The effect of thermal history on the susceptibility of reef-building corals to thermal stress

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SUMMARY
The mutualistic relationship between corals and their unicellular dinoflagellate symbionts (Symbiodinium sp.) is a fundamental component within the ecology of coral reefs. Thermal stress causes the breakdown of the relationship between corals and their symbionts (bleaching). As with other organisms, this symbiosis may acclimate to changes in the environment, thereby potentially modifying the environmental threshold at which they bleach. While a few studies have examined the acclimation capacity of reef-building corals, our understanding of the underlying mechanism is still in its infancy. The present study focused on the role of recent thermal history in influencing the response of both corals and symbionts to thermal stress, using the reef-building coral Acropora aspera. The symbionts of corals that were exposed to 31°C for 48 h (pre-stress treatment) 1 or 2 weeks prior to a 6-day simulated bleaching event (when corals were exposed to 34°C) were found to have more effective photoprotective mechanisms. These mechanisms included changes in non-photochemical quenching and xanthophyll cycling. These differences in photoprotection were correlated with decreased loss of symbionts, with those corals that were not prestressed performing significantly worse, losing over 40% of their symbionts and having a greater reduction in photosynthetic efficiency. These results are important in that they show that thermal history, in addition to light history, can influence the response of reef-building corals to thermal stress and therefore have implications for the modeling of bleaching events. However, whether acclimation is capable of modifying the thermal threshold of corals sufficiently to cope as sea temperatures increase in response to global warming has not been fully explored. Clearly increases in sea temperatures that extend beyond 1–2°C will exhaust the extent to which acclimation can modify the thermal threshold of corals.

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Key words: acclimation, thermal stress, Symbiodinium sp., photoprotective mechanisms, coral bleaching.

INTRODUCTION
Average global sea temperatures have increased approximately 0.6°C since the beginning of the last century and are projected to increase by a further 1.1–6.4°C within the next 100 years (IPCC, 2007). This increase is expected to have substantial impacts on coral reefs and the tens of thousands of organisms that inhabit them (Hoegh-Guldberg, 1999; Hughes et al., 2003). Comparisons of known thermal tolerance levels for corals with projections of sea temperature changes strongly suggest that the frequency and intensity of coral bleaching and mortality is set to increase sharply, leading to declines in coral reefs worldwide (Hoegh-Guldberg, 1999; Hoegh-Guldberg, 2005; Donner et al., 2005). These studies based their conclusions on the assumption that the thermal threshold for a population of corals is relatively constant. It is important, therefore, that we understand the dynamics associated with the thermal threshold, especially when it comes to understanding how thermal history can influence bleaching response.

Reef-building corals are highly dependent on their symbiotic relationship with photosynthetic dinoflagellates (Symbiodinium sp.). As part of this mutualistic endosymbiosis, corals receive the majority of their carbon and energy requirements from Symbiodinium (Muscatine et al., 1984; Falkowski et al., 1993). The transfer of photosynthetic products from the Symbiodinium to the host enables corals to grow and calcify at high rates within the warm and sunlit subtropical and tropical waters (Barnes and Chalker, 1990). The loss of these symbiotic dinoflagellates due to environmental stresses (coral bleaching) is likely to impact the energy and carbon budget of corals, and may result in death if the stress is severe and prolonged (Glynn, 1996). Bleaching [disassociation of the endosymbiosis between coral and Symbiodinium (Hoegh-Guldberg and Smith, 1989)] results in reduced Symbiodinium cell densities and/or their photosynthetic pigments. The loss of Symbiodinium and/or pigments begins with the dysfunction of the photosynthetic apparatus of Symbiodinium (Iglesias-Prieto et al., 1992; Fitt and Warner, 1995; Warner et al., 1996; Iglesias-Prieto and Trench, 1997; Brown et al., 1999; Jones et al., 1998; Downs et al., 2000; Tchernov et al., 2004).

Organisms tend to have thermal tolerances that reflect the environment in which they are found. This can occur either through acclimation, where an organism alters its phenotype, or through adaptation, where propagules are better suited to altered conditions. Corals and their symbionts have adapted to geographical differences in sea temperature through genetic shifts in thermal tolerance over long periods of time (Coles et al., 1976; Hoegh-Guldberg, 1999), which ultimately defines the response of the coral holobiont to stress within a region (Donner et al., 2005). However organisms are also
able to shift their phenotypic responses to a limited extent through acclimation to environmental extremes (Schmidt-Nielsen, 1996). In this respect, many organisms show the ability to acclimate to stressors at both the physiological and molecular levels (Feder and Hofmann, 1999; Tomaneck and Somero, 1999; Sorte and Hoffman, 2005). The coral holobiont is no exception, and several studies have demonstrated that corals (Brown et al., 2002; Coles and Brown, 2003; Castillo and Helnuth, 2005; Dove et al., 2006) and *Symbiodinium* (Iglesias-Prieto and Trench, 1997; Downs et al., 2000) can acclimate to heat and light stress. In addition, variations in the bleaching susceptibility of conspecifics across environmental gradients, for instance latitude, suggest the further potential for corals to acclimate to rising sea temperatures in the field (Coles and Brown, 2003; Donner et al., 2005; Ulstrup et al., 2006). The ability to acclimatize to different local conditions has the potential to play an important role in explaining small- and large-scale patterns in bleaching susceptibility.

The physiological behavior of reef-building corals is actively influenced by their dinoflagellate symbionts (Little et al., 2004; Berkelmans and van Oppen, 2006). Acclimation to light stress in *Symbiodinium* has been shown to occur through changes in peridinin chlorophyll a-binding protein complexes (PCP) and chlorophyll a-chlorophyll c2-peridinin protein complexes (acpPCP), light harvesting pigments (Iglesia-Prieto and Trench, 1997), the efficiency of photosystem II (PS II) and xanthophyll turnover rates (Brown et al., 1999). *Symbiodinium* may acclimate to stressful conditions such as high light and temperature during periods of pre-exposure by the early activation of photo-protective mechanisms. Such mechanisms include changes in the efficiency of the xanthophyll cycle (Brown et al., 1999; Brown et al., 2000), photosynthetic efficiency (Anthony and Hoegh-Gulberg, 2003) and non-photochemical quenching.

The ability of the coral symbiosis to acclimate to stressors has been ignored in much of the recent experimental work on coral bleaching that consists of single thermal stress events, which often do not mirror actual conditions. Corals located on reef crests are often exposed to thermal and light conditions above their predicted thresholds for several hours on consecutive days (R.A.M., personal observations). Few controlled experiments have attempted to determine the effect of short-term elevated temperatures on subsequent bleaching outcomes (Coles and Jokiel, 1978).

The aim of the present study was to explore the influence of thermal history on the response of the coral symbiosis (*Acropora aspera*) to thermal stress. In particular, our study explores the changes in photosystem II efficiency, non-photochemical quenching, xanthophyll cycling and symbiont density in response to thermal stress in pre-exposed (31°C) and control coral populations. In particular, the study investigates the effect of three different prior thermal stress histories on the acquisition of thermal tolerance in order to explore whether the pattern of pre-exposure is important over and above the actual amount of exposure.

**MATERIALS AND METHODS**

**Collection and maintenance of corals**

Branches of the reef-building corals *Acropora aspera* (tan/cream morph (Dove, 2004)) containing *Symbiodinium* clade C3 (LaJeunesse et al., 2004) were collected from three large colonies on the reef flat adjacent to Heron Island Research Station (HIRS, 23°33'S, 151°54'E) in April 2006. Single upward-growing branch tips (3-4 cm long) were removed using wire cutters and transported to the seawater facility at HIRS and placed in racks immersed in running seawater. Coral branches were acclimatized in 4 large experimental aquarium tanks to the mean local ambient temperature (27°C, drawn from the reef crest). Corals were exposed to natural reef flat summer daily light levels (average daily maximum 1271.28±40.07 μmol m-2 s-1, average daily light dosage 21.37±0.88 μmol m-2 day-1) throughout the experiment.

**Experimental treatments**

The experimental system consisted of four 750 l tanks, two of which were heated treatment tanks and two of which were unheated controls. All coral branches were split evenly and placed in the control tanks at the beginning of the experiment. The experimental design involved pre-stressing corals 2 weeks and 1 week prior to a simulated bleaching event (Fig. A). To achieve this, 60 coral branches were moved from the control tanks to the treatment tank at 08:00 h 2 weeks and 1 week before the simulated bleaching event, whereupon the temperature was elevated to approximately 31°C over a 48 h period. After 2 days, corals were returned to the control tanks. Hereafter, those corals that were pre-stressed 2 weeks prior to the simulated bleaching event were called H2, those pre-stressed 1 week prior are called H1, and those that had not been pre-stressed H0. In addition control corals were not subjected to the simulated bleaching event. To control for effects due to handling, all corals were moved...
and handled whenever any corals were moved between tanks. Given that in total there were four different treatments, this experimental design was chosen in an effort to minimize inter-tank differences, which may have confounded comparison between treatments. Light levels were recorded using an ODYSSEY recorder (DATAFLOW Systems Pty Ltd, Christchurch, New Zealand) placed in each aquarium. Water temperature was recorded every 2 min using StowAway TidbiT Loggers placed in each aquarium (Onset Computer Corporation, Bourne, MA, USA).

Five coral branches per treatment were removed each day from the experiment at 18:00 h for measurement of photosynthetic efficiency. One branch per colony per tank for each corresponding treatment was snap-frozen using liquid nitrogen at 12:00 h on each day of treatment exposure and stored in a –70°C freezer. These branches were used to measure *Symbiodinium* cell densities, chlorophyll and xanthophyll pigment concentrations (Fig. 1B).

**Measurements of photosynthetic efficiency**

Effective dark-adapted quantum yield (\(F_{v}/F_{m}\)) is a relative measure of the rate at which PS II can use light to process electrons flowing through the photosynthesis and the photosynthetic efficiency of the light reactions (Hoegh-Guldberg and Jones, 1999). An imaging pulse amplitude modulation (PAM) chlorophyll fluorometer (MAXI Imaging PAM, Walz, Effeltrich, Germany) was used to analyse the photosynthetic efficiency of corals daily at 18:00 h. Five coral branches were measured per treatment per tank (totalling ten branches per treatment) in a glass Petri dish containing seawater from the flow-through system. Corals were dark-adapted for 40 min prior to measuring effective quantum yield (\(F_{v}/F_{m}\)) to assess whether PS II was adversely affected by the treatments (Warner et al., 1996).

Induction recovery curves were also performed to examine the ability of *Symbodiumium* to acclimate to short-term light stress. Coral branches were exposed to 461 g m\(^{-2}\) s\(^{-1}\) for 6 min followed by a dark recovery period of 14 min. Photo-kinetic parameters, including non-photochemical quenching and PS II quantum yield, were measured during the light period using a saturation pulse every second. A saturating pulse was used 16 times integrated over 13 min 58 s during the recovery phase. Dynamic yield and non-photochemical quenching were determined using the calculations of Warner et al. (Warner et al., 1996).

**The extraction of non water-soluble pigment**

Coral tissue from frozen coral branches was removed using an air brush and 5 ml of filtered (0.45 μm) seawater solution. *Symbiodinium* were separated from the coral tissue by centrifugation at 4500 g (4°C) for 5 min. The supernatant was discarded and the pellet resuspended in HPLC grade methanol (1 ml methanol for every 750 μl of sample). Solutions were filtered through a 0.22 μm membrane filter (GSWP04700, Millipore, North Ryde, NSW, Australia) and 50 μl sterilised milli-Q was then added to a 250 μl aliquots prior to use in High Performance Liquid Chromatography (HPLC). Samples were then separated and analysed using the methods of Dove et al. (Dove et al., 2006) and Zapata et al. (Zapata et al., 2000) using a SHIMADZU (Tokyo, Japan) SCL-10 HPLC attached to a SHIMADZU SPD-M10A photodiode array detector.

**Cell densities of Symbiodinium**

One branch per colony for each corresponding treatment within each tank was used to assess dinoflagellate density in *A. aspera*. Density was determined using a SEDGEMICK rafter cell 550 (ProSciTech S8050, Kirwin, Queensland, Australia). Fourteen 1 μl cells were counted within a 1 ml slide, and averaged per sample.
weeks prior to the main experimental period of thermal stress (days 16, 17 and 18, \( P<0.001 \)) (Fig. 2A). Corals that were preheated 2 and 1 weeks prior also experienced a significant decline, but this was significantly less during the last three time points, indicating reduced damage to the efficiency of the PS II reaction centre.

The population density of *Symbiodinium* (cells cm\(^{-2}\)) in those corals that had not been pre-stressed also showed a significant decrease at the final sampling point (day 19) as compared to treatments that did not undergo prior thermal stress (\( P=0.005 \), Fig. 2B). The population density of dinoflagellates in corals preheated 2 weeks and 1 week prior remained relatively constant across the experiment, ranging between 1.3 and 1.7×10\(^6\) cells cm\(^{-2}\), and was not significantly different from unheated controls.

Induction recovery curves were performed to indicate the speed of recovery of the PS II reaction centre from PAR pressure (Fig. 3A). Effective quantum yield measurements for day 18 (Fig. 3B) and dark acclimation period (Fig. 3D) showed a significant effect of treatment (\( P=0.029 \)). Corals preheated 2 weeks prior to the bleaching event had significantly higher NPQ values than those preheated 1 week prior and those not preheated during both actinic illumination (\( P=0.00075 \)) and dark acclimation period (\( P=0.001 \)). The induction recovery curve recorded 2 days prior (data not shown) saw corals preheated 1 week prior having a significantly lower rate of NPQ in comparison to the two other treatments at the end of the acclimation period (\( P=0.023 \)). Therefore, it can be inferred that during the last 3 days of the simulated bleaching event (days 16–18), there is an increase in rate of NPQ in coral branches preheated 2 weeks prior, a decrease in rate of NPQ in corals not preheated, and no difference in rate of NPQ in corals preheated 1 week prior.

**The effect of prior thermal stress treatments on the composition of photosynthetic pigments in *Symbiodinium* sp.**

No significant interaction between ‘Time×Treatment’ was found for the effects on Chl \( a \) cell\(^{-1}\) (Fig. 4B \( F_{9,96}=1.45 \ P=0.24 \)). There was, however, a significant interaction between prior treatment and...
Corals were heat stressed for either 2 weeks, 1 week or not pre-stressed prior to the main experimental period, averaging 34°C. Effective PSII yield, NPQ, *Symbiodinium* density and xanthophyll ratios are displayed in greater detail in the figures. Arrows indicate significant difference (†, increased; †, decreased) from control on the final day of the simulated bleaching event. Double arrows indicate significant difference between treatments.

**DISCUSSION**

Coral reefs have shown large-scale and rapid changes in response to climate change. In this respect, small increases in temperature have been found to have profound effects on the endosymbiotic relationship between reef-building corals and their dinoflagellates (Hoegh-Guldberg, 1999; Hughes et al., 2003). A large number of studies have demonstrated that corals and their symbionts are living close to their current thermal maxima. Projections that combine the behaviour of corals with projections of sea temperature from global climate change models suggest that mass bleaching events will become a yearly phenomenon within the next 30–50 years (Hoegh-Guldberg, 1999). This is expected to lead to a rapid decline in coral cover on tropical reef systems, which may be the basis of the observed decline seen by a number of recent studies (e.g.

### Table 1. Summary of results from physiological parameters measured in *Acropora aspera* taken from three colonies on Heron Island reef crest

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Heated 2 weeks prior</th>
<th>Heated 1 week prior</th>
<th>Not pre-heated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective yield of PS II (Fv/Fm)</td>
<td>‡</td>
<td>††</td>
<td>††</td>
</tr>
<tr>
<td>NPQ</td>
<td>††</td>
<td>††</td>
<td>††</td>
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<tr>
<td><em>Symbiodinium</em> density</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Xanthophyll pool (Dt+Dd)</td>
<td>††</td>
<td>††</td>
<td>††</td>
</tr>
<tr>
<td>Xanthophyll pool/Chl a</td>
<td>††</td>
<td>††</td>
<td>††</td>
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<tr>
<td>Xanthophyll cycling Dt/(Dt+Dd)</td>
<td>††</td>
<td>††</td>
<td>††</td>
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</table>

Corals were heat stressed for either 2 weeks, 1 week or not pre-stressed prior to the main experimental period, averaging 34°C. Effective PSII yield, NPQ, *Symbiodinium* density and xanthophyll ratios are displayed in greater detail in the figures. Arrows indicate significant difference (†, increased; †, decreased) from control on the final day of the simulated bleaching event. Double arrows indicate significant difference between treatments.

Dt, diatoxanthin; Dd, diadinoxanthin.
between the yield of control and pre-stressed corals were only seen to be more thermally tolerant, as indicated by reduced loss of prior to thermal stress beyond the bleaching threshold were found to lead to increased thermal tolerance through acclimation.

temperatures (Fig. 5), we examined if short-term prior thermal stress bleaching is predominantly associated with short-term increases in corals experimentally (Coles and Jokiel, 1978). Given that coral However, only a single study has examined thermal acclimation in and latitudinal (Hoegh-Guldberg, 1999; Ulstrup et al., 2006) scales. (e.g. Brown et al., 2002), colony, reefal (Castillo and Helmuth, 2005) shown to lead to acclimation in coral conspecifics at intra-colony (Hoegh-Guldberg, 2000) and Donner et al. (Donner et al., 2005) estimated that the thermal tolerance of reef-building corals will have to rise by approximately 0.2–1.0°C per decade to keep pace with rising global temperatures. Thermal history has also been shown to lead to acclimation in coral conspecifics at intra-colony (e.g. Brown et al., 2002), colony, reefal (Castillo and Helmuth, 2005) and latitudinal (Hoegh-Guldberg, 1999; Ulstrup et al., 2006) scales. However, only a single study has examined thermal acclimation in corals experimentally (Coles and Jokiel, 1978). Given that coral bleaching is predominantly associated with short-term increases in temperature, ranging from a few hours to a few days, and that these bleaching conditions may be preceded by warmer conditions (Fig. 5), we examined if short-term prior thermal stress leads to increased thermal tolerance through acclimation.

Corals that were subjected to warmer than average temperatures prior to thermal stress beyond the bleaching threshold were found to be more thermally tolerant, as indicated by reduced loss of Symbiodinium compared to corals that had not been pre-stressed (Fig. 2B). While pre-stressed coral symbiont densities were unchanged at the end of the bleaching, symbiont densities in non-prestressed corals declined by approximately 40%. Given that the Symbiodinium photosynthetic apparatus has been suggested as the point of thermal lesion in a number of studies (Iglesias-Prieto et al., 1993; Warner et al., 1996; Jones and Hoegh-Guldberg, 1999), we explored the responses by corals and their symbionts with respect to photosynthetic efficiency and pigment profiles, particularly the xanthophyll pool, which provides the majority of NPQ.

The dark-adapted yield of photosystem II has been used extensively as a measure of damage to photosystem II and has been shown to be a conventionally good proxy for bleaching susceptibility (Warner et al., 1999; Jones et al., 2000; Pitt et al., 2001). Those corals that had not been stressed had significantly lower dark-adapted yields than pre-stressed and control corals in the final 3 days of the bleaching event (Fig. 2A), indicating that pre-stress provided some level of thermal protection to photosystem II. Significant differences between the yield of control and pre-stressed corals were only seen on the final day of the experiment. In the case of corals that were pre-stressed 2 weeks prior to bleaching (H²), tolerance was correlated with higher levels of NPQ (Fig. 2D) and xanthophyll pool to Chl a ratio (Fig. 4B) while the xanthophyll cycling remained unchanged relative to non-prestressed controls (Fig. 4C).

A very different pattern was observed in xanthophyll cycling for those corals pre-stressed 1 week prior to bleaching. Despite bleaching less than non-prestressed corals, these pre-stressed corals did not differ from control corals with respect to xanthophyll pool size (Table 1) and cycling rate (Fig. 4C). The ratio of diatoxanthin to dinoxanthin and diatoxanthin was significantly less than those seen in coral that were pre-stressed 2 weeks prior and those not pre-stressed (Fig. 4C).

The rate of NPQ was also found to be less in corals prestressed 1 week prior compare to other prestressed corals (Fig. 3D). These contrasting patterns of NPQ, xanthophyll levels and xanthophyll cycling indicate that acclimation is a dynamic process, with distinct differences in stress profiles that differed by 7 days. In addition it indicates that a variety of other thermal protective pathways are occurring in the coral holobiont in addition to those examined here. This is not surprising given that acclimation in the holobiont can involve the coral host, Symbiodinium, or a combination of both (Dove, 2004). Both corals (Kortschak et al., 2003) and Symbiodinium (Leggat et al., 2007) have been shown to possess a wide variety of genes that encode for stress response proteins (e.g. heat shock proteins, superoxide dismutase, ubiquitin etc.), which can impart protection, indicating that a more comprehensive study is required to elucidate all of the underlying mechanisms of thermal acclimation.

Although commonly interchanged, the terms acclimation and heat shock refer to very different cellular responses (Bowler, 2005). Acclimation results from long-term exposure, in the order of days to weeks, to new conditions that are within the normal limits of an organism’s response. Acclimation generally results in a variety of cellular and molecular responses such as alteration of lipid composition, protein isoymes and protein expression, which will provide protection from long-term gradual changes in the environment. In contrast heat shock generally refers to a period of short exposure to near lethal temperature that may or may not provide a very distinct cellular and molecular response to those seen in acclimation. Which cellular and biochemical mechanisms occur in the coral holobiont to different conditions will be a future area of important research. An examination of the literature on perhaps the most well-studied invertebrate model for acclimation, Drosophila sp., demonstrates that the ability of an organism to acclimate to various conditions, how it acclimates and, indeed, the costs of acclimation, can vary significantly between con-specifics and species and have profound effects on an organism (for a review, see Hoffman et al., 2003). For example, the induction of acclimatory responses has been shown to have effects on a variety of other performance measures resulting in such disparate responses as increased longevity, increased cold tolerance and decreased fertility, to list a few (Hoffman et al., 2003). A history of exposure to a range of stresses has also been found to alter subsequent higher plant responses where a priming stress activates genes that leave an epigenetic mark, facilitating a quicker response to subsequent stresses (Bruce et al., 2007). What costs and benefits accrue to the coral holobiont will need to be determined.

This study conclusively demonstrates that thermal stress events 2 weeks and 1 week prior to a bleaching event provide significantly increased thermal tolerance to the coral holobiont, suggesting that short time-scale thermal adaptation can have profound effects on coral bleaching. The inclusion of physiological and acclimatory properties into the modeling of climate change on populations and
ecosystems has already been advocated (Helmut et al., 2005); in the case of the coral holobiont, a better understanding of thermal history on bleaching susceptibility may provide insights into what reefs will look like in the near future under altered climate regimes.

**LIST OF ABBREVIATIONS**

- acpPCP: chlor a-chl c–peridinin protein complex
- Chl a: chlorophyll a
- Dd: diadinoxanthin
- Dtt: diatoxanthin
- NPF: non-photochemical quenching
- PAM: pulse amplitude modulation
- PAR: photosynthetically active radiation
- PAM: peridinin chlorophyll a-binding protein complex
- PS II: photosystem II

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**REFERENCES**


**Table S1** Repeated measures ANOVA results for PAM fluorescence yield (dark adapted $F_v/F_m$) across five time points with time, preheat and tank as within factors

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**Table S2.** Repeated measures ANOVA results for xanthophyll ratios $[Dt/(Dt+Dd)]$ across four time points with time, preheat and tank as within factors

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**Table S3** Repeated measures ANOVA results for xanthophyll pool size $(Dt+Dd)$ across four time points with time, preheat and tank as within factors

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**Table S4** Repeated measures ANOVA results for xanthophyll pool to Chl a ratio $[(Dt+Dd)/chl a]$ across four time points with time, preheat and tank as within factors

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<td>preheat</td>
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<td>preheat X time</td>
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<td>9</td>
<td>0.010</td>
<td>3.3</td>
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<tr>
<td>preheat X tank</td>
<td>0.033</td>
<td>3</td>
<td>0.011</td>
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<tr>
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<td>0.063</td>
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<tr>
<td>error (preheat)</td>
<td>0.024</td>
<td>6</td>
<td>0.004</td>
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