

# Consensus thermotolerance ranking for 110 *Symbiodinium* phylotypes: an exemplar utilization of a novel iterative partial-rank aggregation tool with broad application potential

Timothy D. Swain<sup>†,1,2</sup>, John Chandler<sup>†,3</sup>, Vadim Backman<sup>3</sup> and Luisa Marcelino<sup>\*,1,2</sup>

<sup>1</sup>Department of Civil and Environmental Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA; <sup>2</sup>Department of Zoology, Field Museum of Natural History, 1400 South Lake Shore Drive, Chicago, IL 60605, USA; and <sup>3</sup>Department of Biomedical Engineering, Northwestern University, 2145 Sheridan Road, Evanston, IL 60208, USA

## Summary

1. Corals may reduce the effects of heat-induced bleaching through associations with thermotolerant algal symbionts (*Symbiodinium*). Although hundreds of *Symbiodinium* genetic types (phylotypes) are known, thermotolerance has been systematically evaluated for small subsamples within individual reports, making consensus a challenging task.

2. Data on 110 phylotypes were aggregated from 35 reports, each assessing 2–24 phylotypes (median 4). One-third of reports include ties (phylotypes with indistinguishable thermotolerance). Between reports, most phylotypes are unique (74% assessed once), most pairwise comparisons are unknown (relative thermotolerance for 15% of the possible 5995 phylotype pairs are evaluated) and many rankings are inconsistent (52% of 58 phylotype pairs assessed in more than one report are discordantly ranked).

3. Ranking of *Symbiodinium* phylotypes resulted in 64 thermotolerance cohorts (47 are single phylotypes, 17 contain 2–10 phylotypes with a median of 3 phylotypes) and indicates diverse thermotolerance capabilities within clades and among closely related phylotypes.

4. The iterative partial-rank aggregation method (with error estimation) introduced here is broadly applicable to any quantitative consensus rank building problem where qualitative input ranks are not fully comprehensive nor resolved.

5. Reconstructions based on simulated data (mirroring the empirical data set) indicate robust predictive capabilities for resolving rankings (1.28 approximation of the true solution) and inferring unknown pairwise comparisons (83% correctly predicted pair rankings from only 16% known *a priori*).

6. Application to *Symbiodinium* phylotypes represents an additional tool to generate testable hypotheses on the role of symbiont thermotolerance in an ecosystem that is collapsing in the face of climate change and provides the first quantitative index of *Symbiodinium* thermotolerance and its associated uncertainty.

**Key-words:** climate change, coral bleaching, partial-rank aggregation, symbiosis

## Introduction

Coral reefs are important centres of productivity and biodiversity that support more than 500 million people (Wilkinson 2008). The foundation of these ecosystems are mutualistic endosymbioses with *Symbiodinium*

dinoflagellates, which provide photosynthetic products that fulfil the energetic requirements of their coral hosts (Muscatine 1990). Persistence of coral ecosystems is dependent upon the integrity of these mutualisms; when temporarily disrupted through the bleaching response, corals experience increased disease, predation, competition, bioerosion and mortality, and reduced repair, growth and reproduction (Jokiel 2004; Jones 2008). Coral bleaching is induced

\*Correspondence author. E-mail: l-marcelino@northwestern.edu

<sup>†</sup>These authors contributed equally.

by sufficiently sudden, severe, or prolonged temperatures above local historical maxima, which are predicted to occur at increasing frequencies and severities due to global climate change (Wilkinson 2008). The third global bleaching event is currently ongoing and is predicted to affect 38% of reefs and destroy >12 000 km<sup>2</sup> of coral (www.globalcoralbleaching.org).

Bleaching responses vary across colonies, taxa and events (e.g. Loya *et al.* 2001; van Woesik *et al.* 2011), providing critical insight into bleaching determinants and mechanisms that inform our predictive and remediative capabilities. Multiple environmental factors and host–symbiont interactions contribute to differential bleaching susceptibility (e.g. Enríquez, Méndez & Iglesias-Prieto 2005; Middlebrook, Hoegh-Guldberg & Leggat 2008; Baird *et al.* 2009; Mieog *et al.* 2009; Cunning & Baker 2013; Marcelino *et al.* 2013; Hawkins *et al.* 2014; Wangpraseurt *et al.* 2014; Wooldridge 2014). However, the most frequently invoked hypothesis resides in the diversity of *Symbiodinium* physiological capabilities (e.g. Sampayo *et al.* 2008; Grottoli *et al.* 2014; Silverstein, Cunning & Baker 2014; Hume *et al.* 2015). Nine clades (A–I) of *Symbiodinium* taxa have been identified, four of which (A–D) commonly associate with hermatypic corals (Pochon & Gates 2010; Pochon, Putnam & Gates 2014). *Symbiodinium* clades are subdivided into a rapidly increasing number of subcladal phylotypes (409 as of 2011: Franklin *et al.* 2012), identified through DNA sequences of the second internal transcribed spacer (ITS2) region of the nuclear ribosomal RNA gene. This diversity is meaningful for ecosystem function, as assessed phylotypes often display physiological differences that approximate ecological species (Sampayo *et al.* 2007; LaJeunesse *et al.* 2014) and differ in their ability to acquire inorganic nutrients (Baker *et al.* 2013), resist oxidative stress (McGinty, Pieczonka & Mydlarz 2012), produce photosynthetic products (Cantin *et al.* 2009; Starzak *et al.* 2014) and resist thermal stress (LaJeunesse *et al.* 2009). As thermal stress is often the trigger of coral bleaching, differential *Symbiodinium* thermotolerance is thought to be closely tied to (but not the sole cause of) differential bleaching susceptibility by increasing bleaching thresholds by 1–2 °C (Berkelmans & van Oppen 2006).

There is general acceptance that specific *Symbiodinium* phylotypes are exceptionally thermotolerant (e.g. D1, D1-4, C15, A3), while others are thermosensitive (e.g. C3, C7, B17, A13). This perception is derived from distributional surveys and experiments that assess subsamples of phylotypes. Distributional surveys (e.g. Silverstein *et al.* 2011; Tonk *et al.* 2014) can assess dozens of phylotypes across thermal gradients, however are limited by the distribution of hosts and their specificity to symbionts. Experimental studies (e.g. Thornhill *et al.* 2006; McGinty, Pieczonka & Mydlarz 2012) can assess *in hospite* or *in vitro* phylotypes during abnormal thermal events, however are restricted by host and symbiont

specificity (e.g. Fabina *et al.* 2013) and the culturability of phylotypes (Krueger & Gates 2012). None of these approaches are suitable for systematically assessing thermotolerance of all *Symbiodinium*, and most phylotypes have never been compared in a unified way.

A quantitative consensus thermotolerance ranking based on current knowledge would be a useful tool for assessing reef condition, detecting recent bleaching events and predicting future bleaching events while allowing hypothesis testing of the role of *Symbiodinium* thermotolerance in bleaching susceptibility. However, there are challenges that hinder the construction of such an index. Available data sets contain few phylotypes (rarely more than 5) with minimal overlap across data sets (most phylotypes are evaluated by a single study); therefore, most pairwise comparisons must be inferred. Some data sets include phylotypes with indistinguishable thermotolerance, or tied ranks, prohibiting complete resolution and creating a third category (<, =, >) for analysis. Additionally, available data are often qualitative (e.g.  $\alpha < \beta < \gamma$ ) and contain significant ranking inconsistencies across data sets. These challenges might be best addressed by rank aggregation methods; however, no currently available method simultaneously addresses all of these issues.

Rank aggregation methods are used to produce a consensus of multiple input ranking lists; they address a common problem that originated with election schemes and are a key component of internet search engine algorithms (e.g. Google) and other ranking applications (e.g. best colleges). Because the time required to solve rank aggregation problems increases exponentially with the number of elements (here, elements are phylotypes), it is virtually impossible to find exact solutions to even small (*c.* 20 elements) problems and research has focused on approximation algorithms. While *full rank aggregation* requires fully resolved (no ties) input lists containing all elements and has well-established approximation algorithms, *partial-rank aggregation* allows for unresolved and incomplete input lists, but lacks gold-standard approximations and current methods limit the formats and types of partial lists. Approximation algorithms of *partial-rank aggregation* operate on top-*m* rankings or *p*-ratings, which are unsuitable for this study. Top-*m* rankings simplify input lists to rankings of the highest *m* elements (Ailon 2010; Fang, Xiao & Zhu 2010) and assume remaining elements to be lower; however, assuming lower ranks for unevaluated phylotypes is untenable. By comparison, *p*-ratings allow input lists with ties, but require all elements to be present in each list (Ailon 2010); available thermotolerance data are comprised of partial lists. Additionally, relative thermotolerance assessed by different studies is either qualitative (e.g.  $\alpha < \beta < \gamma$ ), or must be made so in order to compare across studies (e.g. phylotype occurrence vs. photosynthetic performance cannot be directly compared). Qualitative comparisons do not reveal the relative separation

distances between phylotype thermotolerances;  $\alpha$  and  $\beta$  may have similar thermosensitivity and  $\gamma$  may be thermotolerant, resulting in a different interpretation of the example ranking ( $\alpha < \beta \ll \gamma$ ), but current methods cannot accept qualitative input lists and report quantitative rankings. Also, existing methods are computationally intensive; although they can evaluate all possible permutations of elements in small data sets (e.g. Kemeny–Young rank aggregation of <10 elements), these problems are non-deterministic polynomial time-hard (NP-hard) and a single search for the optimal aggregated ranking of 110 elements is estimated at *c.*  $10^{85}$  years on a personal computer (Chin *et al.* 2004; van Zuylen & Williamson 2008).

Here, we developed a novel iterative algorithm (based on *partial-rank aggregation*) that can accurately reconstruct consensus rankings from data sets containing inconsistencies, ties and information gaps, while aggregating qualitative lists into quantitative ranks and estimating associated uncertainties for each ranked element. This method is broadly applicable to ranking problems in ecology (e.g. mate choice, prey choice, foraging selection, or dominance), engineering (e.g. prioritizing contaminated sites for remediation or selection of optimal sites for nature preserves) and beyond. The performance of this algorithm is demonstrated using simulated data sets with known rankings, and an empirical data set to reconstruct the first quantitative consensus ranking for *Symbiodinium* phylotype thermotolerance.

## Materials and methods

We searched the available literature for *Symbiodinium* phylotype thermotolerance and used a novel iterated partial-rank aggregation algorithm (validated against simulated data sets) to create a quantitative consensus ranking.

### COLLECTION OF PARTIAL RANKINGS

We identified reports of *Symbiodinium* thermotolerance in the literature (up to February 2015 publication dates) that contained at least two ITS2 phylotypes (or its equivalents) and included data on relative thermotolerance (Table S1, Supporting information). Reports that exclusively used clade-level designations (or are associated with non-scleractinian hosts) were excluded, as clades contain phylotypes with diverse physiological capabilities (e.g. Tchernov *et al.* 2004). See Appendix S1.1 for details on phylotype identification.

Thermotolerance rankings within each report use one of the following approaches (Table S1): (i) prevalence surveys of *in hospite* phylotypes over thermal gradients, (ii) assessments of symbiont physiological performance and/or prevalence surveys of *in hospite* phylotypes relative to abnormal thermal events or (iii) assessments of phylotype physiological performance *in vitro* relative to abnormal thermal events. These diverse approaches (See Appendix S1.2 for specific examples) are used to derive individual partial ranking lists within each report (or method) and are not directly compared across reports. The partial ranking lists of relative thermotolerance are used as input for the rank aggregation algorithm to create a consensus ranking.

While some rankings are explicitly stated, others were extracted from the data within each report under the central assumption (following the original authors) that high physiological performance and/or prevalence of *Symbiodinium* under high temperature is an indication of thermotolerance (Table S1). In this way, we extracted fully or partly resolved partial rankings from each source publication (only Aschaffenburg 2012 yielded three independent input lists because they were determined by different methods) as the basis of the iterative partial-rank aggregation analysis.

### ITERATIVE PARTIAL-RANK AGGREGATION PIVOTING ALGORITHM

Our novel rank aggregation algorithm combines three disparate concepts: (i) ranking based on consensus-based Borda rank aggregation (developed in voting systems), (ii) updating ranks by a pivot element (an element, or in this study, phylotype, that is shared between input lists: Ailon, Charikar & Newman 2008; van Zuylen & Williamson 2008) and (iii) Monte Carlo iterative analysis to achieve convergence on consensus. This algorithm transforms relative qualitative rankings of thermotolerance for multiple *Symbiodinium* phylotype subsets (partial ranking lists) into a quantitative ranking of the entire sampled population (consensus ranking) with an estimation of uncertainty of the ranking score.

Partial ranking data are analysed in order of greatest information content: the Iterative Partial-Rank Aggregation Pivoting Algorithm (IPRAPA) first ranks the partial lists of phylotypes (originating from individual reports) and then ranks all phylotypes. The lists are qualitative comparative rankings (phylotypes ordered by increasing relative thermotolerance:  $\alpha < \beta < \gamma < \delta$ ) and may contain different numbers of cohorts (phylotypes of same rank and indistinguishable thermotolerance, or ties) and phylotypes (e.g. the ranking  $\gamma < \theta, \chi$ , contains two cohorts and three phylotypes). To anchor partial rankings within the larger population, each list must contain at least one phylotype in common with another partial ranking: the pivot phylotype ( $\gamma$  in the previous examples). Prior to the first iteration, all lists are sorted by the number of cohorts and then phylotypes and are assessed in order of decreasing information content, except when encountering lists lacking a pivot phylotype in the previously entered lists. In this case, lower information-content lists containing pivot phylotypes are ranked higher than lists lacking common phylotypes, until every list in the queue has a phylotype in common with a higher-ranked list.

After ranking all lists, IPRAPA iteratively calculates a consensus quantitative score for each phylotype, which is then used to rank all phylotypes. An iteration begins by assigning a score ( $R$ ) to each phylotype ( $k$ ) in the greatest information-content list ( $i = 1$ ) as:

$$R_{1k}^{(1)} = \frac{\Delta R C_{1k}}{C_1}, \quad \text{eqn 1}$$

where  $\Delta R$  is the preset total range of scores (it is convenient to choose  $\Delta R$  as 100 or equal to the total number of elements across all lists; here, we assigned scores on a 0–100 scale),  $c$  is the cohort of the current phylotype  $R_{1k}$ ,  $C$  is the number of cohorts in list 1, and superscript (1) indicates the first iteration. For all subsequent lists ( $i = 2, \dots, I$ , where  $I$  is the total number of partial ranking lists), IPRAPA updates scores using a pivot from previously ranked lists in the current iteration. A pivot phylotype is chosen as the common phylotype in the current list  $i$  and a previous list that has the maximum cohort value in the current list  $i$ . If a number of pivot candidates exist for a given list, the phylotype ( $k$ ) with the lowest cohort uncertainty ( $U_{ik}$ ) is chosen by:

$$U_{ik} = \frac{\sqrt{\sum_{j=1}^{i-1} \left(\frac{\Delta R}{c_j}\right)^2 \delta_{jk}}}{\sum_{j=1}^{i-1} \delta_{jk}}, \quad \text{eqn 2}$$

where function  $\delta_{jk} = 1$  if list  $j$  contains the pivot candidate  $k$  or  $\delta_{jk} = 0$  otherwise. Thus, the summation is done over all previous lists which contain the pivot candidate. Notice that the sorting of lists ensures that any list  $i$  must have at least one common phylotype that can be used for pivoting this list to at least one higher-ranked list  $j < i$ . As can be seen from eqn (2), the current list  $i$  is excluded from this calculation. Once the pivot phylotype is chosen, the pivot score ( $A$ ) is calculated as the mean over previous rankings of the pivot phylotype in the current iteration using:

$$A_i^{(1)} = \frac{\sum_{j=1}^{i-1} R_{jk}^{(1)} \delta_{jk}}{\sum_{j=1}^{i-1} \delta_{jk}}, \quad \text{eqn 3}$$

where  $R_{jk}$  is the score of pivot phylotype  $k$  in a previously evaluated list  $j$  within the current iteration. This pivot value is then used to calculate scores for all other phylotypes ( $k$ ) in the current list ( $i$ ) using:

$$R_{ik}^{(1)} = A_i^{(1)} \frac{c_{ik}}{c_{ik*}}, \quad \text{eqn 4}$$

where  $A_i$  is the score of the pivot for this list and  $c_{ik*}$  is the cohort to which the pivoting phylotype belongs in list  $i$ . Because the pivot phylotype in list  $i$  is determined by scores for this phylotype in prior lists, it does not have an independent score in list  $i$ , and the pivot phylotype in list  $i$  does not get assigned a score in this list. This process is repeated for each list until one iteration is complete. After the first iteration, an average iteration score ( $R_k^{(1)}$ ) is calculated for each phylotype  $k$  and normalized to the range ( $\Delta R$ ) to foster convergence, using:

$$R_k^{(1)} = \frac{\sum_{j=1}^I R_{jk}^{(1)} \delta_{jk}}{\sum_{j=1}^I \delta_{jk}} \left(\frac{\Delta R}{M^{(1)}}\right), \quad \text{eqn 5}$$

where the maximum average score from the current iteration [superscript (1) indicates the first iteration] is:

$$M^{(1)} = \max_k \frac{\sum_{j=1}^I R_{jk}^{(1)} \delta_{jk}}{\sum_{j=1}^I \delta_{jk}}. \quad \text{eqn 6}$$

Each subsequent iteration repeats in the same manner, except that the initial pivot value is chosen based on rankings from the previous iteration (excluding the first list). Iterations can continue for a specified number or convergence tolerance; in this case, convergence was defined as a difference in scores of  $<10^{-3}$  between current and prior iterations. The iterated consensus scores are taken as the average phylotype scores from the last iteration that achieves convergence:

$$R_k = R_k^{(p)}, \quad \text{eqn 7}$$

where  $p$  is the number of the final iteration. The phylotypes are then ranked in the order of their final scores  $R_k$  (0–100, increasing

in thermotolerance) and phylotypes with the same scores (indistinguishable at a predetermined tolerance) are grouped in cohorts sharing the same rank, with the highest thermotolerance phylotype ranked at 1 (with a scale dependent on the final number of cohorts, decreasing in thermotolerance).

While the first iteration may return the best approximation, subsequent iterations explore a wider parameter space and may return a more accurate solution. The best solution is revealed by comparing coherence (relative to all input partial rankings) of potential solutions ( $R_k = R_k^{(1)}$  vs.  $R_k = R_k^{(p)}$ ). Coherence has been used as the optimal metric for evaluating accuracy of partial-rank aggregation approximation (Fang, Xiao & Zhu 2010) and is the counterpart of Kendall Tau distance, which is typically used as the key evaluation metric in full rank aggregation (see *Validation of IPRAPA*, Appendix S1.3). In essence, it compares all pairs between a candidate solution and each input partial ranking and counts all pairs for which the aggregated ranking does not match input lists while taking into account the length of lists (Fang, Xiao & Zhu 2010).

#### UNCERTAINTY OF THERMOTOLERANCE SCORE AND AVERAGE POSITION ERROR ESTIMATION

Uncertainty or error of measurement ( $U$ ) of each phylotype ( $k$ ) in the consensus score is calculated as:

$$U_k = \frac{\sqrt{\sum_{j=1}^I \min_k (R_{jk}^{(p)})^2 \delta_{jk}}}{\sum_{j=1}^I \delta_{jk}} \left(\frac{\Delta R}{M^{(p)}}\right), \quad \text{eqn 8}$$

where  $\min_k (R_{jk}^{(p)})$  equals uncertainty of phylotype  $R_{jk}$  (superscript  $p$  indicates the iteration),  $I$  is the total number of partial ranking lists, function  $\delta_{jk}$  is defined in eqn (2) and  $M$  is defined in eqn (6). Error of measurement ( $U_k$ ) is a complimentary metric to the standard error of the mean,  $\sigma_k$ :

$$\sigma_k = \frac{\text{st.dev.} \cdot_j (R_{jk}^{(p)})}{c_4 \left(\sum_{j=1}^I \delta_{jk}\right) \sqrt{\sum_{j=1}^I \delta_{jk}}} \left(\frac{\Delta R}{M^{(p)}}\right), \quad \text{eqn 9}$$

where st. dev. is standard deviation, and the correction factor  $c_4(n)$  is the established scale mean of the chi-distribution with  $n - 1$  degrees of freedom. The  $c_4$  correction function is close to 1 if the number of lists ranking a given phylotype ( $\sum_{j=1}^I \delta_{jk}$ ) is large

and decreases to  $\sqrt{2/\pi} \approx 0.8$  for  $\sum_{j=1}^I \delta_{jk} = 2$ . Standard error of the mean ( $\sigma_k$ ) already takes into account the effect of error of measurement  $U_k$ ; however, standard deviation, and thus  $\sigma$ , is not defined if the phylotype only appears in a single list. For this reason, total uncertainty of score estimation was approximated as the maximum of two error metrics:

$$\Delta_k = \max(\sigma_k, U_k). \quad \text{eqn 10}$$

Total uncertainty of score estimation  $\Delta$  was used to determine whether the comparison of thermotolerance scores  $R$  between two phylotypes is informative by evaluating their uncertainty intervals (if two phylotypes are within their uncertainty intervals, their scores are not significantly different).  $\Delta$  can also be converted to another metric, average position error ( $P_{\text{err}}$ ), which is the difference in position between a would-be perfect consensus ranking and IPRAPA approximate consensus ranking:

$$P_{\text{err}} \approx \frac{1}{2\sqrt{6}} \Delta \frac{N}{\text{st.dev}_k(R_k)}, \quad \text{eqn 11}$$

where  $\Delta = (1/N) \sum_{k=1}^N \Delta_k$  is the average total uncertainty and, as before,  $\text{st.dev}_k(R_k)$  is the standard deviation of the distribution of all final scores  $R_k$ .

#### VALIDATION, ACCURACY AND BIAS EVALUATION OF IPRAPA

Using simulated data sets with known complete rankings, IPRAPA was assessed for (i) its ability to reconcile ties within and disagreements between input lists by calculating  $\alpha$ , or the ratio between IPRAPA solution and an exhaustive search (' $\alpha$ -approximation') (see Appendix S1.3), (ii) correctly inferred pairwise relationships among phylotype pairs absent in input lists by calculating  $\beta$  (% pairwise comparisons absent in input lists that are correctly inferred) (Appendix S1.4), (iii) probability of mis-ranking as a function of positional errors (Appendix S1.5) and (iv) directional bias by calculating the average of over- or under-estimation of a known rank (Appendix S1.5).

## Results

#### INPUT DATA STRUCTURE

Available data on *Symbiodinium* relative thermotolerance included 110 unique phylotypes from 37 partial rankings obtained from 35 source publications (Table S1). The structure and features of these data present several analysis challenges: (i) data are sparse with few phylotypes evaluated in each partial list (2–24, median 4), minimal overlap across lists (26% of phylotypes were ranked by two or more reports, Fig. S1a) and most pairwise comparisons are unknown (only 15.3% of the possible 5995 unique pairwise comparisons are available); (ii) nearly one-third (30%) of partial rankings include cohorts with ties (Table S1); and (iii) multiple disagreements exist across partial lists, where 30 of 58 repeatedly ranked pairs of phylotypes (52%) are inconsistently ranked across sources (Fig. S1b, Table S2). Another less obvious (and therefore more insidious) challenge is implied disagreements among sources: rankings that do not contain a specific pairwise comparison may become inconsistent when a consensus ranking is attempted. For example, examined reports stipulate that C1 < C21, D1-4 < C1 and C21 < D1-4 (Tables S1 and S2); therefore, the former reports indicate a consensus among these specific sources (D1-4 < C1 < C21) that is inconsistent with the latter (and with the complete consensus ranking); these implied inconsistencies are more numerous than those that are explicit.

#### CONSENSUS THERMOTOLERANCE SCORES, COHORT RANKS AND UNCERTAINTIES

Applying IPRAPA to the empirical *Symbiodinium* data set generated similar consensus rankings achieved by the first iteration  $R_k^{(1)}$ , and by allowing iterations to continue to

convergence  $R_k^{(p)}$  (after 13 iterations). Because  $R_k^{(1)}$  has higher coherence with input partial rankings (coherence:  $R_k^{(1)} = 160.3$  vs.  $R_k^{(p)} = 156.7$  out of a maximum of 209 if there are no inconsistencies), it is the best approximate solution.

IPRAPA yields a quantitative score ( $R$  of 0–100) and an uncertainty of estimation for each phylotype (Table 1) and represents the relative thermotolerance distance (direction and separation) between phylotypes, but not magnitude of differential thermotolerance. For example, C1 ( $R = 21.72 \pm 4.17$ ) and A13 ( $R = 20.22 \pm 7.65$ ) are indistinguishable (as their errors overlap), while D1 ( $R = 43.82 \pm 6.86$ ) is significantly higher, but should not be interpreted as physiologically twice as thermotolerant.

The recovered thermotolerance scores follow a sum of three distinct normal distributions, which may tentatively be interpreted as low, medium and high thermotolerance (Table 1, Fig. 1, inset). The top quartile includes scores >39.8 (and cohort ranks 1–13) and contains the well-known thermotolerant phylotype D1-4 and representatives of A, C, and F clades, while the bottom quartile includes scores <13 (and cohort ranks 53–64) and contains representatives of all clades examined except F, with most phylotypes representing clade C (Table 1, Fig. 1). Furthermore, there is large in-clade score variance for all clades evaluated (Table 1, Fig. 1).

Total uncertainty ( $\Delta$ , Table 1, eqn 10) indicates the error of estimation for  $R$  and ranged from 2.63 to 33.33 (mean of 9.6) in the empirical data. Although the standard error of the mean (eqn 9) includes the error of measurement (eqn 8), standard deviation is not defined for phylotypes occurring in single reports (74% of phylotypes; Fig. S1a, Table S1); therefore, total uncertainty is approximated as the maximum of error metrics (eqn 10, Table 1).

#### IPRAPA PERFORMANCE AND VALIDATION

IPRAPA preformed quickly with all data sets; the estimated time for an exhaustive search (which is NP-hard) of  $N$  elements increases as  $N!$ . IPRAPA (a first-order polynomial time algorithm) typically converges on a solution in <15 iterations and requires *c.* 0.025N seconds on a personal computer; the iterated solution for the thermotolerance data set ( $N = 110$ ) was found in <3 s.

IPRAPA performance was validated for its ability to (i) reconcile disagreements between input lists (52% of phylotype pairs in the empirical data were inconsistently ranked) and (ii) correctly infer relationships among pairs of phylotypes not present (15.3% of the possible 5995 unique pairwise comparisons were available) or tied (30% of phylotypes) within input lists (Appendix S1.4). Because the true ranking is unknown and a would-be perfect ranking is currently intractable, we used simulated data sets. The first evaluation used Kendall Tau distance to compare the ability of IPRAPA and an exhaustive search (Kemeny–Young)

**Table 1.** Thermotolerance scores for 110 *Symbiodinium* phylotypes and their standard errors, uncertainties and rankings. Thermotolerance score ( $R_k$ ) is found by eqn 5, standard error ( $\sigma_k$ ) by eqn 9, error of measurement ( $U_k$ ) by eqn 8 and total uncertainty ( $\Delta$ ) by eqn 10. Cohort rank identifies cohorts (phylotypes with indistinguishable thermotolerance scores, or ties) from most thermotolerant to least (rank 1–64). Percentile rank of the thermotolerance score is the % of scores in the frequency distribution of scores that are equal or lower

Phylotype	Score ( $R_k$ )	Standard error ( $\sigma_k$ )	Error of measurement ( $U_k$ )	Total uncertainty ( $\Delta$ )	Cohort rank	Percentile rank	Phylotype	Score ( $R_k$ )	Standard error ( $\sigma_k$ )	Error of measurement ( $U_k$ )	Total uncertainty ( $\Delta$ )	Cohort rank	Percentile rank
D <sub>Ber06</sub>	100.00	NaN	33.33	33.33	1	100.00	B5	26.56	NaN	8.85	8.85	33	48.60
C116	71.12	NaN	7.90	7.90	2	97.20	D12-13	26.56	8.85	9.90	9.90	33	48.60
C26b <sup>b</sup>	71.12	NaN	7.90	7.90	2	97.20	C3	25.98	3.89	3.83	3.89	34	47.70
C8a	71.12	NaN	7.90	7.90	2	97.20	C42a	25.90	21.51	4.52	21.51	35	46.70
F2	70.83	NaN	11.80	11.80	3	96.30	C31	25.20	17.30	6.62	17.30	36	45.80
C3*	66.67	NaN	33.33	33.33	4	95.40	B40	23.71	NaN	7.90	7.90	37	41.20
C26	63.22	NaN	7.90	7.90	5	93.50	C124	23.71	NaN	7.90	7.90	37	41.20
C3jj	63.22	NaN	7.90	7.90	5	93.50	C128	23.71	NaN	7.90	7.90	37	41.20
A20	59.02	NaN	11.80	11.80	6	92.60	C1c-ft	23.71	NaN	7.90	7.90	37	41.20
C123	55.32	NaN	7.90	7.90	7	88.90	C8b	23.71	NaN	7.90	7.90	37	41.20
C130	55.32	NaN	7.90	7.90	7	88.90	B1	23.63	3.16	4.01	4.01	38	40.30
C1h	55.32	NaN	7.90	7.90	7	88.90	A2	23.02	NaN	7.67	7.67	39	39.40
C40	55.32	NaN	7.90	7.90	7	88.90	C4	22.97	NaN	11.49	11.49	40	38.50
C15-21	53.12	NaN	10.62	10.62	8	85.30	C114	21.95	NaN	4.39	4.39	41	36.60
C15-23	53.12	NaN	10.62	10.62	8	85.30	C3u	21.95	NaN	4.39	4.39	41	36.60
C15-25	53.12	NaN	10.62	10.62	8	85.30	C1	21.72	3.46	4.17	4.17	42	35.70
D1-4	53.12	18.00	4.52	18.00	8	85.30	A13	20.22	7.65	6.34	7.65	43	34.80
A4	45.95	NaN	11.49	11.49	9	84.40	C3n-t	19.83	17.20	4.81	17.20	44	33.90
C3-o	44.27	NaN	8.85	8.85	10	83.40	C1b-c	19.11	4.50	9.39	9.39	45	33.00
D1	43.82	3.40	6.36	6.36	11	82.50	C42a-b	18.52	NaN	9.26	9.26	46	32.10
C21	40.25	7.36	4.50	7.36	12	81.60	C79	17.78	NaN	17.78	17.78	47	31.10
F1	39.84	NaN	13.28	13.28	13	80.70	D5	17.56	NaN	4.39	4.39	48	30.20
C119	39.51	NaN	4.39	4.39	14	71.50	B17	17.25	4.67	5.09	5.09	49	29.30
C125	39.51	NaN	7.90	7.90	14	71.50	B18	15.80	NaN	7.90	7.90	50	27.50
C126	39.51	NaN	7.90	7.90	14	71.50	C3i	15.80	NaN	7.90	7.90	50	27.50
C129	39.51	NaN	7.90	7.90	14	71.50	D15	13.28	4.43	9.90	9.90	51	26.60
C1gg-hh	39.51	NaN	7.90	7.90	14	71.50	C101	13.17	NaN	4.39	4.39	52	25.60
C3h	39.51	NaN	7.90	7.90	14	71.50	E1	12.54	0.74	8.88	8.88	53	24.70
C3n	39.51	NaN	7.90	7.90	14	71.50	C47	11.49	NaN	11.49	11.49	54	23.80
C94a	39.51	NaN	4.39	4.39	14	71.50	A <sub>Mic09</sub>	11.03	NaN	11.03	11.03	55	22.90
D1-11	39.51	NaN	4.39	4.39	14	71.50	D <sub>Abr08</sub>	10.85	NaN	10.85	10.85	56	22.00
D1-4-10	39.51	NaN	4.39	4.39	14	71.50	B10	10.62	NaN	10.62	10.62	57	20.10
F <sub>Pal1</sub>	38.36	NaN	7.67	7.67	15	70.60	C17.2	10.62	NaN	10.62	10.62	57	20.10
C15	37.59	3.77	4.40	4.40	16	69.70	C7	10.36	1.41	5.32	5.32	58	19.20
C33	37.03	NaN	9.26	9.26	17	67.80	B1j	8.85	NaN	8.85	8.85	59	15.50
D1a-c	37.03	NaN	9.26	9.26	17	67.80	C3-p	8.85	NaN	8.85	8.85	59	15.50
A4a	36.86	NaN	12.29	12.29	18	66.90	C7a	8.85	NaN	8.85	8.85	59	15.50
A3	35.86	3.98	8.50	8.50	19	66.00	C81	8.85	NaN	8.85	8.85	59	15.50

(continued)

Table 1. (continued)

Phylotype	Score ( $R_k$ )	Standard error ( $\sigma_k$ )	Error of measurement ( $U_k$ )	Total uncertainty ( $\Delta$ )	Cohort rank	Percentile rank	Phylotype	Score ( $R_k$ )	Standard error ( $\sigma_k$ )	Error of measurement ( $U_k$ )	Total uncertainty ( $\Delta$ )	Cohort rank	Percentile rank
C1c	35.71	19.37	4.00	19.37	20	65.10	C3z	8.78	NaN	4.39	4.39	60	14.60
C35a	35.56	NaN	17.78	17.78	21	64.20	C120	7.90	NaN	7.90	7.90	61	7.30
D13	35.41	NaN	17.71	17.71	22	62.30	C127	7.90	NaN	7.90	7.90	61	7.30
D8	35.41	NaN	17.71	17.71	22	62.30	C161	7.90	NaN	7.90	7.90	61	7.30
B2	34.94	24.09	8.02	24.09	23	61.40	C1hh	7.90	NaN	7.90	7.90	61	7.30
C8	33.10	14.31	10.19	14.31	24	60.50	C37a	7.90	NaN	7.90	7.90	61	7.30
D <sub>Jon08</sub>	33.10	NaN	11.03	11.03	25	56.80	C42	7.90	NaN	7.90	7.90	61	7.30
D <sub>Mie09</sub>	33.10	NaN	11.03	11.03	25	56.80	C7d	7.90	NaN	7.90	7.90	61	7.30
D <sub>Oh11</sub>	33.10	NaN	11.03	11.03	25	56.80	C <sup>Heteroxonia</sup>	7.90	NaN	7.90	7.90	61	7.30
D <sub>Vand05</sub>	33.10	NaN	11.03	11.03	25	56.80	C120a	5.27	NaN	2.63	2.63	62	5.50
A1	32.07	13.23	7.54	13.23	26	55.90	C1m-aa	5.27	NaN	2.63	2.63	62	5.50
C12	31.87	NaN	10.62	10.62	27	55.00	C101a	4.39	NaN	4.39	4.39	63	0.90
C1gg	31.61	NaN	7.90	7.90	28	54.10	C110	4.39	NaN	4.39	4.39	63	0.90
C78	31.30	22.04	10.02	22.04	29	53.20	C1d-t	4.39	NaN	4.39	4.39	63	0.90
D8-12	30.99	4.43	9.90	9.90	30	52.20	C26a	4.39	NaN	4.39	4.39	63	0.90
D1-4-6	30.73	NaN	4.39	4.39	31	51.30	D4-5	4.39	NaN	4.39	4.39	63	0.90
C66	29.21	NaN	14.61	14.61	32	50.40	C3-ff	2.63	NaN	2.63	2.63	64	NaN

algorithm to reconcile disagreements among 10 fully resolved (no ties) rankings of random permutations of 10 phylotypes. The  $\alpha$ -approximation of IPRAPA to Kemeny–Young full rank aggregation for the simulated reduced data set is 1.14–1.46 (mean 1.28 of 10 realizations; Table S3), indicating that results of IPRAPA compare favourably ( $\alpha$  of  $\leq 2$  is considered robust) to the exhaustive search in reconciling disagreements among rankings.

The second evaluation assessed the ability of IPRAPA to correctly reconstruct a full consensus ranking based on sparse partial input ranking lists with ties, using five (increasingly comprehensive) simulated data sets with similar data structure to the empirical data (sparse partial input lists with ties) and determining the accuracy of IPRAPA by calculating (i)  $\beta$ , the % unknown pairwise comparisons correctly inferred, (ii)  $P_{err}$  and (iii)  $\Sigma$ , the % known and unknown pairwise comparisons correctly identified. The additional percentage of pairwise comparisons uncovered by the algorithm was substantial ( $\beta = 80$ –86%, Table S4), position error was reduced with increasing number of known pairwise comparisons in the initial input lists ( $P_{err} = 12.5$ –3.5, Table S4), and total percentage of correct pairwise comparisons increased from 16% to 87% initially known to  $\Sigma = 83$ –98% after application of IPRAPA (Table S4). Regressions of IPRAPA reconstructed rankings on known complete rankings are highly significant (average  $r^2 = 0.90$ , all  $P < 0.001$ ; Fig. S2), indicating that IPRAPA is able to accurately reconstruct consensus rankings using sparse partial rankings.

These simulations also validated approximation equations (eqns 12–15, see text S1.4) used to estimate total uncertainty  $\Delta$  ( $r^2 = 0.97$  between  $\Delta$  predicted by eqn 13 and  $\Delta$  calculated for synthetic data sets using eqn 10), average position error  $P_{err}$  ( $r^2 = 0.91$  between  $P_{err}$  predicted by eqns 11 and 14 vs.  $P_{err}$  calculated by comparing the IPRAPA outputs and true rankings) and  $\Sigma$  ( $r^2 = 0.87$  between  $\Sigma$  predicted by eqn 15 vs. calculated by comparing the IPRAPA outputs and true rankings). Thus, error metrics generated by IPRAPA ( $P_{err}$  and  $\Sigma$ ) can be accurately estimated *a priori* based on the length of input partial rankings.

Equations 12–15 predict accuracy of the consensus ranking for the thermotolerance data set based on the structure of input partial ranking lists. For example,  $P_{err}$  can be converted from total uncertainty  $\Delta$  (eqn 11) or estimated from the total number of phylotypes and average inverse length of rankings ( $\frac{1}{n}$ ) (eqn 14). For the empirical thermotolerance data set of 110 phylotypes,  $\Delta = 9.6$ ,  $\langle \frac{1}{n} \rangle = 0.27$ , and eqns 11, 14 give similar estimates of  $P_{err}$  as 11.3 and 10.5, respectively. These values are consistent with  $\Delta = 9.3$  and  $P_{err} = 12.5$  of the simulated data set that is most similar to the empirical thermotolerance data (simulated data set 1, Table S4).

The average position error  $P_{err}$  is the average absolute distance and is therefore not directional. Positional bias (i.e. over- or under-estimation of ranking) was calculated

as the average of directional position errors and was determined to be zero (for all validation sets, the average of directional position errors did not exceed one position in either direction; Appendix S1.5).

The probability that IPRAPA misranks thermotolerance of a given phylotype decreases exponentially with distance between true and mistaken ranks (positional error distance). For example, for the empirical data set ( $P_{\text{err}} = 10.5$ ) the probability that a phylotype with a thermotolerance rank within the bottom 10 elements is misranked to the top 10 elements (or vice versa) is only *c.* 0.0001 (Fig. S3, Appendix S1.5).

## Discussion

### IPRAPA OVERCOMES SIGNIFICANT CHALLENGES TO BUILD A CONSENSUS OF CURRENT KNOWLEDGE

IPRAPA approximation is an accurate and fast approach to address partial ranking aggregation in its comprehensive form and can be applied to diverse ranking problems in ecology (e.g. species threatened with extinction) and other disciplines (e.g. search engines or rankings of best schools). Here, we quantified the relative score and associated uncertainty of thermotolerance for 110 unique *Symbiodinium* phylotypes from sparse qualitative data with ranking ties, minimal phylotype overlap and inconsistencies between reports. The IPRAPA approximation can be used to further expand this consensus ranking as new information becomes available. Inconsistencies were either explicit (where phylotype pairs assessed in >1 report were discordantly ranked) or implicit (where phylotype pairs not assessed by some reports are discordant when compared across reports). Implicit ranking inconsistencies are particularly insidious for consensus reconstruction, as small subsets of data may be misleading. For example, D1-4 is generally thought of as among the highest thermotolerance; however, if aggregating just two partial rankings where C1 = C3 and D1-4 < C1 (Table S1), D1-4 would rank below C3 (and *c.* 50% of the remaining phylotypes in these partial rankings). In the final consensus, D1-4 ( $52.12 \pm 18.0$ , percentile rank 85.3) ranked significantly above C3 ( $25.98 \pm 3.89$ , percentile rank 47.7) and C1 ( $21.72 \pm 4.17$ , percentile rank 35.7).

Commonly cited phylotypes at the extremes of *Symbiodinium* thermotolerance include thermosensitive C7 and C3 (Warner *et al.* 2006; Jones *et al.* 2008; DeSalvo *et al.* 2010; Silverstein *et al.* 2011; Wang *et al.* 2012; Kemp *et al.* 2014; Keshavmurthy *et al.* 2014) and thermotolerant D1 and D1-4 (Thornhill *et al.* 2006; Kemp, Fitt & Schmidt 2008; McGinley *et al.* 2012; McGinty, Pieczonka & Mydlarz 2012; Wang *et al.* 2012; LaJeunesse *et al.* 2014). These phylotypes are not at the extremes of the reconstructed consensus ranking (Table 1); however, they rank as 19.2, 47.7, 82.5 and 85.3 percentile, respectively. The use of consensus-based (Borda rank aggregation) rather than majority-based ranking can (in part) explain this outcome;

partial lists with uncommon rankings representing a 'significant minority' effect the ultimate outcome and are not simply over-ruled by the majority. Furthermore, larger lists (with the greatest number of cohorts and phylotypes) are aggregated first and are therefore more influential in determining the final consensus ranking. For example, all studies containing F2 ranked it most thermotolerant, A20 was ranked highly in a single report, and D1-4 is often, but not always, ranked as most thermotolerant in any single list (Table S1). Although D1-4 is ranked in the final consensus as highly thermotolerant, it is ranked below A20 and F2 (in part) because a highly comprehensive report ranked D1-4 roughly in the middle of the list (Table S1). Thus, it is possible to develop a more accurate ranking if comprehensive reports with greater inter-ranking overlap are included.

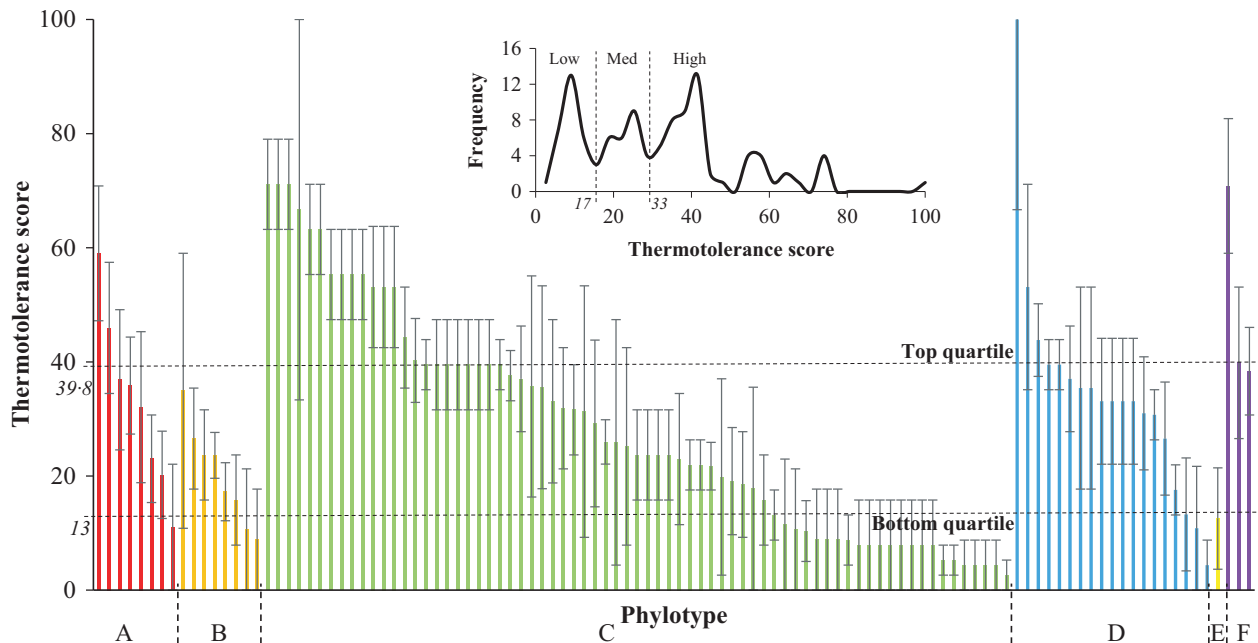
### IPRAPA PROVIDES A UNIFIED METRIC TO DELINEATE DIFFERENTIAL THERMOTOLERANCE

Total uncertainty in thermotolerance score ( $\Delta$ , Table 1, eqn 10) enables assessment of ecological implications of a wide range of phylotype thermotolerance by determining the minimal difference between two scores that are needed to confidently infer their pairwise relationship. If two phylotypes have scores outside their uncertainty intervals, then the approximated thermotolerance is significantly different. For a given phylotype,  $\Delta$  is determined by ranking differences among individual lists (the standard error of the mean, eqn 9), the number of cohorts represented in the lists and the number of lists that rank the phylotype (the latter two factors are accounted for by the standard error of measurement, eqn 8). If a phylotype is inconsistently ranked, is present in few lists, or the lists have few cohorts,  $\Delta$  will increase. For example, C3\* is ranked by a single report and therefore has a relatively high  $\Delta$  of 33.33, D1-4 is ranked by 11 reports (which decreases its error of measurement), but these reports rank it inconsistently, which increases its  $\Delta$  to 18.0 (Table 1). The mean total uncertainty for the empirical thermotolerance data set was  $\Delta = 9.6$ .

### IPRAPA IS ACCURATE AND EXTENSIBLE

IPRAPA yields a consensus ranking that, based on validation with simulated data, is an accurate approximation of the unknown true ranking. Against known rankings, IPRAPA was nearly as capable as an exhaustive search (Kemeny–Young rank) in reconciling disagreements among input rankings (mean  $\alpha = 1.28$ , Table S3). Furthermore, the use of five simulated data sets that mimicked the structure of the empirical data demonstrated accuracy of IPRAPA to correctly reconstruct a consensus ranking based on sparse partial input ranking lists with ties. IPRAPA correctly inferred most of pairwise phylotype comparisons absent in input lists ( $\beta = 80\text{--}86\%$ , Table S4) and, as would be expected, the total number of correctly identified pairwise comparisons increased with the number





**Fig. 1.** *Symbiodinium* phylotype thermotolerance scores ( $R_k$ ) and total uncertainties (error bars =  $\Delta$ , eqn 10) organized by clade (inset: Frequency of thermotolerance scores ( $R_k$ ) for all 110 phylotypes).

of initially known pairs ( $\Sigma = 83\text{--}98\%$  from  $16\text{--}87\%$  initially known, Table S4). For the simulated data that best matched the structure of the empirical thermotolerance data (37 partial lists of 110 total elements with 16% of pairwise comparisons known), IPRAPA was capable of accurately inferring 83% of pairwise comparisons and the consensus ranking closely resembled the true ranking ( $r^2 = 0.70$ ,  $P < 0.001$ ; Fig. S2, Table S4). Values of  $\beta$  and  $\Sigma$  depend on the structure of input partial rankings; generally, more comprehensive partial rankings, greater phylotype overlap among rankings and rankings covering a wider range of score values will increase  $\beta$  and  $\Sigma$ .

Expansion of the consensus ranking by the addition of partial rankings may increase the number of phylotypes and/or improve accuracy of reconstruction. Reports can be evaluated for their effect on accuracy of IPRAPA by estimating the percentage of known and unknown pairwise comparisons correctly identified ( $\Sigma$ , eqn 15) from the length of novel partial ranking lists and the total number of phylotypes in each list. Depending on the data structure of additional reports, there may be a trade-off between ranking more phylotypes and optimizing accuracy ( $\Sigma$ ). For example, adding a new report of few phylotypes could reduce accuracy ( $\Sigma$ , as per eqn 15) if it also contains new phylotypes, but increase accuracy if it does not.

#### LIMITATIONS AND GENERAL CONSIDERATIONS

The ranking generated by IPRAPA uses data collected under diverse goals and methods to return the first approximation of the true *Symbiodinium* thermotolerance ranking based on data that are currently available. Thermotolerance has been assessed in  $<25\%$  of the known diversity of

phylotypes (409; Franklin *et al.* 2012), and adding new phylotypes or new data to the analysis may alter the rank order or delineation of significant differences between reconstructed ranks.

Bleaching response can be context dependent: the host (Abrego *et al.* 2008; Baird *et al.* 2009; Fitt *et al.* 2009; Bellantuono, Hoegh-Guldberg & Rodriguez-Lanetty 2012; Kenkel *et al.* 2013; Marcelino *et al.* 2013; Wooldridge 2014), bacterial symbionts (Gilbert *et al.* 2012) and *Symbiodinium* densities (Cunning & Baker 2013) can all have significant roles in determining holobiont bleaching response, and *Symbiodinium in vitro* may respond differently than those *in hospite* (Bhagooli & Hidaka 2003). Acknowledging that the diversity of conditions and methods will increase total uncertainty of the estimation within each report (Table 1), we accept this uncertainty (and embrace the advantages of averaging rankings across diverse conditions) as necessary to simultaneously maximize genetic resolution and thermotolerance assessment given currently available data, and the likely insurmountable challenges of identically assessing all known phylotypes. Furthermore, this approach should become increasingly useful as more studies assess different phylotypes in multiple coral species with a wide range of different bleaching susceptibilities, because we will be better able to tease apart confounding factors (e.g. host thermotolerance) and identify the role of *Symbiodinium* thermotolerance in bleaching susceptibility.

Phylotypes are identified by the standard molecular marker (ITS2) through a diversity of methods that each has their own resolution capabilities and failure rates (some of which can even be user specific and therefore difficult to quantify). There are several other genetic tools with similar

(species level) or better (population level) resolution (e.g. cp23S, Pochon *et al.* 2010; psbA minicircle, LaJeunesse & Thornhill 2011; or multilocus microsatellite genotyping, Thornhill *et al.* 2014; Pettay *et al.* 2015); however, none are as regularly employed, standardized across clades or widely evaluated for differential thermotolerance (i.e. ITS2 phylotypes simultaneously maximize identity resolution and thermotolerance data availability). Although ITS2 is generally considered to be an ecologically meaningful species-level marker for *Symbiodinium*, intragenomic variation may confound identifications (LaJeunesse *et al.* 2014; Thornhill *et al.* 2014) and populations within phylotypes may have variable thermotolerance (Howells *et al.* 2012). Physiological diversity within phylotypes will increase total uncertainty or may confound estimates of rarely assessed phylotypes, for thermotolerance scores reported here (Table 1). As use of high-resolution markers expands and their relative thermotolerance reported, these data can further refine the consensus thermotolerance ranking.

Limitations of individual partial rankings reinforce the importance of generating a unified consensus ranking. In the current literature, differential *Symbiodinium* thermotolerance is frequently offered as a partial explanation of differential coral bleaching susceptibility; however, that assertion (unless matched to assessments performed with identical conditions and organisms) is based on a qualitative and subjective informal partial aggregation of published partial rankings and is not likely to accurately reflect current knowledge. With the potentially insurmountable challenges of experimentally assessing all known phylotypes, our best (although imperfect) approach may be to provide a quantitative systematic approximation of the true consensus.

#### ECOLOGICAL IMPLICATIONS

This consensus ranking of *Symbiodinium* phylotype thermotolerance represents a tool to generate testable hypotheses of the role of *Symbiodinium* thermotolerance and to aid in the interpretation of distribution, prevalence and dominance of phylotypes over space and time. For example, a recent study by Kemp *et al.* (2015) surveyed *Symbiodinium* phylotypes A3, B1, B17, C3, C7, C7a and D1-4 and identified intercolony and geographic distribution patterns that are correlated with light availability, cardinal direction and depth and commented on the relative thermotolerance of A3, D1-4 and C7 for predicting future bleaching events (with thermosensitive C7-dominated colonies at 'high risk' for bleaching). Thermotolerance scores of D1-4 ( $53.12 \pm 18$ ) and A3 ( $35.86 \pm 8.50$ ) are in the top quartile (39-8); C7a ( $8.85 \pm 8.85$ ), C7 ( $10.36 \pm 5.32$ ) and B17 ( $17.25 \pm 5.09$ ) are within the bottom quartile (13); and C3 ( $25.98 \pm 3.89$ ) and B1 ( $23.63 \pm 4.01$ ) are in between. This may indicate that colonies (studied by Kemp *et al.* 2015) dominated by C7a and B17 or that colonies with high proportions of low thermotolerance symbionts (under the assumption that photobiont thermotolerance will change

proportionally to symbiont composition; Cunning, Silverstein & Baker 2015), could also be designated as 'high risk' for bleaching.

This study by Kemp *et al.* (2015) also highlights the utility of the consensus ranking reported here. Of the seven phylotypes examined, the authors identified A3 and D1-4 as thermotolerant and C7 as thermosensitive, based on their considerable previous experience and knowledge of the literature. The ranking reported here identifies the same three phylotypes as extremes in the thermotolerance continuum, but IPRAPA allows analysis of the ecological implications of the lesser known phylotypes. For example, in the input lists used here, C7a was never directly compared to A3, B17 or C7; without consensus analysis or new experimentation, the relationships between these phylotypes could not be known.

The reputation of D1-4 is well established and appears in eleven input lists in this study. Although D1-4 is within the top quartile of thermotolerance reconstructed by IPRAPA, there are 40 other phylotypes (including phylotypes representing clades A, C & F) within the error of its thermotolerance score (Table 1). Perhaps the reputation of extreme thermotolerance of D1-4 lies not only in its documented resistance to thermal stress, but also (in part) because D1-4 is the most common and geographically widespread of any D phylotype (LaJeunesse *et al.* 2014) and is therefore often the most thermotolerant in any single ecosystem or study.

Distribution of thermotolerance scores themselves may give further indication of the practicality of resolution of molecular markers and categorization of thermal resistance. Within clades, the diversity of scores encompasses large proportions (26-96) of the possible range of thermotolerance (Fig. 1). Among clades, the means and ranges of scores are largely overlapping (Fig. 1) for clades B (mean  $\pm$  SE  $20.17 \pm 3.08$ ; range 9-35) and C ( $28.41 \pm 1.02$ ; 3-71), and A ( $33.00 \pm 5.37$ ; 11-59) and D ( $32.24 \pm 4.55$ ; 4-100), making it clear that clade-level identifications are not informative for elucidating *Symbiodinium* thermotolerance. As population-level data become more widely available, and genotypes are assessed for differential thermotolerance (e.g. Howells *et al.* 2012), the resolution of this ranking can be further improved.

With the ranking presented here, and its capacity for refinement and expansion with new data, our ability to interpret the significance of specific host-symbiont associations and the resilience of reefs may be greatly improved. Surveys of *Symbiodinium* phylotypes can now be interpreted in the light of relative thermotolerance of many phylotypes and can be integrated with coral bleaching response and host-symbiont association data sets to better elucidate the role of *Symbiodinium* thermotolerance in coral bleaching susceptibility. High concentrations of high-thermotolerance phylotypes may be an indication of reefs that experience regular thermal stress fluctuations (when independent temperature data support that conclusion; Stat *et al.* 2013), an indication of recent bleaching events

(when the thermal record does not support regular temperature extremes; Silverstein, Cuning & Baker 2014), or may aid in predicting the severity of future bleaching events (Stat & Gates 2011).

## Acknowledgements

We are grateful to the Biodiversity Synthesis Center of the Field Museum and M. Westneat and R. Bieler for space and support. This work was supported by NSF grants CBET-0937987 and CBET-1240416. The authors declare no conflict of interest.

## Data accessibility

All of the data used in these analyses are contained in the text, tables and appendices. The MATLAB code for IPRAPA is available in MATLAB Central (<http://www.mathworks.com/matlabcentral/fileexchange/55898>).

## References

- Abrego, D., Ulstrup, K.E., Willis, B.L. & van Oppen, M.J.H. (2008) Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 2273–2282.
- Ailon, N. (2010) Aggregation of partial rankings, p-ratings and top-m lists. *Algorithmica*, **57**, 284–300.
- Ailon, N., Charikar, M. & Newman, A. (2008) Aggregating inconsistent information: ranking and clustering. *Journal of the ACM*, **55**, e23.
- Aschaffenburg, M.D. (2012) *The physiological response of Symbiodinium spp. to thermal and light stress: a comparison of different phylotypes and implications for coral reef bleaching*. PhD, University of Delaware, ProQuest LLC, Ann Arbor, MI, USA.
- Baird, A.H., Bhagooli, R., Ralph, P.J. & Takahashi, S. (2009) Coral bleaching: the role of the host. *Trends in Ecology & Evolution*, **24**, 16–20.
- Baker, D.M., Andras, J.P., Jordan-Garza, A.G. & Fogel, M.L. (2013) Nitrate competition in a coral symbiosis varies with temperature among *Symbiodinium* clades. *ISME Journal*, **7**, 1248–1251.
- Bellantuno, A.J., Hoegh-Guldberg, O. & Rodriguez-Lanetty, M. (2012) Resistance to thermal stress in corals without changes in symbiont composition. *Proceedings of the Royal Society B-Biological Sciences*, **279**, 1100–1107.
- Berkelmans, R. & van Oppen, M.J.H. (2006) The role of zooxanthellae in the thermal tolerance of corals: a 'nugget of hope' for coral reefs in an era of climate change. *Proceedings of the Royal Society B-Biological Sciences*, **273**, 2305–2312.
- Bhagooli, R. & Hidaka, M. (2003) Comparison of stress susceptibility of *in hospite* and isolated zooxanthellae among five coral species. *Journal of Experimental Marine Biology and Ecology*, **291**, 181–197.
- Cantin, N.E., van Oppen, M.J.H., Willis, B.L., Mieog, J.C. & Negri, A.P. (2009) Juvenile corals can acquire more carbon from high-performance algal symbionts. *Coral Reefs*, **28**, 405–414.
- Chin, F.Y.L., Deng, X.T., Fang, Q.Z. & Zhu, S.F. (2004) Approximate and dynamic rank aggregation. *Theoretical Computer Science*, **325**, 409–424.
- Cuning, R. & Baker, A.C. (2013) Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change*, **3**, 259–262.
- Cuning, R., Silverstein, R.N. & Baker, A.C. (2015) Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proceedings of the Royal Society B-Biological Sciences*, **282**, e20141725.
- DeSalvo, M.K., Sunagawa, S., Fisher, P.L., Voolstra, C.R., Iglesias-Prieto, R. & Medina, M. (2010) Coral host transcriptomic states are correlated with *Symbiodinium* genotypes. *Molecular Ecology*, **19**, 1174–1186.
- Enríquez, S., Méndez, E.R. & Iglesias-Prieto, R. (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. *Limnology and Oceanography*, **50**, 1025–1032.
- Fabina, N.S., Putnam, H.M., Franklin, E.C., Stat, M. & Gates, R.D. (2013) Symbiotic specificity, association patterns, and function determine community responses to global changes: defining critical research areas for coral-*Symbiodinium* symbioses. *Global Change Biology*, **19**, 3306–3316.
- Fang, Q.Z., Xiao, H. & Zhu, S.F. (2010) Top-d rank aggregation in web meta-search engine. *Frontiers in Algorithmics*, **6213**, 35–44.
- Fitt, W.K., Gates, R.D., Hoegh-Guldberg, O., Bythell, J.C., Jatkar, A., Grottoli, A.G. et al. (2009) Response of two species of Indo-Pacific corals, *Porites cylindrica* and *Stylophora pistillata*, to short-term thermal stress: the host does matter in determining the tolerance of corals to bleaching. *Journal of Experimental Marine Biology and Ecology*, **373**, 102–110.
- Franklin, E.C., Stat, M., Pochon, X., Putnam, H.M. & Gates, R.D. (2012) GeoSymbio: a hybrid, cloud-based web application of global geospatial bioinformatics and ecoinformatics for *Symbiodinium*-host symbioses. *Molecular Ecology Resources*, **12**, 369–373.
- Gilbert, J.A., Hill, R., Doblin, M.A. & Ralph, P.J. (2012) Microbial consortia increase thermal tolerance of corals. *Marine Biology*, **159**, 1763–1771.
- Grottoli, A.G., Warner, M.E., Levas, S.J., Aschaffenburg, M.D., Schoepf, V., McGinley, M. et al. (2014) The cumulative impact of annual coral bleaching can turn some coral species winners into losers. *Global Change Biology*, **20**, 3823–3833.
- Hawkins, T.D., Krueger, T., Becker, S., Fisher, P.L. & Davy, S.K. (2014) Differential nitric oxide synthesis and host apoptotic events correlate with bleaching susceptibility in reef corals. *Coral Reefs*, **33**, 141–153.
- Howells, E.J., Beltran, V.H., Larsen, N.W., Bay, L.K., Willis, B.L. & van Oppen, M.J.H. (2012) Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, **2**, 116–120.
- Hume, B.C.C., D'Angelo, C., Smith, E.G., Stevens, J.R., Burt, J. & Wiedenmann, J. (2015) *Symbiodinium thermophilum* sp. nov., a thermo-tolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Scientific Reports*, **5**, e8562.
- Jokiel, P.L. (2004) Temperature stress and coral bleaching. *Coral Health and Disease* (eds E. Rosenberg & Y. Loya), pp. 401–425. Springer-Verlag, Berlin, Germany.
- Jones, R.J. (2008) Coral bleaching, bleaching-induced mortality, and the adaptive significance of the bleaching response. *Marine Biology*, **154**, 65–80.
- Jones, A.M., Berkelmans, R., van Oppen, M.J.H., Mieog, J.C. & Sinclair, W. (2008) A community change in the algal endosymbionts of a scleractinian coral following a natural bleaching event: field evidence of acclimatization. *Proceedings of the Royal Society B-Biological Sciences*, **275**, 1359–1365.
- Kemp, D.W., Fitt, W.K. & Schmidt, G.W. (2008) A microsampling method for genotyping coral symbionts. *Coral Reefs*, **27**, 289–293.
- Kemp, D.W., Hernandez-Pech, X., Iglesias-Prieto, R., Fitt, W.K. & Schmidt, G.W. (2014) Community dynamics and physiology of *Symbiodinium* spp. before, during, and after a coral bleaching event. *Limnology and Oceanography*, **59**, 788–797.
- Kemp, D.W., Thornhill, D.J., Rotjan, R.D., Iglesias-Prieto, R., Fitt, W.K. & Schmidt, G.W. (2015) Spatially distinct and regionally endemic *Symbiodinium* assemblages in the threatened Caribbean reef-building coral *Orbicella faveolata*. *Coral Reefs*, **34**, 535–547.
- Kenkel, C.D., Goodbody-Gringley, G., Caillaud, D., Davies, S.W., Bartels, E. & Matz, M.V. (2013) Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. *Molecular Ecology*, **22**, 4335–4348.
- Keshavmurthy, S., Meng, P.J., Wang, J.T., Kuo, C.Y., Yang, S.Y., Hsu, C.M. et al. (2014) Can resistant coral-*Symbiodinium* associations enable coral communities to survive climate change? A study of a site exposed to long-term hot water input. *PeerJ*, **2**, e327.
- Krueger, T. & Gates, R.D. (2012) Cultivating endosymbionts – host environmental mimics support the survival of *Symbiodinium* C15 *ex hospite*. *Journal of Experimental Marine Biology and Ecology*, **413**, 169–176.
- LaJeunesse, T.C. & Thornhill, D.J. (2011) Improved resolution of reef-coral endosymbiont (*Symbiodinium*) species diversity, ecology, and evolution through psbA non-coding region genotyping. *PLoS ONE*, **6**, e29013.
- LaJeunesse, T.C., Smith, R.T., Finney, J. & Oxenford, H. (2009) Outbreak and persistence of opportunistic symbiotic dinoflagellates during the 2005 Caribbean mass coral 'bleaching' event. *Proceedings of the Royal Society B-Biological Sciences*, **276**, 4139–4148.
- LaJeunesse, T.C., Wham, D.C., Pettay, D.T., Parkinson, J.E., Keshavmurthy, S. & Chen, C.A. (2014) Ecologically differentiated stress-tolerant endosymbionts in the dinoflagellate genus *Symbiodinium* (Dinophyceae) Clade D are different species. *Phycologia*, **53**, 305–319.

- Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H. & van Woesik, R. (2001) Coral bleaching: the winners and the losers. *Ecology Letters*, **4**, 122–131.
- Marcelino, L.A., Westneat, M.W., Stoyneva, V., Henss, J., Rogers, J.D., Radosevich, A. *et al.* (2013) Modulation of light-enhancement to symbiotic algae by light-scattering in corals and evolutionary trends in bleaching. *PLoS ONE*, **8**, e61492.
- McGinley, M.P., Aschaffenburg, M.D., Pettay, D.T., Smith, R.T., LaJeunesse, T.C. & Warner, M.E. (2012) Transcriptional response of two core photosystem genes in *Symbiodinium* spp. exposed to thermal stress. *PLoS ONE*, **7**, e50439.
- McGinty, E.S., Pieczonka, J. & Myrdlarz, L.D. (2012) Variations in reactive oxygen release and antioxidant activity in multiple *Symbiodinium* types in response to elevated temperature. *Microbial Ecology*, **64**, 1000–1007.
- Middlebrook, R., Hoegh-Guldberg, O. & Leggat, W. (2008) The effect of thermal history on the susceptibility of reef-building corals to thermal stress. *Journal of Experimental Biology*, **211**, 1050–1056.
- Mieog, J.C., Olsen, J.L., Berkelmans, R., Bleuler-Martinez, S.A., Willis, B.L. & van Oppen, M.J.H. (2009) The roles and interactions of symbiont, host and environment in defining coral fitness. *PLoS ONE*, **4**, e6364.
- Muscantine, L. (1990) The role of symbiotic algae in carbon and energy flux in reef corals. *Coral Reefs: Ecosystems of the World* (ed. Z. Dubinsky), pp. 75–87. Elsevier, New York, NY, USA.
- Pettay, D.T., Wham, D.C., Smith, R.T., Iglesias-Prieto, R. & LaJeunesse, T.C. (2015) Microbial invasion of the Caribbean by an Indo-Pacific coral zooxanthella. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 7513–7518.
- Pochon, X. & Gates, R.D. (2010) A new *Symbiodinium* clade (Dinophyceae) from soritid foraminifera in Hawai'i. *Molecular Phylogenetics and Evolution*, **56**, 492–497.
- Pochon, X., Putnam, H.M. & Gates, R.D. (2014) Multi-gene analysis of *Symbiodinium* dinoflagellates: a perspective on rarity, symbiosis, and evolution. *PeerJ*, **2**, e394.
- Pochon, X., Stat, M., Takabayashi, M., Chasqui, L., Chauka, L.J., Logan, D.D.K. *et al.* (2010) Comparison of endosymbiotic and free-living *Symbiodinium* (Dinophyceae) diversity in a hawaiian reef environment. *Journal of Phycology*, **46**, 53–65.
- Sampayo, E.M., Franceschinis, L., Hoegh-Guldberg, O. & Dove, S. (2007) Niche partitioning of closely related symbiotic dinoflagellates. *Molecular Ecology*, **16**, 3721–3733.
- Sampayo, E.M., Ridgway, T., Bongaerts, P. & Hoegh-Guldberg, O. (2008) Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 10444–10449.
- Silverstein, R.N., Cuning, R. & Baker, A.C. (2014) Change in algal symbiont communities after bleaching, not prior heat exposure, increases heat tolerance of reef corals. *Global Change Biology*, **21**, 236–249.
- Silverstein, R.N., Correa, A.M.S., LaJeunesse, T.C. & Baker, A.C. (2011) Novel algal symbiont (*Symbiodinium* spp.) diversity in reef corals of Western Australia. *Marine Ecology Progress Series*, **422**, 63–75.
- Starzak, D.E., Quinell, R.G., Nitschke, M.R. & Davy, S.K. (2014) The influence of symbiont type on photosynthetic carbon flux in a model cnidarian-dinoflagellate symbiosis. *Marine Biology*, **161**, 711–724.
- Stat, M. & Gates, R.D. (2011) Clade D *Symbiodinium* in scleractinian corals: a “nugget” of hope, a selfish opportunist, an ominous sign, or all of the above? *Journal of Marine Biology*, **2011**, 730715.
- Stat, M., Pochon, X., Franklin, E.C., Bruno, J.F., Casey, K.S., Selig, E.R. *et al.* (2013) The distribution of the thermally tolerant symbiont lineage (*Symbiodinium* clade D) in corals from Hawaii: correlations with host and the history of ocean thermal stress. *Ecology and Evolution*, **3**, 1317–1329.
- Tchernov, D., Gorbunov, M.Y., de Vargas, C., Narayan Yadav, S., Milligan, A.J., Haggblom, M. *et al.* (2004) Membrane lipids of symbiotic algae are diagnostic of sensitivity to thermal bleaching in corals. *Proceedings of the National Academy of Sciences of the United States of America*, **101**, 13531–13535.
- Thornhill, D.J., LaJeunesse, T.C., Kemp, D.W., Fitt, W.K. & Schmidt, G.W. (2006) Multi-year, seasonal genotypic surveys of coral-algal symbioses reveal prevalent stability or post-bleaching reversion. *Marine Biology*, **148**, 711–722.
- Thornhill, D.J., Lewis, A.M., Wham, D.C. & LaJeunesse, T.C. (2014) Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution*, **68**, 352–367.
- Tonk, L., Sampayo, E.M., LaJeunesse, T.C., Schrameyer, V. & Hoegh-Guldberg, O. (2014) *Symbiodinium* (Dinophyceae) diversity in reef-invertebrates along an offshore to inshore reef gradient near Lizard Island, Great Barrier Reef. *Journal of Phycology*, **50**, 552–563.
- Wang, J.T., Meng, P.J., Chen, Y.Y. & Chen, C.A. (2012) Determination of the thermal tolerance of *Symbiodinium* using the activation energy for inhibiting photosystem II activity. *Zoological Studies*, **51**, 137–142.
- Wangpraseurt, D., Larkum, A.W.D., Franklin, J., Szabo, M., Ralph, P.J. & Kühl, M. (2014) Lateral light transfer ensures efficient resource distribution in symbiont-bearing corals. *Journal of Experimental Biology*, **217**, 489–498.
- Warner, M.E., LaJeunesse, T.C., Robison, J.D. & Thur, R.M. (2006) The ecological distribution and comparative photobiology of symbiotic dinoflagellates from reef corals in Belize: potential implications for coral bleaching. *Limnology and Oceanography*, **51**, 1887–1897.
- Wilkinson, C. (2008) *Status of the Coral Reefs of the World: 2008*. Global Coral Reef Monitoring Network and Reef and Rainforest Research Centre, Townsville, Qld, Australia.
- van Woesik, R., Sakai, K., Ganase, A. & Loya, Y. (2011) Revisiting the winners and the losers a decade after coral bleaching. *Marine Ecology Progress Series*, **434**, 67–76.
- Wooldridge, S.A. (2014) Differential thermal bleaching susceptibilities amongst coral taxa: re-posing the role of the host. *Coral Reefs*, **33**, 15–27.
- van Zuylen, A. & Williamson, D.P. (2008) Deterministic algorithms for rank aggregation and other ranking and clustering problems. *Approximation and Online Algorithms*, **4927**, 260–273.

Received 8 October 2015; accepted 27 May 2016

Handling Editor: Wolf Blanckenhorn

## Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Fig. S1.** Features of *Symbiodinium* thermotolerance assessments collected from the literature.

**Fig. S2.** Regression analyses for five simulated data sets showing the relationship between the IPRAPA predicted ranking and the *a priori* known complete rankings.

**Fig. S3.** Measured and predicted positional error distance probability distributions.

**Table S1.** *Symbiodinium* thermotolerance input ranking lists and data source information with a matrix of phylotype presence for each report.

**Table S2.** Repeatedly assessed pairs of *Symbiodinium* phylotypes by source matrix.

**Table S3.** Assessment of IPRAPA performance in resolving ranking disagreements among input lists.

**Table S4.** Description of five simulated data sets with *a priori* known complete rankings and assessment metrics of IPRAPA accuracy.

**Appendix S1.** Supplemental methods.