "realized" temperature – not the "intended" temperature as in Fig. 1B.

Does "individual" mean a fragment with two measurements? If so, this conflicts with usage in 1A where it seems to mean "colony" (or "genotype"?).

Fig. 2A: Color is valuable here for identifying nurseries, but stronger primary or more distinctive colors, rather than "pastel" shades, would enhance the plot. The blues of UM and CRF and green RR are particular difficult to distinguish, and the two pinks (NSU, MML) become difficult to separate when both occur in the tails. The nursery color key could be defined in Fig. 1A by coloring the "boxes" labelling each site.

Does "individual" mean a "colony/genotype" or a "fragment"?

Label y-axis as "Number of colonies" rather than "Count" [of what?]

Fig. 2B: Legend is somewhat misleading – while the points are individual colonies, the probability density functions are nursery properties. Grand mean line is too faint. Label y-axis as "Nurseries" and add "ranked by latitude" to legend?

Fig. 2C: Since this figure does not include any genetic or GXE analyses, the legend would be more accurate as [*changes italicized*]: "(C) Adjusted ED50 values, *after subtracting* ~~the~~ variation among nursery environments from ~~the~~ total phenotypic variation; *they were used* to estimate …"? Also, would it be interesting/useful to color code 2C by nursery (as in 2A)?

Fig. 3: Why are latitude, longitude and MMM values on x-axes (in A, B and C) all expressed with different precisions? Why are longitude ranges and 1^{o} interval sizes different in B and D? Why are Dry Tortugas colonies included in B but excluded from D? Why is the region mapped in 3D not identical to that in Fig. 1A?

Fig. 4: The potential problems of distinguishing similar colors (raised for blues and pinks in Fig. 2A) are realized in this figure with its overlapping lines. Some combination of stronger colors and different symbols would aid comparisons among nurseries.

Fig. 5: Since both axes are temperatures, they should be expressed on the same scale, and labelled with the same precision.

Table S1: See Fig.1A notes for suggested expansion and inclusion in the main text. It should certainly include the June measurements.

Fig. S1: This Venn diagram is not particularly informative, and overlapping colors are difficult to interpret. Probably easier to interpret if only the outline of each oval was colored, with all interiors white.

Much more informative would be a tabulation of genotype ID, collection site (lat/long?) of source colony, initial propagation nursery, shared nurseries… Main text or legend should state whether sharing occurred at the time of initial collection of the source genotype, or after growth and propagation at one nursery.

Second sentence of current legend should replace "colonies" with "genotypes" – as a single entity, a colony cannot exist at two sites simultaneously.

Fig. S2: Area mapped should be identical to that in Fig. 1A and Fig. 3D. Does the horizontal line in FWC leads to Dry Tortugas (off the map)? – that site should be included in all maps.

Fig. S3: Information about genotype IDs and source collection sites needs to be available, either in the Table suggested to replace Fig. S1, or by citing appropriate deposited data. Do letters indicate collection sites? (e.g., does KW = Key West?).

Fig. S4: Since both axes are ED50s, they should use same scale, so all plots should be square, not the current rectangles. It would be helpful to insert (N = ?) for each plot. Since plots above and below the diagonal appear to be the same data with axes transposed, it seems unnecessary (and misleading?) to include both halves of the matrix. Since the plots forming the main diagonal appear to be plotting each colony against itself, there is no information to be gained from them – so why are they included?

DEFINITIONS

One potential source of confusion that interferes with reading and comprehension is the way the paper refers to corals by a variety of terms that have many shades of meaning but often seem to be used interchangeably or as synonyms: clone, genotype, genet, population … Some have very specific meanings (not necessarily identical) in ecology, demography, genetics, and/or evolution, while others are very imprecise with meanings largely dependent on context. These words often appear in the same or adjacent sentences referring to the same or different levels of biological organization in ways that are not immediately obvious. In particular,

Coral, colony, individual, fragment are used as synonyms for 1 piece of coral

Genotype and genet seem used interchangeably on multiple scales from a single fragment to all colonies of one genotype. The original definition of "genet" (for diploid organisms) is all tissues derived by mitosis from the same zygote (i.e., all have same genotype). Single isolated pieces of that tissue that are physiologically and ecologically independent (i.e., clonemates from fragmentation, asexual reproduction, parthenogenesis…) are defined as "ramets".

For corals, each colony and each fragment is a ramet, and has the same ecologically also an individual; genetically, individual becomes context-dependent, but it is simplest if the entire genet is defined as the individual, and ramets are described by other terms. Genotype is also context-dependent (every biological unit from cell to fragment to genet has a genotype).

I strongly advise defining a single term for each level of genetic, ecological and or morphological organization and using only that term for that meaning throughout this paper.

"Population" is also used loosely on several scales from in one nursery up to the Florida Reef Tract, but genetically and ecologically, it has precise meanings that probably do not include the scale of the FRT.

MINOR POINTS (by line number)
These are not exhaustive, and many suggested wording changes are intended as examples of common imprecise, low information, confusing or misleading usages that tend to recur elsewhere in the paper. The paper should be checked for grammatical errors, especially frequent mixing of singular and plural nouns and verbs.

Abstract:

28	Replace "highlights the need for" with "is driving development of potential"
30	Replace "target" with "rely on using"
31-32	Plural "challenges" is subject of singular verb "has" – change to "have"
32-33	Capitalize "Coral Bleaching Automated Stress Systems (CBASS)" – as in line 83
34	Replace "across" with "growing in" or "transplanted to" – more informative and precise
35	Replace "coral" with "colony"
36	Is "range" within a nursery, or across all nurseries?
39	Define population: within a nursery? entire reef tract?

Introduction:

57	Replace "help to boost" with "increase"
62	Replace "significant" with "substantial" – unqualified "significant" is vague; better if only used in statistical sense
63	"at scale" is extremely vague and does not indicate direction (or magnitude) of scale
64	Delete "our" – implies restriction to current authors rather than to discipline in general
65	Avoid restrictive "we" and excessive infinitives. One possible alternative: "Overcoming these challenges requires better understanding of the natural variation…"
67	"co-determined" is a legal term meaning a decision-making process involving both management and workers (as more or less co-equals). Better phrasing: "is determined by interactions between a coral's genes, symbionts and environment"
71	Replace "across" with "within"
72	Delete "Corals' "
74-75	Delete "sometimes" and Replace vague "show" with "may have"
75-76	As written, this sentence could just as well be interpreted as evidence of variation in the symbiont's genotype.
82	Replace "determination" with "applications"?
87	Replace "Such efforts" with "CBASS"?

91 Delete “a population of ” – population could have several meanings in the context of this sentence

97 Delete “board”

98 Replace “tolerance” with “tolerances – this is one of many cases where a singular noun refers to plural entities (and *vice versa*)

101-102 Implies all genotypes are common-gardened in all 6 nurseries – instead, some of the genotypes were tested in 2 to 4 nurseries

103-105 Rewrite without first person pronouns implying restriction to these authors.

Methods:

108 Start with brief information about source colonies. Criteria? When established in nurseries? When were some shared with other nurseries (at time of source collection or later?

108 Follow with brief introduction to June on-shore measurements should be introduced here (give rationale for June study? pilot? temporal variation?) State which nurseries visited on each cruise.

110-114 Nursery list need not be repeated here, since it is spelled out in Fig 1 legend. Even better (and much easier to read) if tabulated as the table suggested to complement Fig. 1A

118 Replace multi-meaning “each coral’s” to precise “each fragment”

119 “Replicate” implies within a treatment (e.g., at one temperature). This design does not replicate genotypes at this level – instead, the 8 fragments of each colony are distributed over 8 treatments (1 per temperature). State typical size (dimensions) of fragments.

127 State dimensions of each tank including water depth, and fragment positions (and how mounted?)

133 Was PAR measured at same depth as corals?

153-156 Since nursery codes are already defined, just use abbreviated codes here

160-163 Were livewells shaded or exposed to ambient sunlight? What were ambient and livewell temperatures? How different from initial experimental $30^{O}C$?

163 Dimensions of grid? Spacing of fragments? Does “consistent position” mean all fragments of a genotype were in the same position in each tank (not randomized among tanks)?

170-171 Delete sentence – said earlier in more detail

175 Does this mean randomization within each tank, among tanks, or both?

193 Replace “were then used to model” with “modeled” – example of excessive wordiness

215 Delete “of the climatology”

221 Give reference for original extraction protocol

226 Replace “detect the presence of” with “distinguish”

242-245 Does this describe the June measurements? Augment here with more specifics from the Supplementary methods (e.g., design, dates…)

Results:

258 Add numbers of genotypes at 2 and 3 nurseries (to add up to 229)

259 Insertion “were represented *by ramets* at multiple nurseries”

266 Replace “was not equal across nurseries” with “differed significantly among nurseries”

267 Does “individual” refer to fragments or genotypes?

276-277 What is evidence that there is no relevant genetic variation in *Symbiodinium* among corals?

Discussion:

252-262 Given the relatively simple geomorphology, bathymetry, circulations and climates of the region, are there any reasons (e.g., from other organisms) to expect a different distribution of tolerances? In larger, more complex systems (e.g., Great Barrier Reef) where bleaching differs markedly with both longitude and latitude, thermal tolerance probably does vary on several scales.

The Mean Monthly Maximum oceanic temperature in 5^{O} pixels is likely to be a poor indicator of the diurnal, tidal and lunar extremes and rates of change of temperature in local habitats where retention times of water may be prolonged on different parts (e.g., reef flats, lagoons).

397-398 Indicate main differences with other systems?

References:

1. All *Genus* and *species* names in titles should be italicized (many refs.)
2. Don’t capitalize *species* names (ref 10)
3. Only capitalize proper nouns (names) in titles (refs 10, 19, 42