Title: Warm temperature acclimation impacts metabolism of paralytic shellfish toxins from *Alexandrium* in commercial oysters.

Running head: Temperature effects toxin metabolism in oysters

Authors

Hazel Farrell[1,2,3], Frank Seebacher[4], Wayne O'Connor[5], Anthony Zammit[3], Tim Harwood[6], Shauna Murray[1,2*]

Institute or laboratory of origin:

[1]School Plant Functional Ecology and Climate Change Cluster (C3). University of Technology Sydney, Ultimo, NSW 2007, Australia.

[2]Sydney Institute of Marine Sciences. Chowder Bay Rd, Mosman NSW 2088, Australia.

[3]NSW Food Authority, 6 Avenue of the Americas, Newington NSW 2127, Australia

[4]Integrative Physiology, School of Biological Sciences, Heydon Laurence Building A08, The University of Sydney, NSW 2006 Australia.

[5]Industry & Investment NSW. Port Stephens Fisheries Institute, Locked Bag 1, Nelson Bay, NSW 2315, Australia.

[6]Cawthron Institute, Private Bag 2, Nelson 7010, New Zealand.

Corresponding author: *Correspondence: Shauna Murray, tel +61 2 9514 4006, fax +61 2 9514 4079, email shauna.murray@uts.edu.au

Keywords:

Alexandrium, paralytic shellfish toxins, ocean temperature increases, bivalves, accumulation, depuration, metabolism, metabolic enzymes

vi. Type of paper:

Original research article/Primary research paper

Abstract

Species of *Alexandrium* produce potent neurotoxins termed paralytic shellfish toxins, and are expanding their ranges worldwide, concurrently with increases in sea surface temperature. The metabolism of molluscs is temperature-dependent, and increases in ocean temperature may influence both the abundance and distribution of *Alexandrium* and the dynamics of toxin uptake and depuration in shellfish. Here, we conducted a large-scale study of the effect of temperature on the uptake and depuration of paralytic shellfish toxins in three commercial oysters (*Saccostrea glomerata* and diploid and triploid *Crassostrea gigas,* n=252 per species/ploidy level). Oysters were acclimated to two constant temperatures, reflecting current and predicted climate scenarios (22 and 27 °C), and fed with the paralytic shellfish toxin-producing species *Alexandrium minutum*. While the oysters fed on *Alexandrium minutum* in similar quantities, concentrations of the toxin analogue GTX1,4 were significantly lower in warm-acclimated *S. glomerata* and diploid *C. gigas* after 12 days. Following exposure to *A. minutum*, toxicity of triploid *C. gigas* was not affected by temperature. Generally, detoxification rates were reduced in warm-acclimated oysters. The routine metabolism of the oysters was not affected by the toxins, but a significant effect was found at a cellular level in diploid *C. gigas*. The increasing incidences of *Alexandrium* blooms worldwide are a challenge for shellfish food safety regulation. Our findings indicate that rising ocean temperatures may reduce paralytic shellfish toxin accumulation in two of the three oyster types, however, they may persist for longer periods in oyster tissue.

Introduction

Increases in ocean temperature have widespread effects on the distribution, abundance, physiology and interactions of marine species (IPCC, 2013; Vermeer and Rahmstorf, 2009; Poloczanska *et al.*, 2012; Fig 1a). In Australia, the increase in ocean temperature at mid-latitudes of up to 2.0 °C over the past 100 years (Thompson *et al.* 2009; Ridgway *et al.* 2012) is significantly greater than the global mean, and is related to a southern range extension of the East Australian Current. The majority of animals within the world's oceans are ectotherms that are influenced directly by increases in ambient temperature. Temperature increases lead to thermodynamic changes in physiological function, and the ability of organisms to cope with these changes will depend on the thermal sensitivity of thermal performance curves and their plasticity resulting from developmental and reversible acclimation, or genetic adaptation (Seebacher *et al.* 2010; Wilson *et al.*, 2010; Hoffmann & Sgrò, 2011; Munday *et al.*, 2012; Seebacher & Franklin, 2012). For sessile intertidal organisms, acclimation is the most feasible response to rapid ocean warming (Harley, 2011).

Ocean temperature change can also indirectly impact marine invertebrates due to its effects on phytoplankton distribution and abundance (Hobday *et al.*, 2006; Hallegraeff, 2010; Thomas *et al.*, 2012), specifically, changes to the abundance and distribution of harmful algal bloom forming taxa (Glibert *et al.*, 2014). The increase in temperature in the East Australian current region is likely to cause an earlier timing of peak production and an increase in the seasonal window of species of *Alexandrium* and *Gymnodinium catenatum* (Hallegraeff, 2010), which produce paralytic shellfish toxins (PSTs). PSTs produced by species of *Alexandrium* include more than 20 known analogues, of which saxitoxin (STX), neosaxitoxin (NEO) and the gonyautoxins (GTX1, GTX2, GTX3, GTX4) are the most potent (Llewellyn *et al.*, 2006). PSTs have severe impacts on humans, and a broad range of marine organisms, including

77 mammals, birds, fish, molluscs and crustaceans, by selectively blocking voltage-gated Na+
78 channels in excitable cells, affecting neural impulse generation (Catterall, 1980). In the
79 context of human wellbeing, negative effects of climate change on valuable food species are
80 of particular concern. Global annual mollusc food production is approximately 2.1 x 10^7 t, of
81 which oyster production comprises around 22% (FAO, 2014). Pacific oysters (*Crassostrea*
82 *gigas*) are produced worldwide, and in Australia the indigenous Sydney rock oyster,
83 *Saccostrea glomerata*, is one of the main species produced (Fig. 1c). Since 2005, there has
84 been an increase in blooms of *Alexandrium* in south eastern Australian coastal waters (Farrell
85 *et al*., 2013; Fig. 1b), which have resulted in over 50% of algal-related shellfish harvest area
86 closures.
87
88 The thermal dependence of bivalve physiological responses to *Alexandrium,* and the way in
89 which this impacts the total PST concentrations in species, has never been assessed. This
90 information is crucial given the current and predicted rates of ocean temperature increases.
91 Based on experimental feeding studies, variation between bivalve species in the rate of and
92 the total uptake and depuration of PSTs has been found (Bricelj *et al*., 1990; Sekiguchi *et al.*
93 2001; Chen and Chou, 2002; Blanco *et al*., 2003; Lassus *et al*., 2005; Li *et al.*, 2005; Kwong
94 *et al*., 2006; Asakawa *et al.*, 2006; Hégaret *et al*., 2007; Lassus *et al.,* 2007; Galimany *et al.,*
95 2008; Murray *et al*., 2009; Haberkorn *et al*., 2011; Contreras *et al.,* 2012; Fernandez-Reiriz *et*
96 *al.,* 2013; Bricelj *et al.*, 2014; Haberkorn *et al*., 2014). This was hypothesized to be due to
97 differences in the feeding level on *Alexandrium* among bivalve species (Hégaret *et al*., 2007;
98 Contreras *et al.,* 2012). Some studies have used relatively small samples sizes or pooled
99 samples, which may not take into account the substantial differences found between bivalve
100 individuals in PST toxin levels (Lassus *et al*., 2005; Kodama, 2010).
101

Our aim was to determine the temperature dependence of the dynamics of *Alexandrium* feeding, PST accumulation and depuration, and physiological and enzyme responses of oysters (*S. glomerata* and diploid and triploid *C. gigas*), using a large scale study, to take into account large individual variability. As the ploidy level in *C. gigas* has been shown to impact metabolic rate (Haberkorn *et al*. 2010, Guéguen *et al*. 2012), we tested both diploid and triploid *C. gigas*. We hypothesised that oysters held at a predicted higher temperature (27 °C) relative to their current range (22 °C), would not differ significantly in their rate of toxin accumulation, as they would acclimate their metabolic processes accordingly. To test these hypotheses, we temperature acclimated the oysters (n=252 per species/ploidy level) and fed them with cultures of toxic *Alexandrium minutum* over a period of 12 days. We examined toxin dynamics, routine metabolic rate and metabolic enzyme activity.

Materials and methods

Study species and acclimation treatments

Adult *S. glomerata* and diploid and triploid *C. gigas* were sourced from farms in Port Stephens, NSW, during September and October 2012, and all experiments were conducted at the NSW Dept. Primary Industries, Port Stephens Fisheries Institute. Sea surface temperatures at the time of collections were 18-19 °C. Shell size and body mass were measured before the experiment to ensure that the specimens were of marketable condition (see supplementary information Table S1). Oysters were cleaned to remove fouling and held in 400 L aerated tanks (ca. 100 individuals $tank^{-1}$), containing 1 µm-filtered seawater from their estuary of origin (salinity ~ 35 g L^{-1}). Over 5-7 days, tank water temperatures were gradually increased to either 22 or 27 °C (± 0.5 °C) and 100 oysters per genotype or species were held in each acclimation treatment. The oysters were held at the final constant acclimation temperatures for two weeks. Seawater changes took place every two days and no mortalities occurred during the acclimation period.

Phytoplankton culture growth and maintenance

All algal cultures were grown at 23 °C with a 12/12-h light:dark photoregime at 60 μm^2 s^{-1}. Non-toxic live feed comprising *Isochrysis* aff. *galbana* (CS-177), *Pavlova lutheri* (CS-182) and *Chaetoceros muelleri* (CS-176) were grown in f/2 medium (Guillard & Ryther, 1962). During the acclimation period (described above), the oysters were fed daily with a mixed algal diet of late exponential phase non-toxic feed (2×10^9 cells $oyster^{-1}$ day^{-1}). *Alexandrium minutum* culture CS-324/16 was obtained from the CSIRO National Algae Culture Collection. This strain was originally isolated from Adelaide, South Australia. The toxin profile of the strain was characterized as containing primarily gonyautoxins GTX1,4 and low levels of GTX2,3 and STX (Negri *et al*., 2003). *A. minutum* cultures were grown in GSe medium and

harvested during late exponential phase. To confirm the presence of PSTs, two samples (200 mL of ~200,000 cells mL^{-1}, 400 mL of ~120,000 cells mL^{-1}) of late exponential phase *A. minutum* were collected and centrifuged at 5,000 rpm. The supernatant was removed and the pellet was frozen at -80 °C for later quantification of toxins.

Feeding experiments

A. minutum feeding experiments were carried out in 200 L aquaria. For each species and temperature combination, 252 oysters were distributed randomly across 12 tanks (21 oysters $tank^{-1}$), a total of 756 oysters. For 12 days, 6 tanks received a mixed non-toxic algal diet only. The remaining 6 tanks received the mixed algal diet, plus late exponential phase cultures of *A. minutum* (300 cells mL^{-1}) were added three times daily. *A. minutum* cell concentrations in the tanks were checked twice daily. Salinity was ~35g L^{-1} and water temperatures were maintained at 22 °C and 27 °C ± 0.5 °C. Seawater changes took place every two days. Water samples were collected directly from each water tank, preserved with lugol's iodine and examined, via light microscopy, in order to determine *A. minutum* clearance (feeding) rate by the oysters. *A. minutum* cells were added to maintain a minimum tank concentration of 300 cells mL^{-1} to ensure maximum consumption (Bricelj *et al*, 1990, Murray *et al*., 2009). On day 0, before introduction of the *A. minutum*, and on days 6 and 12, three oysters were collected from each tank. This was equivalent to 36 individuals per time point for each oyster species/ploidy level. Following the seawater change on day 12, the remaining oysters of all treatments were fed the mixed algal diet only to allow depuration of toxins. Depuration was carried out for 7 days and further sampling (3 oysters $tank^{-1}$) was carried out on days 13 and 19. At the time of sampling, body mass, shell length, breadth and height of each oyster were recorded. After euthanising, the shell and tissue wet mass were also recorded for each oyster, and tissue was retained at -80 °C for toxin analysis.

Toxin analysis

The PST content was measured in 300 individual oysters (n = 9 oysters per toxic treatment and n=30 non-toxic control oysters), according to Lawrence *et al*. (2005) and Harwood *et al*. (2013). Briefly, homogenised (Omni Tissue Homogeniser, Omni International, USA) oyster tissue was vortexed with 3 mL of 1% acetic acid solution. The mixture was heated at 100 °C for 5 min, re-vortexed and then cooled and incubated at 4 °C for 5 min. Following centrifugation (10 min at 4,500 rpm), the supernatant was collected. The pellet was re-suspended with a further 3 mL of 1% acetic acid and re-centrifuged (10 min at 4,500 rpm). The resulting supernatant was added to the original quantity and diluted with deionised water to 10 mL. The *A. minutum* cell pellet was extracted according to the same method with slight modifications. Initially, the cell pellets were freeze-thawed to ensure cell lysis. Also, after both supernatants were combined, the dilution step to gain a final volume of 10 mL was excluded to gain a higher toxin yield. Prior to analysis, a SPE C18 clean up (GracePure SPE C18-Max 500 mg/3 mL, Alltech Associates (Australia) Pty Ltd) was carried out on 1 mL of each extract. The pH of the final 4 mL effluent was adjusted to 6.5 with 1 M NaOH.

Quantification of toxins by ultra-performance liquid chromatography (UPLC) and fluorescence detection (FD) was carried out as per Harwood *et al.* (2013). Analytical certified reference standards were sourced from the National Research Council of Canada. The UPLC chromatogram of the extract from the *A. minutum* pellet had two peaks that corresponded to the analogues GTX1,4 and a third peak that fit the retention times of both GTX1,4 and GTX2,3 (refer supplementary information; Fig. S1b). The GTX1,4 concentration was determined from the second peak (Fig. S1b), while the concentration of GTX2,3 was verified by an additional peroxide oxidation (Harwood *et al.,* 2013). Extracts from oysters that had not

been exposed to *A. minutum* were spiked with known quantities of GTX1,4 and GTX2,3, to estimate the method's recovery factor. Recovery factors were incorporated into the final estimates of toxin levels.

Routine metabolic rate

On day 0 and day 12, the routine metabolic rate (RMR) of individual oysters (3 oysters $tank^{-1}$) was measured using a closed respirometry system according to Parker *et al.* (2012). In total the measurement procedure was carried out on 216 individual oysters (n=9 oysters $treatment^{-1}$ $sampling\ point^{-1}$). Measurements were carried out at either 22 °C or 27 °C, depending on the treatment temperature. During RMR measurements, one control (non-toxic diet) diploid *C. gigas* (27 °C) and one replicate (toxic diet) *S. glomerata* (27 °C) failed to respire during the analysis. RMR represents the level of metabolism for normal, unrestricted activity. In this case, the valve of the oysters was unhindered, and the shells could open freely. Digestion was ongoing, evidenced by the production of faecal pellets during the measurement process. This differed from measurements of standard or resting metabolic rate, where minimal activity, independent of digestion, is quantified (Willmer *et al.*, 2009) A fiber-optic probe (PreSens dipping probe DP-PSt3, AS1 Ltd, Palmerston North, New Zealand) was fitted to an airtight 500 mL chamber. Individual oysters (displacement volume < 50 mL) were submerged gently in seawater within the darkened chamber. Estimates of RMR were based on the time taken for the percentage oxygen saturation of seawater in the chamber to reduce from 100 to 80%, because of respiration by the oyster. The oxygen probe was calibrated based on a two-point calibration (0% and 100%). Values for RMR (mg O_2 g^{-1} DTM h^{-1}) were calculated as

$$RMR = \frac{V_r \times \Delta C_w 0_2}{\Delta t \times DTM} \qquad (1)$$

where V_r (L) is the volume of the chamber minus the displacement volume of the oyster ΔC_wO_2 (mg O_2 L^{-1}) is the measured change in oxygen concentration over time Δt (h), and values were normalized to 1g of dry tissue mass (DTM, g).

Metabolic enzyme activities

After RMR measurements, the 216 individual oysters (n=9 oysters treatment^{-1} sampling point^{-1}) were euthanised and 50 mg of tissue from the adductor muscle and digestive gland were dissected, placed in 1.5 ml Eppendorf tubes and flash frozen with liquid nitrogen. All samples were stored at -80 °C until further enzyme analysis. The remaining oyster tissue was freeze-dried for 48hrs (Alpha 2-4 LSC plus, Martin Christ Gefriertrocknungsanlagen GmbH, Germany) to determine dry tissue mass (DTM).

Activities of citrate synthase (CS), cytochrome c oxidase (COX) and the combined activity of lactate, glycine and β-alanine dehydrogenase (LDH), were measured as indicators of tricarboxylic acid cycle, mitochondrial electron transfer, and anaerobic ATP production, respectively. All assays were conducted according to published protocols (Seebacher *et al*. 2003; Sinclair *et al*. 2006). Briefly, digestive gland or adductor muscle tissue (0.05g) was homogenised in nine volumes of extraction buffer (50 mmol l^{-1} imidazole/HCl, 2 mmol l^{-1} $MgCl_2$, 5 mmol l^{-1} ethylene diamine tetra-acetic acid (EDTA), 1 mmol l^{-1} reduced glutathione and 1% Triton X-100). All samples were kept on ice during homogenisation. For COX and CS assays, homogenates from digestive gland tissue, were further diluted by a factor of 10. All assays were measured spectrophotometrically in an UV/visible spectrophotometer (Ultrospec2100 pro, Biochrom, UK) with a temperature controlled cuvette holder (Seebacher *et al*., 2003). Each assay was performed in duplicate at two temperatures, which coincided with acclimation temperatures (22 and 27 °C).

Statistical analyses

Clearance rate (CR) of *A. minutum* was calculated according to

$$CR = \frac{(lnC_o - lnC) \times V}{t} \quad (2)$$

where C_o and C are the initial and final *A. minutum* cell concentrations, respectively, V is the volume of suspension (holding tank volume) and t is time (Coughlin, 1969).

On selected days (n=5 oyster species/ploidy level^{-1} treatment^{-1}), the final cell concentration for each replicated was estimated over 24 hour periods, following tank seawater changes. Clearance rates were normalised to 1 g oyster tissue wet weight based on the mean values in Table S1 (Bricelj et al., 1990). To determine any significant difference between oyster species/ploidy level and acclimation temperature on clearance rates, data were analysed by a two-way permutation analysis of variance (ANOVA).

In order to determine the effect of temperature on the accumulation and depuration of PSTs (GTX1,4 and GTX2,3) in oysters, we examined toxicity on day 12, the period of maximum exposure to *A. minutum*, and day 13, following 24 hours of the oysters receiving a non-toxic diet only (depuration). Analysis of PSTs in each oyster species/ploidy level was by two-way permutation ANOVA with exposure treatment (days 12 and 13, as above) and acclimation temperature as factors.

Also on day 12, RMR data for each oyster species/ploidy level (n = 9 oysters treatment^{-1}) were analysed by a two-way permutation ANOVA with diet (toxic or non-toxic) and acclimation temperature as factors. Enzyme activities were analysed (n = 9 oysters treatment^{-1}) with a three-way permutation ANOVA with acclimation temperature and diet as factors and test temperature as a repeated measure.

All analyses were carried out in R (R.app GUI 1.63, 2012). Permutation analysis were carried out using the lmPerm package (Wheeler, 2014), Results are expressed as mean ± standard error mean (SEM). For all statistical analyses, the significance level was set at the $p \leq 0.05$ alpha-level. For each multifactorial analysis, the highest significant interaction was examined. Significant main effects were only examined when no significant interactions were reported. *Post hoc* analysis of means was by Tukey's Honest Significant Difference (HSD). For analyses where nominal variables had only two levels, no *post hoc* analysis was carried out.

Results

Uptake of A. minutum

Temperature did not affect the rate at which the different oyster species/ploidy levels fed on toxic *A. minutum* (Fig. 2, Table 1, S2).

Toxicity of A minutum and oysters

The PST analogues GTX 1,4 (1,112 ± 208 ng mL^{-1}) and GTX 2,3 (22.21 ± 4.01 ng mL^{-1}) were found in the culture of *A. minutum*. These concentrations corresponded to 0.59 ± 0.08 pg GTX1,4 $cell^{-1}$ and 0.012 ± 0.003 pg GTX2,3 $cell^{-1}$. All oysters that were exposed to a toxic diet of *A. minutum* accumulated both GTX1,4 and GTX2,3 during the 12-day treatment (Fig. 3). PSTs were not found in the control (non-toxic diet) or day 0 samples (data not shown).

GTX1,4 concentrations were lower (exposure treatment × acclimation temperature; Tables 1 and 2) in warm-acclimated diploid *C. gigas* (Fig. 3a) and *S. glomerata* (Fig. 3c) following 12 days of exposure to *A. minutum*. Following 24 hours of receiving a non-toxic algal diet only, warm-acclimated diploid *C. gigas* (Fig. 3a) and *S. glomerata* (Fig. 3c) had slower detoxification of GTX1,4 (exposure treatment × acclimation temperature, Tables 1 and 2). GTX2,3 concentrations after the 12-day exposure treatment and 24-hour depuration process were unaffected by temperature for both of these species (Tables 1 and 2, Fig. 3b,d).

GTX1,4 concentrations after the 12-day exposure treatment and 24-hour depuration process were unaffected by temperature for triploid *C. gigas* (Tables 1 and 2, Fig. 3e). Similarly, temperature did not influence concentrations of GTX2,3 in triploid *C. gigas* at the end of the 12-day exposure to a toxic diet. However, following the 24-hour depuration treatment,

detoxification was reduced in warm-acclimated triploid *C. gigas* (exposure treatment × acclimation temperature interaction; Tables 1 and 2, Fig. 3f).

Routine metabolic rate and metabolic enzyme activities

Diet and acclimation temperature did not have a significant influence on RMR in diploid and triploid *C. gigas* (Tables 1 and 3, diploid *C. gigas*: Fig. 4a,b; triploid *C. gigas*: Fig. 4e,f). RMR was higher in warm-acclimated *S. glomerata*, independent of diet (Table 3, Fig. 4c,d).

LDH activity was elevated in digestive gland tissue from warm-acclimated diploid *C. gigas* (Tables 1 and 4, Fig. S2). Lower LDH activity was observed in adductor muscle of diploid *C. gigas* exposed to a toxic diet (Table 5, Figure S3).

CS activity was reduced in digestive gland tissues from warm-acclimated *S. glomerata* (Tables 1 and 4, Fig. S4). The acclimation temperature × diet × test temperature interaction was significant for LDH activity in adductor muscle samples from *S. glomerata* (Table 5, Fig. S5). The interpretation of this interaction was unclear based on very little discernable differences in the graphed results. However, samples from warm-acclimated oysters had greater LDH activity (Tables 1 and 5, Fig S5).

Triploid *C. gigas* digestive gland samples had a reduced response for LDH and CS activities in warm-acclimated oysters (Tables 1 and 4, Fig. S6). Triploid *C. gigas* adductor muscle tissue had elevated LDH activity in warm-acclimated oysters at the 27 °C test temp (acclimation temperature × test temperature interaction) (Tables 1 and 5, Fig. S7), whereas CS and COX activities were reduced in the adductor muscle of warm-acclimated triploid *C. gigas* (Table 5, Fig. S7).

319

320 Where test temperature produced a significant main effect (Tables 4 and 5), the majority of
321 increased responses were at 27 °C (LDH: diploid *C. gigas* adductor muscle (Fig. S3); CS: all
322 oyster species/ploidy level digestive gland (Figs. S2, S4, S6), *S. glomerata* and triploid *C.*
323 *gigas* adductor muscle (Figs. S5, S7); COX: *S. glomerata* and triploid *C. gigas* adductor
324 muscle (Figs. S5, S7). LDH activity for triploid *C. gigas* digestive gland was elevated at the
325 22 °C test temperature (Table 4, Fig. S6).

Discussion

Filter-feeding bivalves face a combination of stressors from climate change, because the metabolic and physiological responses of these ectotherms are modulated by water temperature (Hawkins, 1995, Angilletta *et al*., 2002, Peck *et al*., 2004, Clarke and Fraser, 2004), and also because the distribution and abundance of toxin producing dinoflagellate species are likely to change (Hallegraeff, 2010, Glibert *et al*., 2014). South-eastern Australia in particular is considered a climate change "hotspot" due to decadal increases in temperature of ~0.2 °C since the 1940s, accompanied by a southern range expansion of the Eastern Australian Current (Ridgway, 2007, Ridgway & Hill, 2012; Wu *et al*. 2012), and has a substantial bivalve shellfish aquaculture industry.

We conducted the first large-scale experiment examining toxin uptake and depuration dynamics in three oyster species/ploidy levels to determine the combined impact of PSTs and temperature. While differences in the feeding efficiency on *A. minutum* by the different oyster types were not apparent (Table 1), significant differences in the concentration of PST analogues were observed between oyster types and acclimation temperatures after the 12-day exposure treatment (Table 1). In particular, diploid *C. gigas* and *S. glomerata* contained less GTX1,4, the more potent of the two PST congeners present, at warmer temperatures (Table 1). There was no apparent influence of temperature in the accumulation of GTX1,4 by triploid *C. gigas* or GTX2,3 by any of the three oyster types, at the end of the 12 day exposure treatment. PST analogues, identical to that in the *Alexandrium minutum*, were detected in all individual oysters after 12 days of exposure to a toxic diet (Fig. S1). Biotransformation of PST analogues, in which the toxin analogues or their proportions in bivalves differs from that of the *Alexandrium* culture, commonly occurs in clams, scallops, and mussels (Kwong *et al,* 2006; Sagou *et al.*, 2005; Bricelj & Shumway 1998; Bricelj *et al* 2014), but appears to be less

commonly reported in oysters (Bricelj & Shumway 1998; Murray *et al.*, 2009), in line with our findings.

In our study, triploid *C. gigas* and *S. glomerata* were found to be approximately 50% less toxic than diploid *C. gigas* at the end of the period of exposure to a toxic diet. Experimental feeding studies using PST-producing dinoflagellates have been conducted on mussels (Bricelj *et al*., 1990, Blanco *et al.,* 2003, Li *et al*., 2005, Kwong et al., 2006, Galimany *et al*., 2008), scallops, cockles and clams (Bricelj et al., 1990; Sekiguchi et al., 2001; Chen and Chou, 2001, 2002; Kodama, 2010; Higman and Turner, 2010; Contreras *et al.,* 2012;) and oysters (Lassus *et al.,* 2005*,* 2007; Murray *et al*., 2009; Haberkorn *et al*., 2010*,* 2011*,* 2014). Most studies analysed a single bivalve species or strain, but for those studies that compared total toxicity among species or ploidy levels, given the same experimental conditions, significant differences were generally found (Haberkorn *et al*. 2011, Contreras *et al.,* 2012). This was attributed to differences in feeding levels amongst bivalve species (Hegaret et al., 2007; Higman and Turner, 2010, Contreras *et al.*, 2012) or ploidy levels (Haberkorn et al., 2010). In our study, differences in total PST toxicity were found among species (Table 1) and at certain temperature treatments despite the fact that feeding levels on *Alexandrium* were not significantly different. This indicates that differences in PST metabolism rates may be instead contributing to these differences.

Both diploid and triploid *C. gigas* acclimated their RMR to their respective treatment temperatures independently of diet, which indicated that the exposure to *Alexandrium minutum* did not incur a metabolic cost. However, the reduced response in LDH activity suggested an increase in potential for aerobic metabolism in diploid *C. gigas* that were fed with *A. minutum*. Aerobic metabolism in oysters is associated with increased circulation and

376 filtration of seawater (Lucas, 2012). This finding may explain how, overall, the greatest
377 toxicity was observed in diploid *C. gigas*, at both temperatures. Previously, faster
378 accumulation of PSTs has been found in triploid *C. gigas* compared to diploid strains, and
379 was considered to be correlated to their faster metabolic rates (Haberkorn *et al*., 2010),
380 depending on sexual maturity (Guéguen *et al*., 2012).
381
382 We had anticipated that oysters would adjust their metabolic processes with increasing
383 temperatures. This was not the case for *S. glomerata* on day 12, as the elevated RMR,
384 independent of diet, implied a higher metabolic maintenance cost associated with warmer
385 conditions. Temperature-dependent responses of some metabolic enzyme activities (CS and
386 LDH) promoted the potential for anaerobic metabolic pathways in warm-acclimated *S.*
387 *glomerata*. While all three oyster species/ploidy levels experienced some effects of higher
388 temperature acclimation on metabolic enzyme activity, a greater number of responses were
389 noted in triploid *C. gigas*. The majority of these suggested a greater potential for anaerobic
390 metabolic scope (Table 1). Multiple stressors (temperature and cadmium or temperature and
391 elevated CO_2) have been found to have inhibitive effects on the aerobic scope of *C. gigas and*
392 *Crassostrea virginica* (Lannig *et al*. 2006; 2010). Our findings on *S. glomerata* and diploid *C.*
393 *gigas* were similar, although the responses were fewer than in triploid *C. gigas*.
394
395 In both diploid *C. gigas* and *S. glomerata*, warm-acclimated oysters had a slower depuration
396 of GTX1,4, following the dietary change from *A. minutum* to a diet of non-toxic algae only,
397 while there was no effect of temperature on the detoxification of GTX2,3 for either of these
398 species. For triploid *C. gigas*, the detoxification rate of GTX1,4 was unaffected by
399 temperature, however, warm-acclimated triploid *C. gigas* experienced slower reduction of
400 GTX2,3. Detoxification rates have been found previously to vary between species (Mons *et*

401 *al*., 1998, Kwong *et al*., 2006). While exposure to a toxic diet had a significant effect at a
402 cellular level in diploid *C. gigas*, the temperature-dependent responses of metabolic enzyme
403 activities to warmer conditions suggested that predicted changes to ocean temperatures will
404 influence toxin accumulation and depuration dynamics in all three oyster types.
405
406 By simulating an *Alexandrium* bloom under two temperature scenarios, using a large scale
407 study, we have shown differential toxin uptake and depuration in three oyster species/two
408 ploidy levels. Our findings indicate that both *S. glomerata* and diploid *C. gigas* may have
409 lower GTX1,4 concentrations in warmer waters given the same density of *A. minutum* bloom,
410 while detoxification will be slower. However, the current trend of increasing abundance and
411 distribution of PST producing species of *Alexandrium* (Anderson *et al*., 2012) will add a layer
412 of complexity to determining future risk of PSTs in commercial bivalves.
413
414

Acknowledgements

This work was supported by an Australian Research Council Linkage grant number LP110100516. This is contribution number xx from the Sydney Institute of Marine Science. The authors wish to thank the staff at the DPI Port Stephens Fisheries Centre for technical support during the experiment. Thanks to Alex Little for assistance with enzyme sample analysis. The advice of research and technical staff at the Cawthron Institute, New Zealand on toxin analysis is acknowledged. HF would like to thank Paul Parton for assistance with figure formatting.

References

Anderson DM, Alpermann TJ, Cembella AD, Collos Y, Masseret E, Montresor M (2012) The globally distributed genus *Alexandrium*: Multifaceted roles in marine ecosystems and impacts on human health. *Harmful Algae,* **14**, 10-35.

Angilletta MJ, Niewiarowski PH, Navasc CA (2002) The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**, 249-268.

Blanco J, Reyero MI, Franco J (2003) Kinetics of accumulation and transformation of paralytic shellfish toxins in the blue mussel *Mytilus galloprovincialis*. *Toxicon,* **42**, 777-784.

Bricelj VM, Lee JH, Cembella AD, Anderson DM (1990) Uptake kinetics of paralytic shellfish toxins from the dinoflagellate *Alexandrium fundyense* in the mussel *Mytilus edulis*. *Marine Ecology Progress Series,* **63**, 177-188.

Bricelj VM, Connell L, Konoki K, MacQuarrie SP, Scheuer T, Catterall WA, Trainer VL (2005) Sodium channel mutation leading to saxitoxin resistance in clams increases risk of PSP. *Nature,* **434**, 763-766.

Bricelj VM, Shumway S (1998) Paralytic shellfish toxins in bivalve molluscs; occurrence, transfer kinetics and biotransformation. *Reviews in Fisheries Science,* **4**, 315-383

Bricelj VM, Cembella AD, Laby D (2014) Temperature effects on kinetics of paralytic shellfish toxin elimination in Atlantic surf clams, *Spisula solidissima. Deep Sea Research II: Topical Studies in Oceanography,* **103**, 308-317

Catterall WA (1980) Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. *Annual Review of Pharmacology and Toxicology*, **20**, 15-43.

Chen CY, Chou HN (2002) Fate of paralytic shellfish poisoning toxins in purple clam Hiatula rostrata, in outdoor culture and laboratory culture. *Marine Pollution Bulletin*, **44**, 733-738.

Chen CY, Chou, HN (2001) Accumulation and depuration of paralytic shellfish poisoning toxins by purple clam *Hiatula rostrata* Lightfoot. *Toxicon,* **39**, 1029-1034.

448 Clarke A, Fraser KPP (2004) Why does metabolism scale with temperature? *Functional*
449 *Ecology*, **18**, 243-251.
450 Contreras AM, Marsden ID, Munro MH (2012) Effects of short-term exposure to paralytic
451 shellfish toxins on clearance rates and toxin uptake in five species of New Zealand bivalve.
452 *Marine and Freshwater Research*, **63**, 166-174.
453 Coughlin J (1969) The estimation of filtering rate from the clearance of suspension. *Marine*
454 *Biology,* **2**, 356-358.
455 FAO (2014) *Global Aquaculture Production 1950-2012 (online query)*
456 ***http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en***
457 Farrell H, Brett S, Ajani P, Murray S (2013) Distribution of the genus *Alexandrium* (Halim)
458 and paralytic shellfish toxins along the coastline of New South Wales, Australia. *Marine*
459 *Pollution Bulletin*, **72**, 133-145.
460 Galimany E, Sunila I, Hégaret H, Ramón M, Wikfors GH (2008) Experimental
461 exposure of blue mussel (*Mytilus edulis*, L.) to the toxic dinoflagellate *Alexandrium*
462 *fundyense*: histopathology, immune response, and recovery. Harmful Algae, 7 (5), 702-711.
463 Glibert PM, Allen JI, Artioli Y, Beusen A, Bouwman L, Harle J, Holmes R, Holt J (2014),
464 Vulnerability of coastal ecosystems to changes in harmful algal bloom distribution in
465 response to climate change: projections based on model analysis. *Global Change Biology*, **20**,
466 3845-3858.
467 Guéguen M, Baron R, Bardouil M, Haberkorn H, Soudant P, Truquet P, Lassus P (2012)
468 Influence of *Crassostrea gigas* (Thunberg) sexual maturation stage and ploidy on uptake of
469 paralytic phycotoxins. *Toxicon,* **60**, 40-43.
470 Guillard RL, Ryther, JH (1962) Studies of marine planktonic diatoms. *Canadian Journal of*
471 *Microbiology,* **8**, 229-239.

IPCC (2013) *Fifth Assessment Report - Climate Change 2013: The Physical Science Basis.* Summary for Policy Makers. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM) (eds.), pp. 1-30. Cambridge University Press.

Haberkorn H, Lambert C, Le Goïc N et al (2014) Cellular and biochemical responses of the oyster *Crassostrea gigas* to controlled exposures to metals and *Alexandrium minutum.* *Aquatic Toxicology* **147**, 158-167.

Haberkorn H, Lambert C, Le Goïc N et al. (2010) Effects of *Alexandrium minutum* exposure upon physiological and hematological variables of diploid and triploid oysters, *Crassostrea gigas*. *Aquatic Toxicology,* **97**, 96-108.

Haberkorn H, Tran D, Massabuau J-C, Ciret P, Savar V, Soudant P (2011) Relationship between valve activity, microalgae concentration in the water and toxin accumulation in the digestive gland of the Pacific oyster *Crassostrea gigas* exposed to *Alexandrium minutum.* *Marine Pollution Bulletin*, **62**, 1191-1197.

Hallegraeff GM (2003) Harmful algal blooms: a global overview. In *Manual on Harmful Marine Microalgae* (eds Hallegraeff GM, Anderson DM, Cembella AD), pp. 25-49. Monographs on Oceanographic Methodology, 11, UNESCO

Hallegraeff GM (2010) Ocean climate change, phytoplankton community responses, and harmful algal blooms: a formidable predictive challenge. *Journal of Phycology*, **46**, 220-235.

Hallegraeff GM (2014) Harmful Algae and their Toxins Progress, Paradoxes and Paradigm Shifts. In *Toxins and Biologically Active Compounds from Microalgae, Volume 1* (ed Rossini, GP), pp3-20. CRC Press.

496 Harley CD (2011) Climate change, keystone predation, and biodiversity loss. *Science*, **334**,
497 1124-1127.
498 Harwood DT, Boundy M, Selwood AI, van Ginkel R, MacKenzie L, McNabb PS (2013)
499 Refinement and implementation of the Lawrence method (AOAC 2005.06) in a commercial
500 laboratory: Assay performance during an *Alexandrium catenella* bloom event. *Harmful Algae*,
501 **24**, 20-31.
502 Hawkins AJ (1995) Effects of temperature change on ectotherm metabolism and evolution:
503 metabolic and physiological interrelations underlying the superiority of multi-locus
504 heterozygotes in heterogeneous environments. *Journal of Thermal Biology*, **20**, 23-33.
505 Hégaret H, Wikfors GH, Shumway SE (2007) Diverse feeding responses of five species of
506 bivalve mollusc when exposed to three species of harmful algae. *Journal of Shellfish*
507 *Research*, **26**, 549-559.
508 Hobday AJ, Okey TA, Poloczanska ES, Kunz TJ, Richardson AJ (eds) (2006). *Impacts of*
509 *climate change on Australian marine life: Part A. Executive Summary. Report to the*
510 *Australian Greenhouse Office, Canberra, Australia. September 2006.*
511 Hoffmann AA, Sgrò CM (2011) Climate change and evolutionary adaptation. *Nature*, **470**,
512 479–485.
513 Kodama M (2010) Paralytic shellfish poisoning toxins: biochemistry and origin. *Aqua*
514 *Bioscience Monographs*, **3,** 1-38.
515 Kwong RWM, Wang W-X, Lam PKS, Yu PKN (2006) The uptake, distribution and
516 elimination of paralytic shellfish toxins in mussels and fish exposed to toxic dinoflagellates.
517 *Aquatic Toxicology*, **80**, 82–91.
518 Lannig G, Eilers S, Pörtner HO, Sokolova IM, Bock C (2010) Impact of ocean acidification
519 on energy metabolism of oyster, *Crassostrea gigas* - changes in metabolic pathways and
520 thermal response. *Marine Drugs,* **8**, 2318-2339.

521 Lannig G, Flores JF, Sokolova, IM (2006) Temperature-dependant stress response in oysters
522 *Crassostra virginica*: pollution reduces temperature tolerance in oysters. *Aquatic Toxicology,*
523 **79**, 278-287.
524 Lassus P, Bardouil M, Baron R, Berard JB, Masselin P, Truquet P, Pitrat JP (2005) Improving
525 detoxification efficiency of PSP contaminated oysters (*Crassostrea gigas* Thunberg).
526 *Aquaculture Europe,* 3-6.
527 Lassus P, Amzil Z, Baron R et al. (2007) Modeling the accumulation of PSP toxins in Thau
528 Lagoon oysters (*Crassostrea gigas*) from trials using mixed cultures of *Alexandrium catenella*
529 and *Thalassiosira weissflogii*. *Aquatic Living Resources,* **20**, 59-67.
530 Lawrence JF, Niedzwiadek B, Menard C (2005) Quantitative determination of paralytic
531 shellfish poisoning toxins in shellfish using prechromatographic oxidation and liquid
532 chromatography with fluorescence detection: collaborative study. *Journal of AOAC*
533 *International*, **88**, 1714-1732.
534 Li AMY, Yu PKN, Hsieh DPH, Wang W-X, Wu RSS, Lam, PKS (2005) Uptake and
535 depuration of paralytic shellfish toxins in the green-lipped mussel, *Perna iridis*: a dynamic
536 model. *Environmental Toxicology and Chemistry,* **24**, 129-135.
537 Llewellyn L, Negri A, Robertson A (2006) Paralytic shellfish toxins in tropical oceans. *Toxin*
538 *Reviews*, **25**, 159-196.
539 Lucas JS (2012) Bivalve Molluscs. In *Aquaculture, Farming Aquatic Animals and Plant,*
540 *Second edition* (Lucas JS Southgate PC eds), pp. 541-566. Blackwell Publishing Ltd.
541 Mons MN, van Egmond HP, Speijers, GJA (1998) *Paralytic shellfish poisoning: a review.*
542 RIVM Report, The Netherlands.
543 Munday PL, McCormick MI, Nilsson GE (2012) Impact of global warming and rising CO_2
544 levels on coral reef fishes: what hope for the future? *The Journal of Experimental Biology*,
545 **215**, 3865-3873.

546 Murray SA, Wiese M, Stüken A, Brett S, Kellmann R, Hallegraeff G, Neilan BA (2011)
547 sxtA-Based Quantitative Molecular Assay To Identify Saxitoxin-Producing Harmful Algal
548 Blooms in Marine Waters. *Applied and Environmental Microbiology*, **77**, 7050-7057.
549 Murray SA, O'Connor WA, Alvin A, Mihali TK, Kalaitzis J, Neilan BA (2009) Differential
550 accumulation of paralytic shellfish toxins from *Alexandrium minutum* in the pearl oyster,
551 *Pinctada imbricata*. *Toxicon,* **54**, 217-223.
552 Parker L, Ross P, O'Connor W, Borysko L, Raftos D, Portner H (2012) Adult exposure
553 influences offspring response to ocean acidification in oysters. *Global Change Biology,* **18**,
554 82-92.
555 Poloczanska ES, Hobday AJ Richardson AJ (eds) (2012). *Marine Cliimate Change in*
556 *Australia, Impacts and Adaptation Responses.* 2012 Report Card.
557 Negri A, Llewellyn L, Doyle J, Webster N, Frampton D, Blackburn S (2003). Paralytic
558 shellfish toxins are restricted to few species among Australia's taxonomic diversity of
559 cultured microalgae. *Journal of Phycology*, **39**, 663-667.
560 Peck LS, Webb KE, Bailey DM (2004) Extreme sensitivity of biological function to
561 temperature in Antarctic marine species. *Functional Ecology,* **18**, 625-630.
562 Ridgway K, Hill K (2012) East Australian Current. In *A Marine Climate Change Impacts and*
563 *Adaptation Report for Australia 2012* (eds Poloczanska ES, Hobday AJ, Richardson AJ)
564 Retrieved from www.oceanclimatechange.org.au [22 December 2014].
565 Ridgway KR (2007) Long-term trend and decadal variability of the southward penetration of
566 the East Australian Current. *Geophysical Research Letters*, **34**, L13613,
567 doi:10.1029/2007GL030393, 2007.
568 Seebacher F, Franklin CE (2012) Determining environmental causes of biological effects: the
569 need for a mechanistic physiological dimension in conservation biology. *Philosophical*
570 *transactions of the Royal Society of London. Series B, Biological sciences*, **367**, 1607-1614.

571 Seebacher F, Brand MD, Else PL, Guderley H, Hulbert AJ, Moyes CD (2010) Plasticity of
572 oxidative metabolism in variable climates: molecular mechanisms. *Physiological and*
573 *Biochemical Zoology*, **83**, 721-732.
574 Seebacher F (2003) Seasonal acclimatisation of muscle metabolic enzymes in a reptile
575 (*Alligator mississippiensis*). *Journal of Experimental Biology*, **206**, 1193-1200.
576 Sagou R, Amanhir R, Taleb H, Vale P, Blaghen M, Loutfi M (2005) Comparative study on
577 differential accumulation of PSP toxins between cockle (*Acanthocardia tuberculatum*) and
578 sweet clam (*Callista chione*). Toxicon **46**, 612-618.
579 Sekiguchi K, Sato S, Kaga S, Ogata T, Kodama M (2001) Accumulation of paralytic shellfish
580 poisoning toxins in bivalves and an ascidian fed on *Alexandrium tamarense* cells. *Fisheries*
581 *science*, **67**, 301-305.
582 Sinclair EL, Thompson MB, Seebacher F (2006) Phenotypic flexibility in the metabolic
583 response of the limpet *Cellana tramoserica* to thermally different microhabitats. *Journal of*
584 *Experimental Marine Biology and Ecology*, **335**, 131-141.
585 Thomas MK, Kremer CT, Klausmeier CA, Litchman E (2012) A global pattern of thermal
586 adaptation in marine phytoplankton. *Science*, **338**, 1085-1088.
587 Thompson P, Baird ME, Ingleton T, Doblin MA (2009) Long-term changes in temperate
588 Australian coastal waters: implications for phytoplankton. *Marine Ecology Progress Series,*
589 **394**, 1-19.
590 Vermeer M, Rahmstorf S (2009) Global sea level linked to global temperature. *Proceedings*
591 *of the National Academy of Sciences of the United States of America*, **106**, 21527-21532.
592 Wheeler B (2014) *Permutation tests for linear models*. February 11 2014. http://cran.r-
593 project.org/src/contrib/Archive/lmPerm/
594 Willmer P, Stone G, Johnston I (2009) *Environmental physiology of animals*. John Wiley &
595 Sons.

596 Wilson SK, Adjeroud M, Bellwood DR et al. (2010) Crucial knowledge gaps in current
597 understanding of climate change impacts on coral reef fishes. *The Journal of Experimental*
598 *Biology*, **213**, 894-900.
599 Wu L, Cai W, Zhang L et al. (2012) Enhanced warming over the global subtropical western
600 boundary currents. *Nature Climate Change,* **2**, 161-166.

Supporting information legends

Fig. S1 Example chromatographs showing the toxin peaks for the extracts from the A. minutum pellet (a) and the corresponding peaks observed in extracts from oyster tissue (b and c).

Fig. S2 Activities (µmol g^{-1} min^{-1}) of lactate, glycine and β-alanine dehydrogenase (LDH; a-b), citrate synthase (CS; c-d), cytochrome c oxidase (COX; e-f) from diploid *C. gigas* digestive gland. Data for oysters exposed to either a toxic or non-toxic diet are shown separately. Each panel shows results from oysters acclimated at current mean summer water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature is shown on the x-axis; n = 9; bars = SEM. There were no significant interaction terms.

Fig. S3 Activities (µmol g^{-1} min^{-1}) of lactate, glycine and β-alanine dehydrogenase (LDH; a-b), citrate synthase (CS; c-d), cytochrome c oxidase (COX; e-f) from diploid *C. gigas* adductor muscle. Data for oysters exposed to either a toxic or non-toxic diet are shown separately. Each panel shows results from oysters acclimated at current mean summer water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature is shown on the x-axis; n = 9; bars = SEM.

Fig. S4 Activities (µmol g^{-1} min^{-1}) of lactate, glycine and β-alanine dehydrogenase (LDH; a-b), citrate synthase (CS; c-d), cytochrome c oxidase (COX; e-f) from *S. glomerata* digestive gland. Data for oysters exposed to either a toxic or non-toxic diet are shown separately. Each panel shows results from oysters acclimated at current mean summer water temperature (22

°C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature is shown on the x-axis; n = 9; bars = SEM. There were no significant interaction terms.

Fig. S5 Activities (µmol g^{-1} min^{-1}) of lactate, glycine and β-alanine dehydrogenase (LDH; a-b), citrate synthase (CS; c-d), cytochrome c oxidase (COX; e-f) from *S. glomerata* adductor muscle. Data for oysters exposed to either a toxic or non-toxic diet are shown separately. Each panel shows results from oysters acclimated at current mean summer water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature is shown on the x-axis; n = 9; bars = SEM.

Fig. S6 Activities (µmol g^{-1} min^{-1}) of lactate, glycine and β-alanine dehydrogenase (LDH; a-b), citrate synthase (CS; c-d), cytochrome c oxidase (COX; e-f) from triploid *C. gigas* digestive gland. Data for oysters exposed to either a toxic or non-toxic diet are shown separately. Each panel shows results from oysters acclimated at current mean summer water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature is shown on the x-axis; n = 9; bars = SEM. There were no significant interaction terms.

Fig. S7 Activities (µmol g^{-1} min^{-1}) of lactate, glycine and β-alanine dehydrogenase (LDH; a-b), citrate synthase (CS; c-d), cytochrome c oxidase (COX; e-f) from triploid *C. gigas* adductor muscle. Data for oysters exposed to either a toxic or non-toxic diet are shown separately. Each panel shows results from oysters acclimated at current mean summer water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature is shown on the x-axis; n = 9; bars = SEM.

650 **Table S1** Summary of oyster species/ploidy level and weight ranges used for the controlled
651 feeding experiment.
652
653 **Table S2** Analysis of clearance rate of *A. minutum* by each oyster species/ploidy level
654 (diploid and triploid *C. gigas* and *S. glomerata*) at each acclimation temperature across the
655 12-day exposure period. This was a two-way permutation ANOVA based on estimates of *A.*
656 *minutum* clearance rate for 24 hours after each tank seawater change (n=5 species/ploidy
657 level^{-1} temp^{-1}) with oyster species/ploidy level and acclimation temperature as factors.

658 ***Tables***

659 **Table 1** Summary of significant (interaction or main effect) responses by warm (27 °C)
660 acclimated oysters (diploid and triploid *C. gigas* and *S. glomerata*) to experimental measures
661 of *A. minutum* clearance rate, paralytic shellfish toxin (PST) concentrations after 12-days of
662 exposure to toxic diet and 24 hour depuration, routine metabolic rate and metabolic enzyme
663 activity (lactate, glycine and β-alanine dehydrogenase (LDH), citrate synthase (CS),
664 cytochrome c oxidase (COX)).

Experiment		Oyster species/ploidy level (27 °C treatment) diploid *C. gigas*	*S. glomerata*	triploid *C. gigas*	Ref.
Clearance rate of toxic *A. mintutum*		-	-	-	Fig. 2, Table S2
PSTs after 12-day exposure					
GTX1,4		↓	↓	-	Table 2,
GTX2,3		-	-	-	Fig. 3
PSTs after 24hr depuration					
GTX1,4		↓	↓	-	Table 2,
GTX2,3		-	-	↓	Fig. 3
Routine metabolic rate (irrespective of diet)		-	↑	-	Table 3 Fig. 4,
Metabolic enzymes					
Digestive gland	LDH	↑	-	↓	
	CS	-	↓	↓	Table 4, Figs S2, S4, S6
	COX	-	-	-	
Muscle	LDH	-	↑	↑	
	CS	-	-	↓	Table 5, Figs S3, S5, S7
	COX	-	-	↓	

Key: ↑ increased response; ↓ reduced response; - no significant response

665

666

667 **Table 2** Summary of two-way permutation ANOVA for accumulation of paralytic shellfish
668 toxins (GTX1,4 and GTX2,3) in each oyster species/ploidy level, with exposure treatment
669 (maximum exposure to *A. minutum*, and 24 hours of depuration) and acclimation temperature
670 as factors.

			diploid *C. gigas*		**triploid *C. gigas***		***S. glomerata***	
PST analogue	**Source of Variation**	**df**	**F**	***p***	**F**	***p***	**F**	***p***
GTX 1,4	Exposure	1	63.99	<0.001	1.93	0.318	21.48	<0.001
	Temperature	1	15.03	<0.002	0.00	0.902	9.02	0.002
	Exposure*Temperature	1	5.96	0.014	0.00	1.000	4.19	0.035
	Error	32						
GTX 2,3	Exposure	1	9.94	0.004	0.75	0.400	10.22	0.000
	Temperature	1	0.02	0.922	12.30	0.001	0.88	0.390
	Exposure*Temperature	1	0.39	0.554	5.88	0.016	1.79	0.121
	Error	32						

671
672

673 **Table 3** Summary of two-way permutation ANOVA for routine metabolic rate, RMR (mg 0_2
674 g^{-1} DTM h^{-1} ± SEM), for each oyster species/ploidy level on day 12, the maximum period of
675 exposure to A. minutum. Acclimation temperature and diet (toxic or non-toxic) were factors
676 for each analysis.

	diploid *C. gigas*			**triploid *C. gigas***			***S. glomerata***		
Source of Variation	**df**	**F**	***p***	**df**	**F**	***p***	**df**	**F**	***p***
Diet	1	1.49	0.115	1	0.01	0.922	1	0.15	0.804
Temperature	1	1.58	0.322	1	0.03	0.863	1	4.78	0.024
Diet*Temperature	1	1.96	0.171	1	0.66	0.548	1	1.46	0.413
Error	31			32			31		

677

678 **Table 4** Summary of three-way permutation ANOVA for lactate, glycine and β-alanine
679 dehydrogenase (LDH), citrate synthase (CS), cytochrome c oxidase (COX) from diploid and
680 triploid *C. gigas* and *S. glomerata* digestive gland. Acclimation temperature (Acc), diet (toxic
681 vs. non-toxic) and test temperature (Test; repeated measure) were factors for analysis.

			diploid *C. gigas*		*S. glomerata*		triploid *C. gigas*	
Assay	**Source of Variation**	**df**	**F**	***p***	**F**	***p***	**F**	***p***
	Between subject effects							
	Acc	1	5.58	0.012	1.25	0.200	3.19	0.030
	Diet	1	0.03	0.980	0.03	0.902	0.15	0.745
	Acc *Diet	1	0.04	0.784	0.12	0.726	0.60	0.391
	Error	32						
LDH	*Within subject effects*							
	Test	1	6.22	0.161	1.27	0.222	28.96	0.040
	Acc*Test	1	1.82	0.162	0.06	0.667	0.20	0.961
	Diet*Test	1	0.84	0.473	0.05	0.824	0.00	0.863
	Acc*Diet*Test	1	0.05	0.961	0.82	0.273	0.30	0.527
	Error	32						
	Between subject effects							
	Acc	1	0.12	0.941	7.36	0.002	3.74	0.050
	Diet	1	1.06	0.267	0.36	0.452	1.40	0.236
	Acc *Diet	1	1.48	0.216	0.36	0.824	0.12	0.980
	Error	32						
CS	*Within subject effects*							
	Test	1	55.10	<0.001	132.88	<0.001	208.70	<0.001
	Acc*Test	1	0.09	0.922	0.08	0.824	0.16	0.745
	Diet*Test	1	2.55	0.765	0.19	0.594	0.80	0.686
	Acc*Diet*Test	1	0.01	0.922	0.09	1.000	0.32	0.882
	Error	32						
	Between subject effects							
	Acc	1	1.13	0.220	1.40	0.414	2.42	0.191
	Diet	1	0.03	0.686	2.01	0.201	3.38	0.058
	Acc *Diet	1	0.55	0.385	0.47	0.478	1.39	0.667
	Error	32						
COX	*Within subject effects*							
	Test	1	10.01	0.452	10.34	0.065	0.60	0.070
	Acc*Test	1	0.00	0.686	1.58	0.324	0.06	0.643
	Diet*Test	1	0.01	1.000	3.64	0.065	0.23	0.444
	Acc*Diet*Test	1	0.20	0.863	0.78	0.706	0.00	1.000
	Error	32						

682 **Table 5** Summary of three-way permutation ANOVA for lactate, glycine and β-alanine
683 dehydrogenase (LDH), citrate synthase (CS), cytochrome c oxidase (COX) from diploid and
684 triploid *C. gigas* and *S. glomerata* adductor muscle. Acclimation temperature (Acc), diet
685 (toxic vs. non-toxic) and test temperature (Test; repeated measure) were factors for analysis.

			diploid *C. gigas*		*S. glomerata*		triploid *C. gigas*	
Assay	**Source of Variation**	**df**	**F**	***p***	**F**	***p***	**F**	***p***
	Between subject effects							
	Acc	1	0.02	0.941	15.32	<0.001	4.74	0.038
	Diet	1	7.81	0.014	0.27	0.500	1.09	0.236
	Acc *Diet	1	0.41	0.373	0.27	0.592	0.53	0.638
	Error	32						
LDH	*Within subject effects*							
	Test	1	25.68	0.007	145.06	<0.001	89.96	<0.001
	Acc*Test	1	0.56	0.686	7.55	0.006	5.85	0.022
	Diet*Test	1	1.35	0.193	0.38	0.444	0.05	0.922
	Acc*Diet*Test	1	0.82	0.660	4.58	0.044	3.48	0.059
	Error	32						
	Between subject effects							
	Acc	1	1.13	0.295	0.44	0.368	19.19	<0.001
	Diet	1	0.01	1.000	0.97	0.258	0.47	0.411
	Acc *Diet	1	0.23	0.686	2.55	0.165	0.70	0.667
	Error	32						
CS	*Within subject effects*							
	Test	1	22.88	0.061	69.99	0.000	164.47	<0.001
	Acc*Test	1	2.54	0.082	1.15	0.396	0.32	0.633
	Diet*Test	1	0.64	0.583	0.21	0.660	1.11	0.304
	Acc*Diet*Test	1	3.67	0.069	0.01	1.000	0.12	1.000
	Error	32						
	Between subject effects							
	Acc	1	0.06	0.784	0.88	0.394	3.22	0.042
	Diet	1	0.17	0.554	0.24	0.583	0.65	0.288
	Acc *Diet	1	0.97	0.346	0.88	0.371	0.32	0.554
	Error	32						
COX	*Within subject effects*							
	Test	1	2.26	0.571	65.36	0.006	8.51	0.615
	Acc*Test	1	0.57	0.765	1.22	0.321	3.35	0.170
	Diet*Test	1	1.94	0.147	0.10	0.726	0.85	0.302
	Acc*Diet*Test	1	1.29	0.396	0.10	0.980	1.52	0.231
	Error	32						

686 ***Figure Legends***

687 **Fig. 1** (a) Estimated annual mean surface temperature change between 1986–2005 and
688 2081–2100 for two climatic model scenarios (RCP2.6: low forcing and RCP8.5: high
689 emission levels) (adapted from IPCC, 2013). (b) Total number of months each year where
690 positive *Alexandrium*-related PSTs were reported along the NSW coastline between February
691 2005 and December 2014 (NSW Food Authority, 2014). (c) Global distribution of production
692 of *C. gigas* (FAO, 2014) and *S. glomerata* (NSW DPI, 2014) overlain on reported incidence
693 of PSTs worldwide 1970 (Hallegraeff, 2003) and current (Hallegraeff, 2014).

694

695 **Fig. 2** Average daily clearance rate (mL min^{-1} g^{-1} wet tissue) of *A. minutum* for each oyster
696 type and temperature treatment across the feeding trial. Results are shown from oysters
697 acclimated at current mean summer water temperature (22 °C; white bars) and predicted
698 warmer conditions (27 °C; black bars), and oyster species/ploidy level is shown on the x-axis;
699 n = 5; bars = SEM.

700

701 **Fig. 3** Paralytic shellfish toxin content in oysters after the maximum period of exposure (day
702 12) to *A. minutum* and 24 hours after feeding with toxic cells had ceased (day 13).
703 Concentrations (µg 100g $tissue^{-1}$) of the analogues GTX1,4 (a, c, e) and GTX2,3 (b, d, f) are
704 shown for each oyster species/ploidy level: diploid *C. gigas* (a, b), triploid *S. glomerata* (c, d),
705 *C. gigas* (e, f). Each panel shows results from oysters acclimated at current mean summer
706 water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars),
707 and *A. minutum* exposure treatment is shown on the x-axis; n = 9; bars = SEM. For GTX1,4,
708 there were significant interactions between exposure treatment and acclimation temperature
709 for diploid *C. gigas* (a) and S. glomerata (c). For GTX2,3, there was a significant interaction
710 between exposure treatment and acclimation temperature for triploid *C. gigas* (f).

Fig. 4 Routine metabolic rate, RMR, (mg 0_2 g^{-1} DTM h^{-1}± SEM) on day 12, the maximum period of exposure in oysters that received *A. minutum* (a, c, e) and those that were fed a non-toxic diet only (b, d, f) are shown for each oyster species/ploidy level: diploid *C. gigas* (a, b), triploid *C. gigas* (c, d), *S. glomerata* (e, f). Each panel shows results from oysters acclimated at current mean summer water temperature (22 °C; white bars) and predicted warmer conditions (27 °C; black bars), and test temperature, which corresponded to the acclimation temperatures, is shown on the x-axis; n = 9, with the exception of non-toxic diploid *C. gigas* and toxic *S. glomerata* at 27 °C, where n=8 (see text); bars = SEM. There was a significant main effect of temperature for *S. glomerata* (e, f).

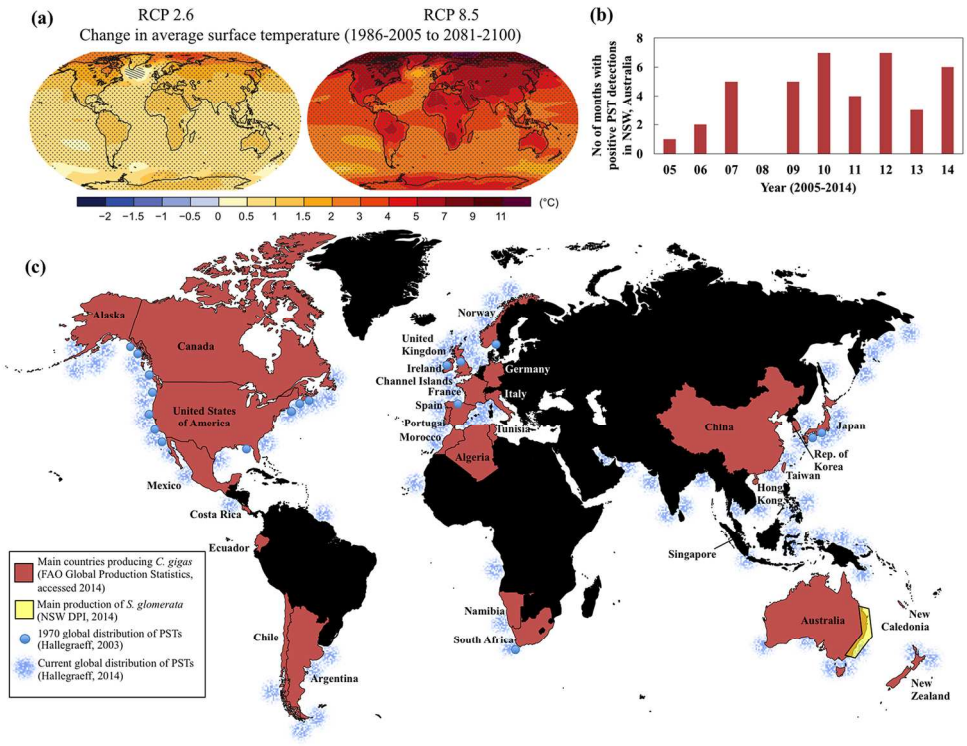
Supporting Information

Table S1 Summary of oyster species/ploidy level and weight ranges used for the controlled feeding experiment.

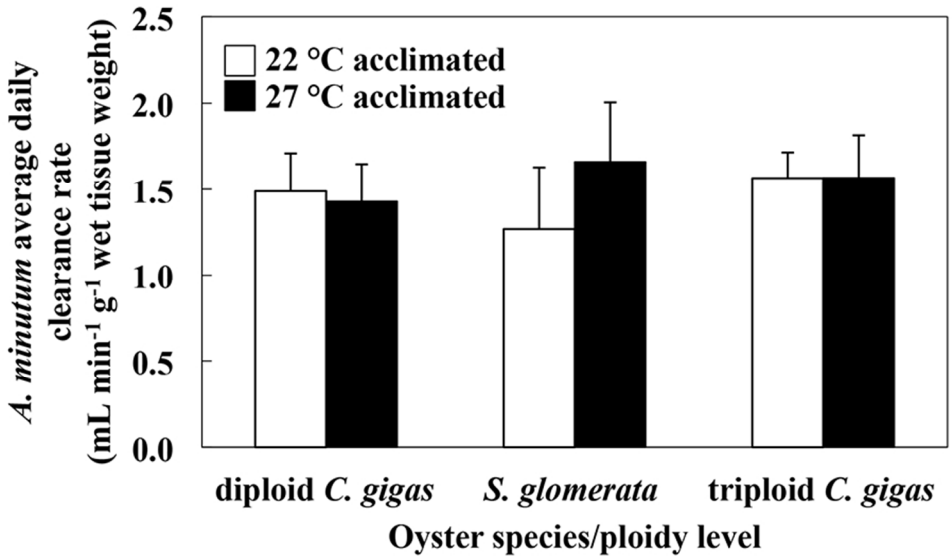
Oyster species/ploidy level (n=216)	Total Weight (g) (mean ± SD)	Total Tissue (g) (mean ± SD)
Diploid *C. gigas*	33.29±7.52	4.89±1.47
Triploid *C. gigas*	66.59±13.34	10.01±2.79
S. glomerata	29.49±4.95	4.64±1.02

Table S2 Analysis of clearance rate of *A. minutum* by each oyster species/ploidy level (diploid and triploid *C. gigas* and *S. glomerata*) at each acclimation temperature across the 12-day exposure period. This was a two-way permutation ANOVA based on estimates of *A. minutum* clearance rate for 24 hours after each tank seawater change (n=5 species/ploidy level^{-1} temp^{-1}) with oyster species/ploidy level and acclimation temperature as factors.

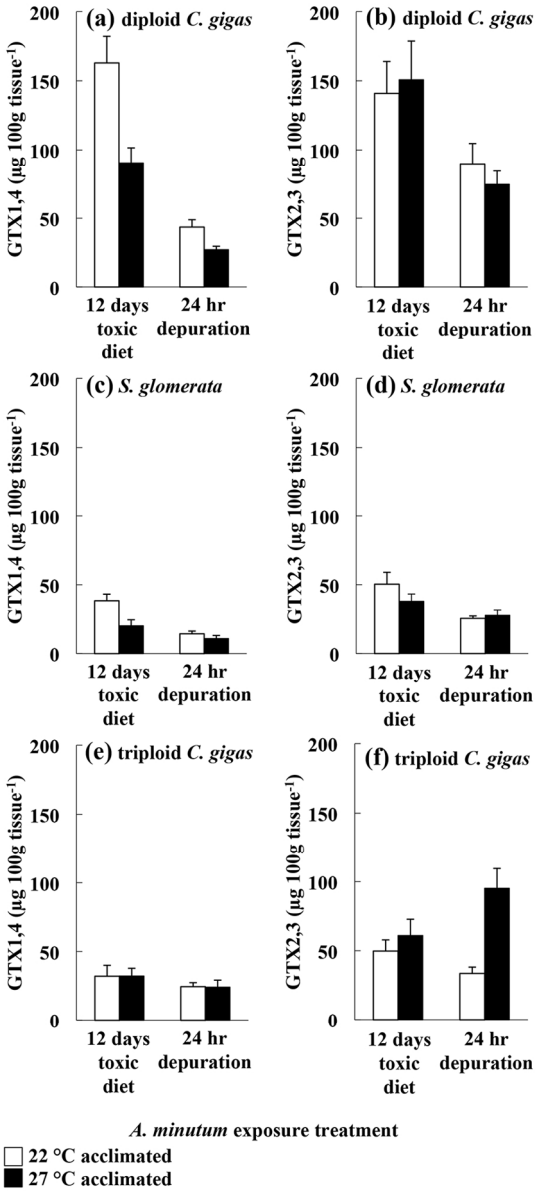
Source of Variation	**df**	**F**	***p***
Temperature	1	0.26	0.478
Oyster	2	0.10	0.961
Oyster*Temperature	2	0.42	0.737
Error	24		



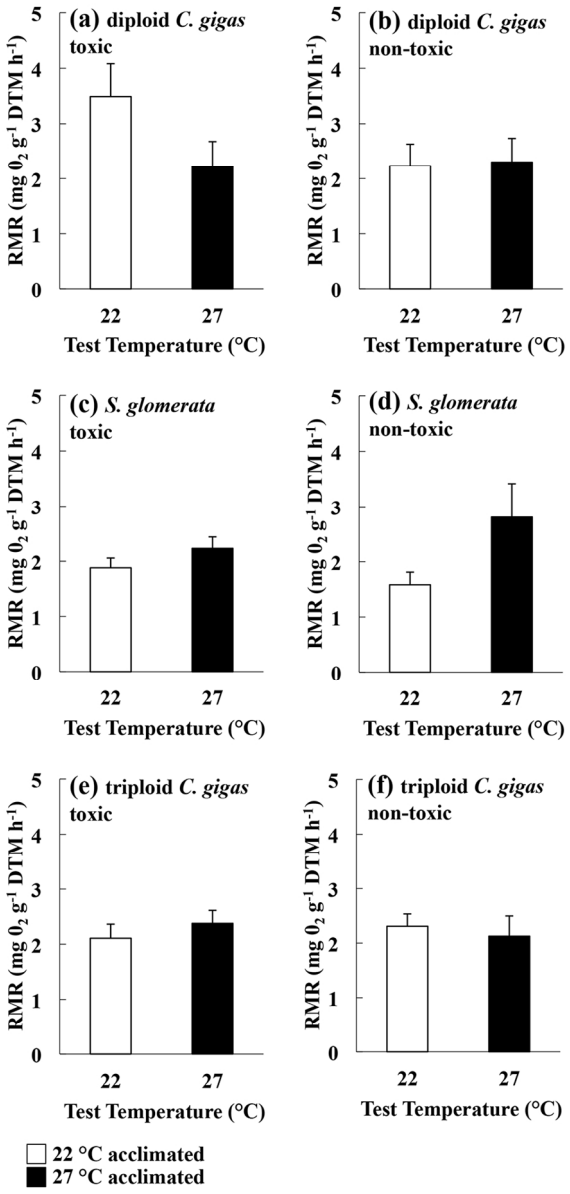
168x126mm (300 x 300 DPI)



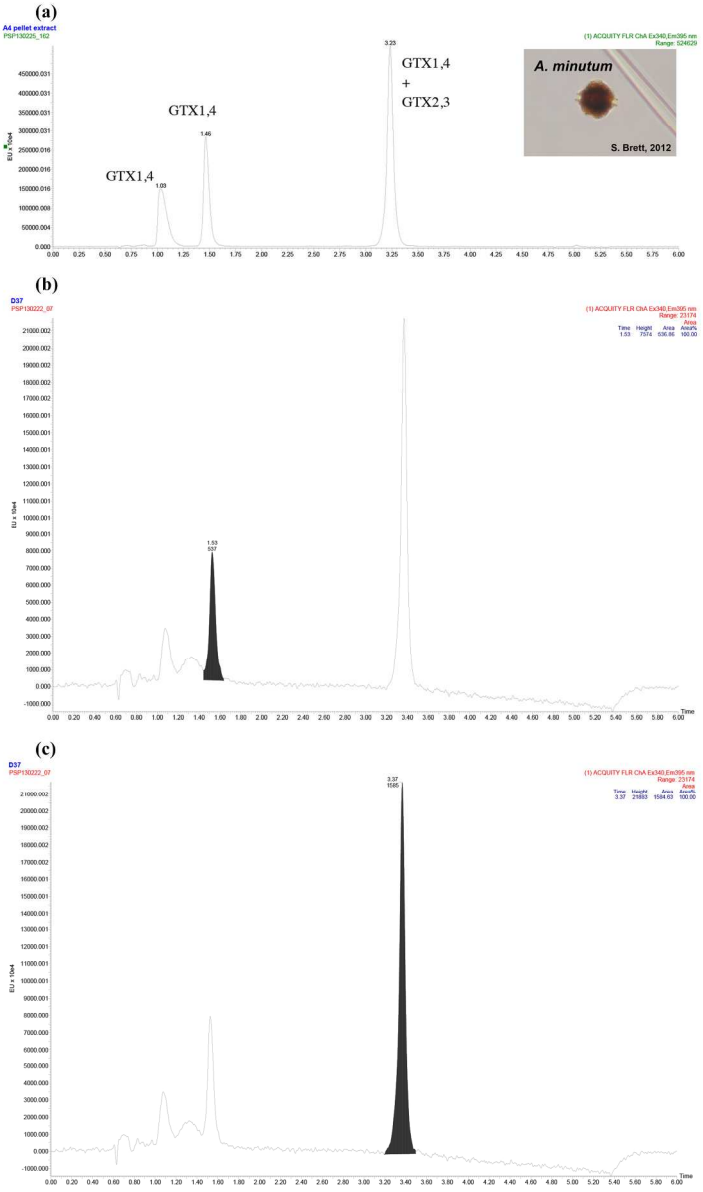
80x46mm (300 x 300 DPI)



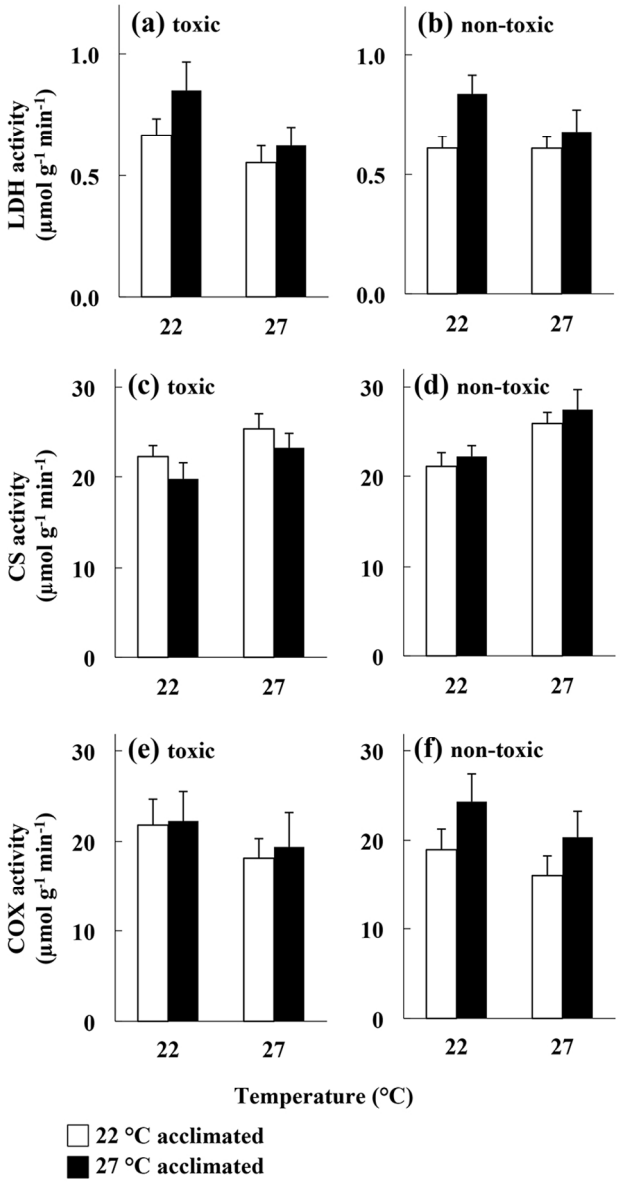
80x171mm (300 x 300 DPI)



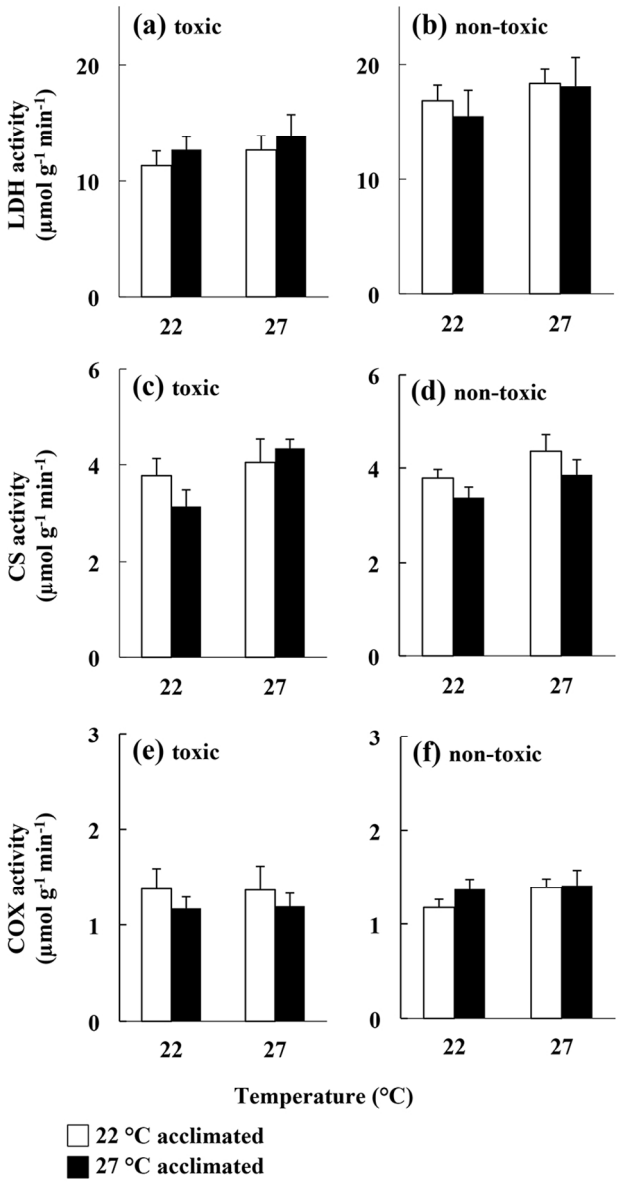
80x164mm (300 x 300 DPI)



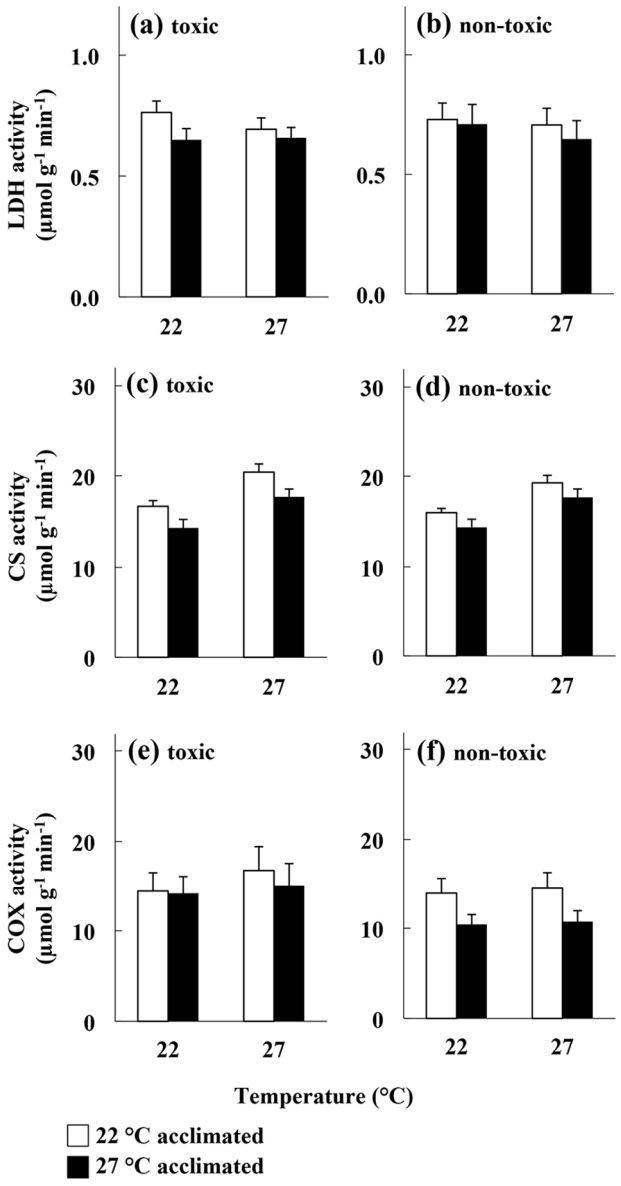
168x270mm (300 x 300 DPI)



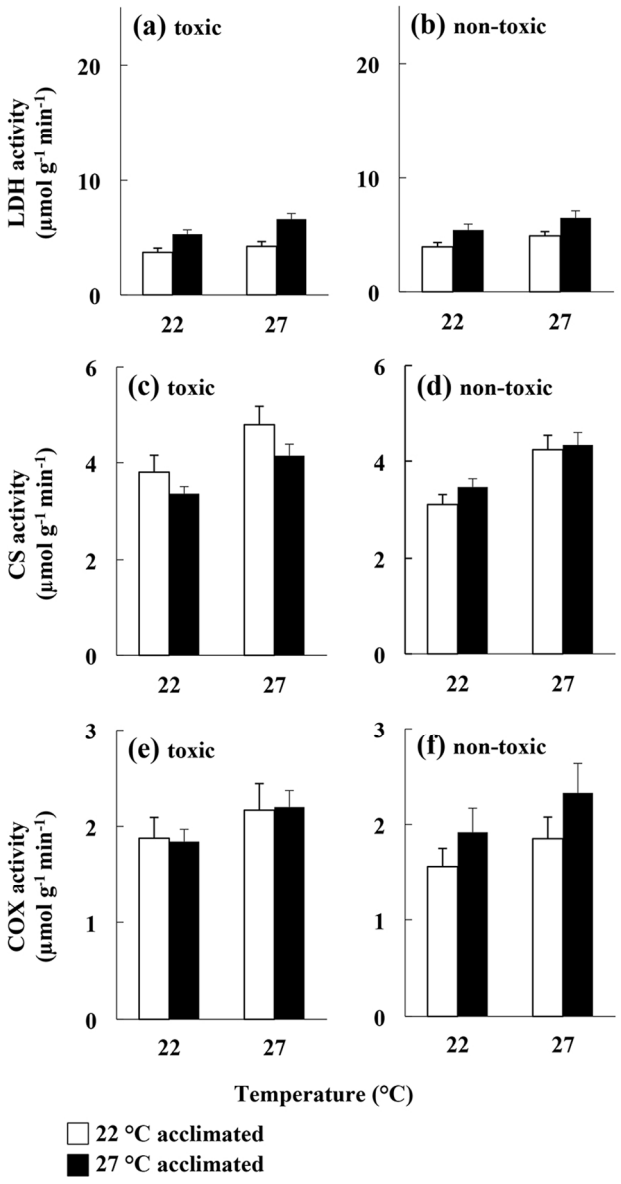
80x144mm (300 x 300 DPI)



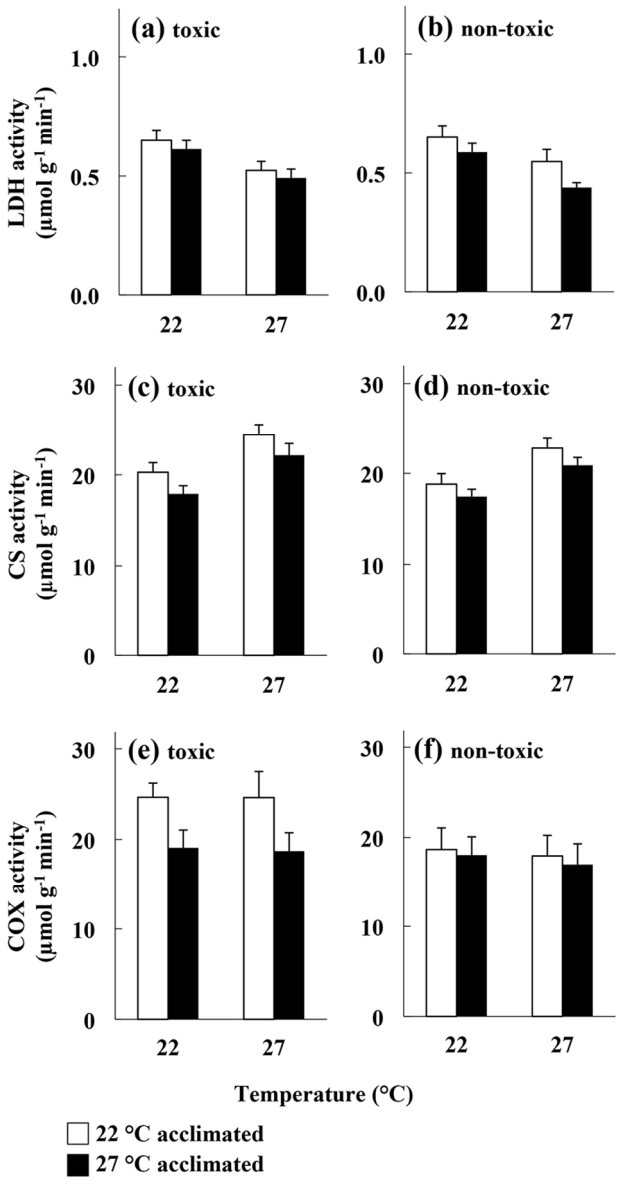
80x144mm (300 x 300 DPI)



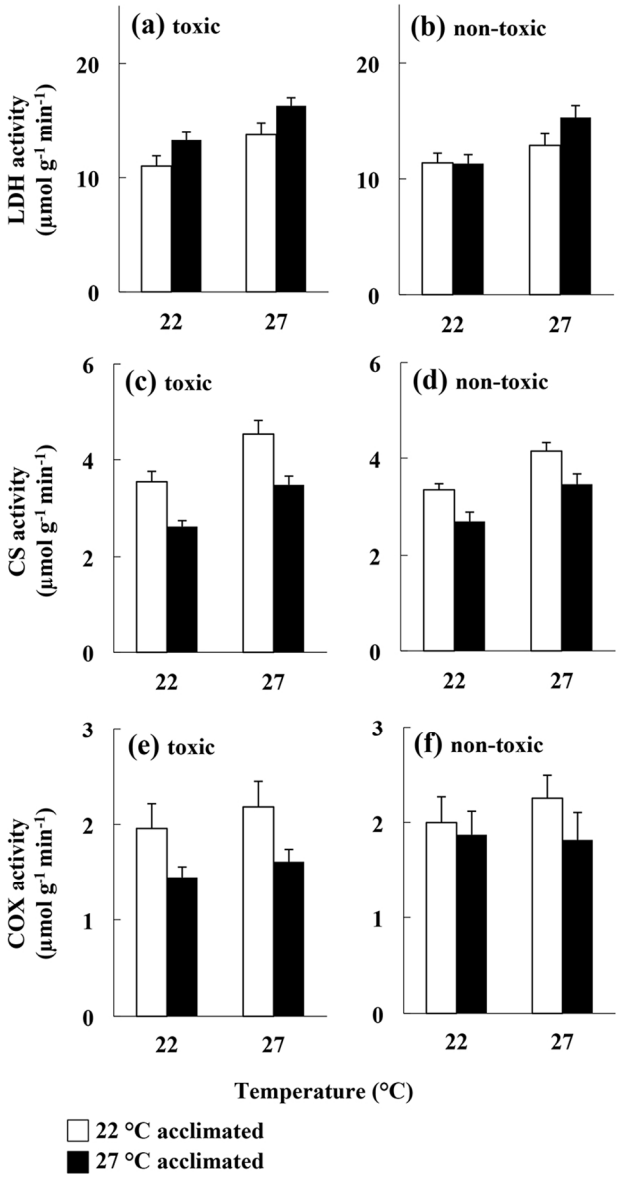
80x144mm (300 x 300 DPI)



80x144mm (300 x 300 DPI)



80x144mm (300 x 300 DPI)



80x144mm (300 x 300 DPI)