2 Expression of common biomarkers in Antarctic krill (*Euphausia* 3 *superba*) exposed to an organochlorine contaminant

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7 **Abstract** Persistent organic pollutant (POP) contamination
8 of Polar Regions continues to present a major ecological
9 challenge and an environmental stressor to local biota.
10 Antarctic krill (*Euphausia superba*) are a keystone species
11 of the Antarctic sea ice ecosystem. Krill have repeatedly
12 been found to accumulate a diverse array of POPs and
13 thereby serve as vectors of these to the remainder of the
14 Antarctic food web. *p,p'*-Dichlorodiphenyldichloroethylene
15 (*p,p'*-DDE) is a dominant POP compound accumulating in
16 Antarctic krill and higher trophic level predators. Recently,
17 *p,p'*-DDE uptake dynamics, associated behavioural and
18 developmental toxicity were evaluated in this species. The
19 present study investigated the response of enzymes with
20 known roles in detoxification (glutathione *S*-transferase,
21 GST and cytochrome P450 2B, CYP2B), neurotoxicity
22 (acetylcholinesterase, AChE) and oxidative stress (glu-
23 tathione peroxidase GPx) in Antarctic krill exposed to *p,p'*-
24 DDE. CYP2B was not detectable in Antarctic krill. No
25 strong concentration responses resulted from the exposure
26 to *p,p'*-DDE. These findings do not provide evidence for an
27 activated detoxification response to this compound via the
28 tested biochemical pathways in Antarctic krill. This is the
29 first time that GST, AChE and GPx have been characterised

in this species following pollutant exposure. Further
research with additional pollutants and compound mixtures
is necessary to assess the practical role of these enzymes as
biomarkers of pollutant exposure in Antarctic krill. These
first exploratory findings present a valuable contribution to
a critical knowledge gap in polar ecotoxicology, namely the
comparative sensitivity of polar organisms relative to tem-
perate and tropical counterparts.

Keywords Crustacean · Antarctic krill · Antioxidant
enzymes · Biomarker · Detoxification · Persistent organic
pollutant

Introduction

Despite the apparent pristine nature of Antarctica and its
remoteness from industry, persistent organic pollutants
(POPs) have been detected in Antarctic biota since the
1960s (George and Frear 1966; Sladen et al. 1966). The
presence of POPs so far from their sources can largely be
attributed to long range environmental transport pathways
and more recently in situ human activities (Wild et al.
2015). Once deposited at high latitudes, the volatility of
POPs is markedly reduced (Wania and MacKay 1996),
with the Earth's poles proposed as major "environmental
sinks" for most of the world's remaining POPs.

Antarctic krill, *Euphausia superba*, a swarming Euphausiid crustacean, are a commercially valuable Southern Ocean keystone species. Krill are a sympagic species that grazes on phytoplankton, with their abundance and distribution closely linked to the Antarctic sea ice extent (Flores et al. 2012). Despite being one of the most abundant species on the planet, there are concerns for the long-term survival of krill stocks in the face of climate change and over-fishing. Several studies

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have documented decreased krill abundance and density linked with reduced sea ice extent, whilst ocean acidification is projected to have a dramatic effect on recruitment and species survival if carbon emissions are left unmitigated (Kawaguchi et al. 2011). The pivotal ecological role of krill means that a biomass decline would have far-reaching repercussions for the reliant ecosystem (Atkinson et al. 2004; Nicol et al. 2008).

Diverse profiles and notable concentrations of POPs, including organochlorine compounds, have repeatedly been detected in Antarctic krill (Corsolini et al. 2002a, b, 2006; Bengtson Nash et al. 2008). *p,p'*-Dichlorodiphenyldichloroethylene (*p,p'*-DDE) is one of the dominant compounds accumulating in Antarctic krill and higher trophic level Antarctic predators (Bengtson Nash et al. 2008, 2013; Waugh et al. 2014). *p,p'*-DDE occurs in the environment as the most stable metabolite of the organochlorine compound dichlorodiphenyltrichloroethane, which is more commonly known as the pesticide DDT. Despite the basal position of krill in the Antarctic food web, investigations of the toxicological sensitivity of the species remain limited. Recently, the development of a series of krill-based toxicological exposure assays permitted the first evaluation of *p,p'*-DDE toxicity in both larval and adult krill stages (Poulsen et al. 2011, 2012b, 2013). Antarctic krill exposed to *p,p'*-DDE exhibited behavioural responses and altered larval development (Poulsen et al. 2011, 2012a, b, 2013). Nothing is currently known about the underlying biochemical mechanisms of *p,p'*-DDE toxicity in Antarctic krill and their capacity for detoxification. It has often been proposed that the evolutionary isolation and underdeveloped detoxification systems of Antarctic species leave them vulnerable to the toxicity of anthropogenic contaminants (Chapman and Riddle 2005; Corsolini 2009). Further, polar organisms are characterised by gigantism and slow metabolism, making conventional toxicity evaluation time frames unsuitable and limiting comparison to temperate or tropical counterparts (King and Riddle 2001).

In crustaceans, the metabolism of foreign contaminants, or xenobiotics, predominantly occurs in the digestive gland and it is thought to proceed in a similar manner as in mammals. Detoxification is often described in three functional stages. Phase I, where xenobiotics are identified and transformed into more soluble metabolites, is primarily governed by a superfamily of specialised enzymes (cytochrome P450, CYPs). Phase II detoxification is performed by a variety of non-specific detoxification enzymes with the role of further increasing metabolite solubility. Finally Phase III metabolism ensures effective excretion of by-products (Livingstone 1991; Rewitz et al. 2006).

Glutathione *S*-transferases (GST) are a family of generalist Phase II detoxifying enzymes. Up-regulation of GST activity has previously been observed in association with

insecticide resistance (Enayati et al. 2005) and exposure to POPs (Hoarau et al. 2001; Gaume et al. 2014). Throughout the detoxification process, as well as through general metabolism, reactive oxygen species are produced which carry the potential to cause oxidative stress. Oxidative stress can induce up-regulation of antioxidant enzymes, for example glutathione peroxidase (GPx), which are responsible for neutralising reactive oxygen species to prevent associated harmful effects such as lipid peroxidation and DNA damage (Livingstone 1991).

DDT is a neurotoxin which affects crustacean sodium channels, subsequently leading to hyperactivity and death (Sánchez-Bayo 2012). A typically used marker for neurotoxicity is acetylcholinesterase (AChE) activity. The inhibition of AChE impairs the transmission of signals between neurons, and is commonly known to be specific to organophosphate and carbamate pesticides (Hassall 1990). However previous research has reported on AChE inhibition in response to organochlorine compounds, including DDT (Martínez-Tabche et al. 1999; Galindo-Reyes et al. 2000; Bhavan and Geraldine 2001). AChE inhibition may thus provide an easily measurable marker for exposure to DDT and its metabolites in krill.

Enzymes have been widely used as biomarkers of chemical exposure, with CYPs, GST, GPx and AChE some of the most frequently used. Biochemical screening is often more cost-effective than chemical analysis, permitting broad scale application. Further, molecular change signals a biological response to chemical exposure and serves as an early warning of possible toxic effects (Bengtson Nash et al. 2006). Successful implementation of the biomarker approach requires that a direct relationship is established between chemical exposure and expression of the target biomarker (Jemec et al. 2010). Nothing is currently known about the response of sub-cellular biomarkers to chemical exposure in Antarctic krill. The purpose of this exploratory study is to investigate the response of four commonly used biomarkers GST, CYP2B, GPx and AChE to sublethal *p,p'*-DDE exposure in Antarctic krill. The suitability of these enzymes as potential biomarkers of sub-cellular effects in Antarctic krill is discussed, thereby contributing new knowledge to the critical research gap surrounding the detoxification capabilities of endemic Polar species.

Methods

Exposure design

The experimental design used for exposure of Antarctic krill to *p,p'*-DDE is further described in (Poulsen et al. 2012a). Adult Antarctic krill were collected in the austral summer of 2006 from the eastern Antarctic sector (66°S

80°E). Animals were housed at the Australian Antarctic Division krill culturing facilities, Tasmania, Australia until *p,p'*-DDE exposure in 2007. The weight of exposed krill ranged from 364 to 927 mg wet weight. Prior to exposure krill were acclimatised for 24 h under experimental conditions without food. Five krill were housed in 5-L glass beakers. Exposure seawater was collected from Bruny Island, Tasmania, and pre-filtered to 0.2 µm. Exposure solutions were spiked with *p,p'*-DDE using acetone as a solvent carrier (in a final concentration of 0.03 mL L⁻¹). Antarctic krill were exposed for 96 h using five *p,p'*-DDE exposure treatments (1, 5, 10, 15, and 20 µg L⁻¹), plus an additional seawater-only and a seawater-acetone (0.03 mL L⁻¹) control. Exposure media was renewed every 24 h. The setup included three replicate 5-L test beakers for each treatment and control to ensure statistical power. Krill were not fed for the duration of the experiment. Upon the end of the experiment all animals were euthanised by placing in liquid nitrogen and samples were stored at -80 °C until the time of analysis. The experiment was duplicated within 1 month, with krill from the first experiment used for GST, GPx and AChE analysis and krill from the second experiment used for CYP2B analysis.

Internal concentrations

The actual concentrations of seawater and those accumulated by krill were not measured in the present experiment. We confirmed in a supporting experiment conducted under identical conditions that actual seawater concentrations were in accordance with nominal concentrations (Poulsen et al. 2012a). We also confirmed in the supporting experiment that krill uptake of *p,p'*-DDE took place throughout 96 h of exposure by measuring the concentrations in krill bodies (Poulsen et al. 2012a). The uptake was concentration dependent and linear over 96 h. The method used to measure *p,p'*-DDE in krill bodies is destructive and would have rendered enzyme analysis impossible. The concentrations of *p,p'*-DDE in exposure media and internal body residues at 96 h as measured in the supporting experiment are given in Table 1. As the concentrations were not measured in the present experiment, the exposure concentrations will continue to be referred to by their nominal concentrations from here on (1, 5, 10, 15 and 20 µg L⁻¹).

Sample preparation of sub-cellular fractions

Cytosolic fraction

Crustacean digestive glands are highly autolytic. In order to minimise the chances of enzyme degradation during

Table 1 Concentration of *p,p'*-DDE in exposure seawater media and the accumulated internal body burden of Antarctic krill (*Euphausia superba*) measured in a supporting experiment

Nominal exposure concentration µg L ⁻¹	nM	Exposure media (2 h) ^a		Exposure media (24 h) ^a		Internal body residue (µmol kg ⁻¹ w.w.) ^b		Internal body residue (mmol kg ⁻¹ l.w.) ^b	
		µg L ⁻¹	nM	µg L ⁻¹	nM	A	B	A	B
1	3.1	1.1 (0.083)	3.5 (0.26)	1.1 (0.51)	3.4 (1.6)	8.6 (0.99)	13.6 (0.51)	0.28 (0.03)	0.66 (0.02)
5	15.7	4.4 (0.80)	13.9(2.5)	3.9 (0.69)	12.2 (2.2)	31.0 (7.7)	64.5 (2.2)	1.0 (0.25)	3.2 (0.11)
10	31.4	10.7 (1.5)	33.8 (4.7)	12.9 (5.7)	40.6 (17.9)	58.8 (8.8)	117 (3.5)	1.9 (0.28)	5.7 (0.17)
15	47.2	15.1 (1.5)	47.6 (4.7)	16.8 (3.6)	52.7 (11.3)	90.7 (8.3)	157 (8.4)	2.9 (0.27)	7.7 (0.41)
20	62.9	22.0 (5.4)	69.0 (17.0)	42.7 (21)	135 (66.0)	110 (7.2)	169 (24)	3.5 (0.23)	8.3 (1.2)
								Average ^c	Average ^c
								11.1 (3.5)	47.8 (24)
								87.7 (41)	5.7 (0.17)
								124 (47)	7.7 (0.41)
								140 (42)	8.3 (1.2)
									5.9 (3.4)

The supporting exposure experiment was carried out under identical conditions and in duplicate (test A and B). Body residues are reported in wet weight (w.w) and lipid weight (l.w) (reproduced with some modification from Poulsen et al. 2012a)

^aAverage (± SD) seawater concentration measured in test A (n = 4 days, three replicates each)

^bAverage (± SD) krill body residues (n = 3, five individual measurements each)

^cAverage (± SD) between tests A and B

sample preparation, whole body extracts were used for enzyme analysis. Two krill from each replicate of the first experiment were pooled for GST and GPx biochemical assays. The krill were decapitated; heads were kept for AChE analysis. The bodies were homogenised in approximately 10 mL phosphate buffer (100 mM potassium phosphate pH 7.4, 0.1 mM phenylmethylsulfonyl fluoride, 100 mM potassium chloride and 1 mM ethylenediaminetetraacetic acid, modified from Koenig et al. (2013)) with a hand-held electric homogeniser. The homogenate was centrifuged at $12,000\times g$ for 90 min at 4 °C, and the supernatant was re-centrifuged at $100,000\times g$ for 90 min at 4 °C. The resulting supernatant (cytosol) was kept on ice until use.

Post-mitochondrial fraction

Krill heads were pooled to determine AChE activity (approximately 0.24 g wet weight). Homogenisation was carried out according to Minutoli et al. (2002). Samples were homogenised in a 0.1 M Tris-HCl pH 8, 0.1% triton buffer with 1 mL for every 0.06 g tissue. The homogenate was centrifuged for $12,000\times g$ for 10 min at 4 °C and the resulting supernatant (post-mitochondrial fraction) was stored on ice until needed.

Microsomal fraction

Five krill from each replicate of the second experiment were pooled for CYP2B activity. Microsomes were prepared as per the cytosolic fraction, and the resulting pellet (microsomes) from the $100,000\times g$ centrifuge was kept on ice until use.

Biomarker assays

Biomarker assays were carried out in triplicate with positive and negative controls using a BioRad microplate spectrophotometer or a PolarStar Optima microplate reader. GST activity was quantified by measuring the reaction of 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM reduced glutathione (Habig et al. 1974) with 150 μ L of sample. The absorbance at 340 nm (25 °C) was recorded for 5 min. GPx was determined using a commercial assay kit (Cayman Chemical). The decrease in absorbance was measured at 340 nm (25 °C) for 5 min. AChE activity was determined by measuring the reaction of 0.425 mM 5,5'-dithiobis(2-nitrobenzoic acid), 1 mM acetylthiocholine iodide and 10 μ L of sample at 405 nm (25 °C) for 10 min (Ellman and Courtney 1961). Blank activity was measured in the absence of post-mitochondrial fraction. CYP2B activity was determined using the pentoxyresorufin *O*-dealkylase assay. The increase in the fluorescence of 0.25 mM of NADPH, 6.2 μ M pentoxyresorufin and 50 μ L of sample was measured at 30 °C for

10 min (λ excitation = 537 nm and λ emission = 583 nm). The reaction was calculated with a resorufin sodium salt standard curve with seven concentrations (0–80 nM) (Koenig et al. 2013). Protein content was determined using bovine serum albumen as a standard, ranging from 0 to 1000 mg mL⁻¹. The reaction was measured at 562 nm.

Statistical analysis

All measurements are reported as mean $\pm$ standard error (SE) unless otherwise stated. GST and GPx activity is expressed as nmol min⁻¹ mg protein⁻¹; AChE activity is expressed in pmol min⁻¹ mg protein⁻¹. Homogeneity of variance was assessed using a Levene Test and data were checked for normality using a Shapiro–Wilk test. AChE activity was analysed using a Kruskal–Wallis test. GST activity was analysed using Welch's ANOVA with Dunnett's post hoc and GPx responses before exposure concentrations were analysed using a one-way ANOVA with a Tukey post hoc test. Pearson's or Spearman's correlation was used to evaluate linear relationships between concentrations and enzyme responses. All statistical analyses were performed in SPSS, Inc., version 20.0 with *p* values of < 0.05 considered to be statistically significant. The acetone solvent control did not differ significantly from the seawater control; therefore both controls were pooled for the analysis (student's *t* test, GST *t*16 = -0.26, *p* = 0.800, GPx *t*16 = -1.18, *p* = 0.280, AChE *t*9.5 = -1.4, *p* = 0.193).

Results

There was no mortality of any krill throughout the exposure period. Of the enzymes measured, none produced a *p,p'*-DDE concentration-dependent response at the range tested in Antarctic krill (*r* = 0.190, *n* = 63, *p* = 0.135; *r* = 0.246, *n* = 63, *p* = 0.052; *rs* = -0.02, *n* = 57, *p* = 0.887 for GST, GPx and AChE, respectively). CYP2B was not detectable in these specimens. Glutathione *S*-transferase activity was elevated at all *p,p'*-DDE exposure concentrations compared to the control (47.5 ± 2.67) (Fig. 1); however, no treatment induced a significant increase in GST activity in Antarctic krill ($F(5,22.33) = 1.92$, *p* = 0.130). Interestingly the 1 and 20 μ g L⁻¹ concentrations expressed higher GST activity than the 5, 10 and 15 μ g L⁻¹. Treatment activity ranged from 48.71 ± 2.34 for exposure to 15 μ g L⁻¹ to 63.24 ± 5.93 for 20 μ g L⁻¹. Acetylcholinesterase activity was not correlated with *p,p'*-DDE exposure concentration. All exposure concentrations displayed lower activity than the control; however, no treatment was significantly inhibited by *p,p'*-DDE in Antarctic krill ($H(5) = 5.03$, *p* = 0.412, range 0.22 ± 0.01 to 0.25 ± 0.02). Glutathione peroxidase activity ranged from 14.8 ± 1.24 to

Fig. 1 Activity (mean $\pm$ S.E) of the three enzymes, Glutathione S-transferase (GST), Acetylcholinesterase (AChE) and Glutathione peroxidase (GPx), in response to *p,p'*-DDE exposure in Antarctic krill (*Euphausia superba*). The pooled control is represented by the 0 $\mu\text{g L}^{-1}$ treatment. Graphics generated in Graphpad Prism 7

19.06 $\pm$ 0.98. There was no significant change in activity for any treatment ($F(5,57) = 1.97$, $p = 0.097$). GPx and GST followed similar trends throughout the treatment responses,

possibly suggesting that there was an underlying factor, such as gender, which remained unaccounted for.

Discussion

Glutathione S-transferase

The observation of a slight induction of GST activity may suggest that a generic detoxification response was triggered in the krill, however with limited capacity. Indeed a basic capacity for detoxification of chemical exposure may explain the declining potency of *p,p'*-DDE noted during the exposure period (Poulsen et al. 2012a). After initial exposure to *p,p'*-DDE, krill mobility was observed to decrease and as the experiment progressed the response lessened (Poulsen et al. 2012a). *p,p'*-DDE is the most stable metabolite of DDT and it is unknown if this product is further metabolised in Antarctic krill, as is the case for some freshwater invertebrates (Lotufo et al. 2000; Lydy et al. 2000). Based on the present assay results, it does not appear that GST plays a major role in *p,p'*-DDE detoxification in this species. These findings contrast the DDT detoxification pathways known in other species. For example GST causes insecticide resistance and is responsible for the detoxification of DDT to *p,p'*-DDE, in insects and freshwater invertebrates (Clark and Shamaan 1984; Livingstone 1991). Further, increased GST activity is exhibited by molluscs exposed to *p,p'*-DDE, in similar exposure concentrations to those used in this study (Hoarau et al. 2001). Although, the latter study found that only one specific class of GSTs were induced by *p,p'*-DDE, these have not yet been described in Antarctic krill (Clark et al. 2011).

A drawback of the use of GST as a biomarker is the variability of baseline levels demonstrated in previous studies (e.g. Koenig and Solé 2012). Being a generalist family of enzymes, GST induction is triggered by many endogenous and exogenous stimuli such as fasting, gender, vertical migration, season, metabolic rate, etc. (Jemec et al. 2010, 2012; Tremblay et al. 2010). This variability can mask the response GST to xenobiotic exposure therefore further characterisation is needed, but was beyond the scope of this study. The effects of environmental stimuli can be reduced by sufficient acclimation to stable laboratory conditions (Jemec et al. 2010). The present experiment included a 24 h acclimation period to test conditions. It cannot be ruled out that the variability exhibited in krill in this study resulted from environmental factors. More likely the observed variation was caused by biological factors such as age or gender, for which a baseline has not been established in Antarctic krill.

355 Acetylcholinesterase

356 Acetylcholinesterase activity between crustacean species
357 seems to be inversely proportional to size. Previous studies
358 have reported low basal activity of AChE in *Euphausia*
359 *superba*, compared to smaller krill species (Minutoli et al.
360 2002). AChE activity exhibited in this study was slightly
361 lower compared to reports from similarly sized crustacean
362 species (Key and Fulton 2002; Bolton-Warberg et al. 2007;
363 Lavarias et al. 2011). In the present study, the individual
364 responses measured most likely reflect baseline levels.
365 AChE is responsible for the breakdown of neurotransmitters
366 allowing for normal neurological function. Although the
367 inhibition of AChE is not typically associated with DDT and
368 its metabolites, AChE inhibition has previously been
369 observed in crustaceans following DDT exposure (Martinez-
370 Tabche et al. 1999; Galindo-Reyes et al. 2000). There is a
371 small body of research that brings into question the speci-
372 ficity of AChE inhibition to organophosphate and carbamate
373 pesticides. Non-dose-dependent inhibition of AChE was
374 noted in response to polycyclic aromatic hydrocarbon mix-
375 tures in Antarctic scallops (Bonacci et al. 2009), and metals
376 have also been shown to inhibit AChE activity (Frasco et al.
377 2005). It is therefore recommended that AChE activity in *E.*
378 *superba* be examined further, particularly in light of the low
379 basal activity exhibited in this study. It may be valuable for
380 future work to test if other organochlorine contaminants,
381 including the parent metabolite DDT, and known AChE
382 inhibitors such as chlorpyrifos, which was recently detected
383 in Antarctic air and seawater (Bigot et al. 2016), can inhibit
384 this enzyme in Antarctic krill.

385 Glutathione peroxidase

386 The absence of concentration-dependent response for GPx
387 activity indicates the detection of baseline levels of GPx.
388 Up-regulation of antioxidant enzymes would be expected if
389 krill were experiencing oxidative stress relating to *p,p'*-
390 DDE exposure, i.e. oxidative stress would trigger increased
391 production of antioxidant enzymes. In mammalian systems,
392 *p,p'*-DDE is known to cause oxidative stress through
393 the generation of reactive oxygen species (Pérez-Maldon-
394 ado et al. 2005). This response has also been noted in
395 bivalves, for which the production of reactive oxygen
396 species was suggested to be the main mode of toxicity
397 (Dowling et al. 2006). The absence of a significant increase
398 in GPx activity suggests that either: (a) GPx is unable to
399 respond to the high level of *p,p'*-DDE-induced reactive
400 oxygen species in Antarctic krill i.e. an inefficiency of the
401 system potentially capped by a limiting component such as
402 glutathione co-factor (as noted in response to other
403 organochlorines e.g. Numan et al. 1990) or, (b) the krill are
404 not experiencing oxidative stress induced by *p,p'*-DDE. As

the assessment of oxidative stress indicators (such as lipid
peroxidation or protein carbonyl content) were outside the
scope of this study, it is difficult to conclude whether the
krill were experiencing oxidative stress. Generally the
antioxidant capacity of polar invertebrates is quite high
(Regoli et al. 2000; Abele and Puntarulo 2004). Antarctic
krill have been found to experience very little oxidative
stress in response to hypoxia despite the absence of sig-
nificant up-regulation of antioxidant enzymes, including
GPx (Tremblay and Abele 2015). Previous studies have
found that Antarctic krill exhibit high levels of low
molecular weight antioxidants, reduced glutathione and
vitamin E (Dunlap et al. 2002), which afforded adequate
reactive oxygen species elimination through scavenging. It
is possible that Antarctic krill may be able to cope with a
p,p'-DDE-mediated increase in reactive oxygen species
under normal basal GPx expression due to the abundance
of these other antioxidants.

Detoxification capabilities of *Euphausia superba*

The applied exposure concentrations, although in line with
routine toxicity testing protocols, were appreciably higher
than those measured in Antarctic seawater (Bigot et al.
2016). Interestingly, despite these artificially elevated con-
centrations, Phase II enzyme activity did not provide support
for an induction of a detoxification response in the species.
These findings correspond well with the observation of toxic
effects expressed throughout the original behavioural
experiment (Poulsen et al. 2012a). Enzymes of the cyto-
chrome P450 complex are known to be induced by DDT and
p,p'-DDE in mammals (Nims et al. 1998), and may be better
candidates for studying *p,p'*-DDE detoxification in krill.
Furthermore, CYPs have been associated with organochlo-
rine insecticide resistance in crustaceans (Brausch and Smith
2009). DDT-resistant fairy shrimp treated with cytochrome
P450 inhibitors became more sensitive to DDT-induced
toxicity. It is possible that CYPs played a role in the apparent
declining toxicity of *p,p'*-DDE throughout the behavioural
experiment, perhaps more so than the limited capacity
expressed by GST. Despite the non-detectability of CYP2B,
cytochrome P450 is a large and diverse group of enzymes
and further study of other CYPs may provide insights into
krill detoxification mechanisms.

An in-depth comparison of the detoxification capabili-
ties between analogous species or contaminants is not
possible due to an almost complete lack of published data
in this field. Very little is known about polar crustacean
detoxification capabilities. It has been suggested that polar
species may be more susceptible to foreign chemical con-
taminants compared to tropical or temperate species
(Chapman and Riddle 2005; Corsolini 2009; de Hoop et al.
2011), and that the detoxification capabilities of polar

species are lower than temperate and tropical counterparts. This seems to be the case for fish (e.g. Strobel et al. 2015), but the limited number of studies on polar crustaceans present conflicting results. A recent study compared the GST detoxification capabilities of Arctic and sub-Arctic copepods exposed to marine diesel. Interestingly the Arctic species expressed an almost 25 times increase in GST following diesel exposure, whereas GST in the sub-Arctic species only expressed a twofold increase (Hansen et al. 2013). By contrast, a study on the same two species of copepod found the opposite trend when exposed to artificially weathered marine diesel (Hansen et al. 2011). Compared to temperate species, adaption to a polar climate has led to reduced metabolic rates (Peck 2002), which effectively delay the onset of toxicity (King and Riddle 2001; Payne et al. 2014). Reduced metabolism is thus also expected to delay detoxification, possibly explaining the absence of induced GST activity found in this study over a 96 h exposure timeframe. Wild krill are likely to be exposed over a long period of time. Chronic exposure studies may be needed to determine if the absence of significant up-regulation of GST is a function of delayed detoxification.

Conclusions

As a result of their unique adaptations to a cold climate, many biomarker responses for tropical or temperate species may not be directly comparable to polar species. True polar bio-indicator species are rare. To the authors' knowledge, this is the first time that the detoxification response of any polar crustacean has been assessed in response to organochlorine compound exposure. The findings presented here provide an important baseline for future work to establish the mechanisms of organochlorine toxicity and further our understanding of Antarctic krill detoxification capabilities. The activity of glutathione *S*-transferase, glutathione peroxidase and acetylcholinesterase do not respond to *p,p'*-DDE in a concentration-dependent manner in Antarctic krill.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest. All procedures performed involving animals were in accordance with the ethical standards of the institution at which the studies were conducted.

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