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EFFECTS OF THE BOSCALID FUNGICIDE FILAN® ON THE MARINE AMPHIPOD *ALLORCHESTES COMPRESSA* AT ENVIRONMENTALLY RELEVANT CONCENTRATIONS

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Abstract

Fungicides are widely used in agriculture to control fungal diseases. After application, fungicides can be transported offsite to surface and groundwater and ultimately enter estuarine and marine environments. The presence of fungicides in the marine environment may pose risks to marine organisms, but little is known about fungicide effects on these organisms, especially invertebrates. The present study investigated the effects of the commonly used boscalid fungicide Filan® on life history traits, feeding rate, and energy reserves (lipid, glycogen, and protein content) of the marine amphipod *Allorchestes compressa* over 6 wk under laboratory conditions. Amphipods were exposed to 3 concentrations of Filan (1 µg, 10 µg, and 40 µg active ingredient [a.i.]/L), with 5 replicates per treatment. Lipid content and reproduction were the most sensitive measures of effect, with lipid content reduced by 53.8% at the highest concentration. Survival, growth, and other energy reserves of amphipods were also negatively affected by Filan,

and the effects were concentration dependent. Antennal deformities were incidentally observed on the amphipods at a concentration of 40 µg a.i./L. The results of the present study indicate comprehensive effects of the boscalid fungicide Filan on *A. compressa* at environmentally relevant concentrations. The decline or absence of *A. compressa* in marine ecosystems could impair the ecosystem function because of their important role in trophic transfer and nutrient recycling. The authors' results suggest that even though the use of fungicides is often regarded as posing only a minor risk to aquatic organisms, the assessment of their long-term effects is critical.

Keywords: Fungicide, Marine invertebrates, Reproduction, Energy reserves, Filan®

This article includes online-only Supplemental Data.

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INTRODUCTION

Fungicides are used in agriculture and industry or even domestically to control fungal infections, which are increasingly recognized as presenting a threat to global food security [1]. After application, fungicides can be transported off site via air, soil, and water, and therefore, potentially contaminate ground waters, surface waters, freshwaters [2,3], and marine and estuarine environments [4,5]. Although fungicides are designed to kill or inhibit fungal pathogens, their modes of action are not specific to fungi [6]. Therefore, the presence of fungicides in waterways may pose risks to aquatic organisms.

Fungicides are often used as a prophylactic crop protectant that is applied at higher frequencies but at lower application rates than other types of pesticides [2]. Fungicides,

therefore, are often detected in surface water in areas of intense fungicide use at low concentrations but high frequencies [2,3]. Consequently, aquatic organisms are likely to be chronically and repeatedly exposed to fungicides at relatively low concentrations, especially during the application season. Furthermore, the migration of fungicides from these areas to marine or estuarine ecosystem via streams and rivers could lead to relatively low concentrations as a result of dilution. For example, Smalling et al. [5] reported that the fungicide boscalid was detected in 100% of water samples in in a coastal estuary (California, USA) throughout the year, but maximum concentrations were lower than laboratory-derived aquatic life benchmarks for fish and invertebrates. However, the fungicide concentrations could vary because of environmental conditions (e.g., dry season vs wet season) or increase in sediment because some of them are persistent in aquatic environments. For instance, the highest concentration of the fungicide azoxystrobin detected in a coastal estuary (California, USA) in the dry season was 20.2 µg/L, but in the storm season it was detected at concentrations as high as 4550 µg/L [4]. In Western Port (Victoria, Australia), boscalid was the most frequently detected fungicide, with the highest concentration of 22 µg/kg [7]. Fungicides are usually considered to have low toxicity to aquatic animals compared with other pesticides. Limited knowledge is available about the levels of fungicides in the marine environment as well as the chronic effects of fungicides on marine species. A few studies have shown that fungicides can affect marine invertebrates at relatively low concentrations [8–10]. When exposed to sublethal concentrations (367–825 µg/L) of the fungicide propiconazole, the shrimp *Litopenaeus vannamei* showed morphological deformities of the rostrum, pereopods, and uropods [8]. The fungicide carbendazim altered the malondialdehyde level, glutathione, and antioxidant activity of the marine bivalve *Donax faba* at concentrations ranging from 52.65 µg/L to 842.6 µg/L [10]. However, these 2 fungicides

occurring commonly at such high concentrations in the environment is unlikely. In contrast, the fungicide 2-methoxyethylmercuric chloride exhibited broader toxicity at very low concentrations (1 µg/L) across every critical life transition and stage of the broadcast-spawning coral *Acropora millepora* [9]. However, interpreting the ecological impacts of fungicides on aquatic ecosystems without examining the effects of a range of fungicides at environmentally relevant concentrations is difficult.

Boscalid is a systemic fungicide that is active against a broad range of fungal pathogens and has been used in a wide range of crops [3]. It is resistant to most environmental degradation and is expected to be environmentally persistent [11]. Boscalid has been commonly detected in both freshwater [2,3,12] and estuarine environments [4]. In Western Port, Australia, boscalid was often detected in water samples, and the highest concentration was recorded at 3.3 µg/L (Centre for Aquatic Pollution Identification and Management, School of Biosciences, The University of Melbourne, Victoria, Australia, unpublished data, 2013). It is also 1 of the most frequently detected pesticides (in greater than 90% of the samples) in 3 main coastal estuaries in California, USA, with concentrations as high as 36 µg/L [4]. Boscalid has been found in estuarine fish and crabs [5], which could absorb the fungicide directly from the environment or through eating contaminated prey. Boscalid is expected to be present in the marine environment, and therefore organisms in this ecosystem have the potential to be exposed to it. To our knowledge, no current data are available on the sublethal effects of boscalid on marine organisms.

The amphipod *Allorchestes compressa* is abundant and widely distributed along the shores of southeast [13] and southwest [14] Australia. *Allorchestes compressa* is a semi-aquatic amphipod because it inhabits detached macrophytes in the intertidal regions of the shores, and at certain times during the day (e.g., low tide) the amphipod may not be submerged. It is an

important food source for various fish species [15] and plays an important role in the trophic transfer and nutrient recycling in marine ecosystems along the Australian coast [14]. This species has been used in both acute [13,15] and chronic toxicity tests [16], and they are suitable test organisms for studying toxicant effects on growth and reproduction under laboratory conditions [16].

In the present study, we investigated the effects of a commonly used fungicide, boscalid fungicide Filan®, on the marine amphipod *A. compressa* at a range of concentrations that have been found to occur in some natural estuarine environments [4,7]. Although these concentrations do not reflect a realistic environmental situation but rather an unusual event (e.g., caused by accidental releases), investigating how such rare situations could affect a key marine species is necessary. Furthermore, as a semi-aquatic species, *A. compressa* could be exposed directly to relatively high toxicant concentrations from agricultural or urban runoff. Under laboratory conditions, we measured sublethal biochemical (lipid, protein, glycogen content) and physiological (feeding rate) biomarkers as well as life history traits (growth, reproduction, and survival). Previous studies have shown that fungicides had indirect effects on amphipods through changing the microbial composition or biomass on the leaves that the amphipods consume, thereby reducing the palatability of their food [17,18]. Oxygen consumption correlated with bacterial growth [19], so changes in microbial respiration could reflect changes to bacterial biomass. Therefore, we measured microbial respiration in the seagrass used to feed the amphipods as a way to assess the indirect effects of Filan on the microbial community. We are aware that different groups of microorganisms could contribute differently to the nutrient quality of conditioned leaves [17], but that determination is beyond the scope of the present study.

MATERIALS AND METHODS

Chemicals

Boscalid was applied using the commercially available product Filan (Nufarm, Australia, 500 g active ingredient [a.i.]/kg) instead of pure active ingredient to avoid adding further solvents. Filan was dissolved in deionized water to make a stock solution with a nominal concentration of 50 mg a.i./L. The stock solution was then diluted to achieve nominal Filan concentrations of 1 µg a.i./L, 10 µg a.i./L, and 40 µg a.i./L. Both stock and test medium were prepared immediately before use. Water samples were collected before the experiment and after 1 wk exposure and sent to Advanced Analytical Australia Pty (North Ryde, NSW, Australia) for analysis of boscalid concentrations. Sample concentrations were determined using liquid chromatography–tandem mass spectrometry (MS/MS). Water samples were diluted in 30/70 water/(methanol + 0.1% formic acid). An aliquot of diluted water samples was injected onto an Aglient 1260 Infinity-HPLC (ESI positive mode), and components were separated on a Phenomenex-Gemini C18 (150 × 2 × 3um) column at 35 °C. The detector was a Varian-320 MS/MS, set at a temperature of 300 °C and a scan time of 2 s. The binary mobile phase was 5 mM ammonium formate (pH ~3.5) and acetonitrile to methanol (4:1) + 0.2% formic acid, using an initial gradient of 30%, which increased to 100% in 3 min, with a flow rate of 0.15 mL/min. Boscalid concentrations were quantified by multiple reaction monitoring of 343 m/z >307 m/z, 272 m/z, by external standard quantification. Boscalid reference material (Novachem) was of 98% purity. The limit of reporting for boscalid was 0.1 µg/L. A spike recovery was performed with the analytical batch, on sample 22 (reported unspiked at less than the limit of reporting), and the recovery was 72%. Reported results were not corrected for recovery. The measured boscalid concentrations were within 10% of nominal experimental concentrations if these were corrected for the reported recovery.

Test species

Allorchestes compressa and its food, the seagrass *Zostera muelleri*, were collected from Clifton Springs beach, Victoria, Australia, which is considered to be at low risk of pollution [20]. Amphipods were maintained in ambient seawater in the laboratory in groups of 500 at experimental conditions: temperature (20 ± 1 °C), salinity (34 ± 2‰), and at a 16:8-h light:dark photoperiod in 20-L glass aquaria under constant aeration. The seawater was obtained from a circulating seawater system in School of BioSciences, the University of Melbourne. Animals were given dry seagrass as food and water was changed weekly. After the acclimatization period (14 d), gravid females were separated into clean 2-L glass beakers containing ambient seawater. One week later, the resulting juveniles were transferred to fresh 2-L glass beakers and maintained as described previously. Amphipods used in the experiment were less than 6 wk old.

Preconditioned seagrass

During the experiment, amphipods were given preconditioned seagrass as food. To precondition the seagrass, the freshly collected seagrass was cleaned with tap water and air-dried before use. Approximately 25 mg dried seagrass was weighed and placed in nutrient-enriched seawater (5 mg P as K_2HPO_4, 20 mg N as $[NH_4]_2SO_4$ per 1 L seawater) [21] in 600-mL glass beakers for 1 wk. This process was carried out weekly to provide freshly preconditioned seagrass for the amphipods throughout the present study.

Experimental setup

Twenty *A. compressa* individuals were randomly placed 1 by 1 in 600-mL glass beakers containing 400 mL aerated ambient seawater with the respective Filan concentrations, with ambient seawater as controls, and preconditioned seagrass was added to each beaker. Each treatment had 5 replicates. The experiment ran for 6 wk, using the same conditions as described

previously in the section *Test species*. To account for microbial and abiotic seagrass loss during the experiment, an additional replicate per treatment was included without amphipods and treated like the other replicates. Every week, surviving amphipods were gently transferred by plastic pipettes to freshly made seawater medium and fresh preconditioned seagrass. The remaining seagrass was cleaned with deionized water, dried at 60 °C for 24 h, and weighed to determine the animal's feeding rate.

At the end of the experiment, the number of surviving adults and produced juveniles was recorded. Three healthy nongravid females per replicate were randomly selected and frozen at –20 °C for lipid, glycogen, and protein analysis. The remaining surviving adults were preserved in 70% ethanol for further examination using a Leica MS5 microscope with an ocular micrometer. Specimens preserved in ethanol were sexed and head length was measured (from the rostrum tip to the posterior margin of the head) [22] to determine growth based on the final size, with the assumption that the mean size of amphipods per replicate was the same at the beginning of the experiment, because the amphipods were the same age. The number of gravid females and the number of embryos produced per gravid female were recorded.

A second experiment was also set up without the amphipods to assess the effects of fungicide Filan on microbial respiration on the seagrass used to feed the amphipods. Preconditioned seagrass was exposed to the same nominal fungicide concentrations as used in the main experiment for 1 wk. Each treatment had 5 replicates. After 1 wk, the microbial respiration of the seagrass was measured.

Determination of feeding rate

Feeding rate was expressed as milligrams seagrass mass consumed per amphipod per day calculated as follows [23]:

$$C = (Lb \times K - La)/(N \times T)$$

where Lb and La are initial and final dry mass of seagrass, respectively; N is the number of surviving amphipods (the dead organisms could contribute to the seagrass consumption, but we did not account for this in the present study because the time of death was not recorded daily), T is the feeding time in days, and K is the leaf change correction factor given by

$$K = \frac{\Sigma(\frac{LCa}{LCb})}{n}$$

where LCb and LCa are the initial and final dry mass of seagrass in the control replicates without amphipods, n is the number of replicates.

Determination of lipid, glycogen, protein content, and microbial respiration

The lipid, glycogen, and protein assays were carried out using a Synergy 2 microplate reader (Biotek Instruments). Lipid and glycogen content were measured following the method described by Van Handel [24,25], using commercial vegetable oil and glucose as the standards and modified for the use of a microplate reader. The volume of solution in each well was 60 µL; absorbance is measured at 490 nm for lipid and 625 nm for glycogen.

Protein content was determined using a modified Lowry assay (Bio-Rad DC method), with bovine serum albumin as the standard [26].

Microbial respiration on seagrass was measured using changes in oxygen concentration followed the method descried by Carlisle and Clements [27]. Dissolved oxygen was measured with a water quality meter (smartCHEM-LAB, TPS, QLD, Australia) at the beginning and end of a 24-h incubation period.

Statistical analysis

Treatment effects on survival, growth, reproduction, feeding rate, microbial respiration, and energy reserves (lipid, glycogen, and protein content) were analyzed using one-way analysis

of variance followed by Dunnet's pairwise comparisons. Simple and multiple linear regressions were performed to determine the relationship between energy reserves and female amphipod survival and growth. Statistical analysis was performed using SPSS Ver 22 (IBM).

RESULTS

Survival

Survival in the control treatments after 6 wk was 86 ± 1.9 % (mean ± standard error [SE]). Survival decreased with increasing Filan concentrations (Figure 1). Significant differences were found between survival of amphipods from the control and Filan treatments ($F_{3,16} = 4.631$, $p = 0.016$). Survival of the control was significantly different from survival at 10 µg a.i./L and 40 µg a.i./L Filan ($p = 0.046$ and $p = 0.034$, respectively), whereas approximately 70% of animals survived.

Growth

The size of both male and female amphipods was reduced with increasing fungicide concentrations (Figure 2). Significant effects on females were found at all Filan treatments 1 µg a.i./L, 10 µg a.i./L, and 40 µg a.i./L ($p = 0.008$, $p < 0.001$, and $p < 0.001$, respectively), and female head lengths at the highest concentration were reduced by 12.6% compared with those in the control treatments. For males, significant effects were observed at 10 µg a.i./L and 40 µg a.i./L ($p = 0.012$ and $p = 0.025$, respectively) but not at 1 µg a.i./L ($p = 0.496$), and the highest Filan concentration only reduced size by 6.8%.

Reproduction

Filan exposure had strong adverse effects on *A. compressa* reproduction. Neither gravid females nor offspring were present in any of the Filan treatments. Gravid females were first observed in the control at the beginning of week 4. The average number of offspring per

replicate in the control was 7.6 ± 0.68 (mean ± SE). The average number of offspring per single female was 0.58 ± 0.04 (mean ± SE). The average number of gravid females per replicate in the control was 1.4 ± 0.51 (mean ± SE). The average number of embryos per gravid female in the control was 5.14 ± 0.37 (mean ± SE).

Feeding rate

Filan exposure had no significant effect on *A. compressa* feeding rates throughout the 6-wk exposure period (all $p > 0.05$). As expected, the feeding rates in the control and treatments increased from week 1 to week 6 (Supplemental Data Figure S1).

Energy reserves

Energy reserves of the amphipod *A. compressa* decreased with increasing Filan concentrations (Figure 3). Filan had significant effects on lipid content at all concentrations of 1 µg a.i./L, 10 µg a.i./L, and 40 µg a.i./L ($p = 0.001$, $p = 0.002$, and $p < 0.001$, respectively), but only at the highest concentration of 40 µg a.i./L for glycogen and protein content ($p = 0.013$ and $p = 0.007$, respectively). A simple linear regression showed that all 3 types of energy positively correlated to the female size (Figure 4), with lipid content having the highest correlation ($R = 0.705$), then glycogen content ($R = 0.637$) and protein content ($R = 0.560$). However, a multiple linear regression performed on all 3 types of energy reserves simultaneously showed that only lipid and protein content significantly contributed to the predicted model (Supplemental Data Table S1) and had a significant increase in the coefficient of determination ($R^2 = 0.724$, $F_{3,16} = 13.99$, $p < 0.001$). No significant relationship was seen between energy reserves and survival (all $p > 0.05$).

Microbial respiration

Microbial respiration increased with increasing Filan concentrations (Supplemental Data Figure S2). However, no significant difference was seen in microbial respiration in the seagrass between the control and Filan treatments ($F_{3,16}$ = 3.125, p = 0.055).

Deformities

Some deformities were observed in the antennae of amphipods when head length measurements were conducted. The antennae were either missing (Figure 5B) or shortened (Figure 5B and C). No deformities were observed in the control. Malformations only occurred in the 40 µg a.i./L Filan treatment in 13 individuals of 68 examined amphipods.

DISCUSSION

Effects of boscalid exposure on A. compressa at different endpoints

Filan had effects at environmentally relevant concentrations on almost all endpoints measured in the present study, except the feeding rate. However, the levels of effect were different among endpoints.

At the organism level, reproduction was the most sensitive endpoint. No female reproduction occurred in all Filan treatments. This finding is in agreement with the results of previous studies on chronic effects of toxicants on marine and estuarine amphipods that showed that reproduction was delayed [28] or significantly reduced [29], and was a much more sensitive metric than survival [30]. The significant effects of Filan on *A. compressa* reproductive success could be partially explained by the reduction in growth of female amphipods. Body size is a determining factor for the onset of the reproductive phase of amphipods [31], because they have to reach a certain size before reproduction can occur [32]. Therefore, reduced growth can lead to reduced reproduction. The relationship between female size and reproductive output has been documented for some amphipod species such as *Hyalella azteca* [33] and *Gammarus minus* [34].

The effects of Filan on amphipod reproduction at environmentally relevant concentrations should be considered in fungicide risk assessments, because a delay in reproduction could have strong negative effects on the viability of the population at an ecological scale.

Filan exposure also had a significant effect on the growth of *A. compressa*. Growth is routinely used as a sublethal endpoint in chronic toxicity studies, and it is often affected by contaminant exposure [35]. A few studies have shown that female amphipods were more sensitive than males [36,37]. The results of the present study were consistent with previous studies, because we found significant effects on female growth in all Filan treatments whereas only at the higher concentrations for males. The difference in sensitivity of growth in sexually mature male and female amphipods may be partially explained by the increase in energy requirements during oogenesis and brooding in females compared with the less energy-demanding process of spermatogenesis in males [36]. This will result in less energy being available for growth and to cope with toxic stress in females.

As expected, the survival endpoint was less sensitive than reproduction and growth. Significant effects of boscalid fungicide Filan on amphipod survival occurred at concentrations of 10 µg/L and 40 µg/L. To our knowledge, no chronic toxicity data are available for boscalid on marine invertebrates. *A. compressa* seems to be more sensitive to boscalid compared with other invertebrates. For example, a 21-d chronic exposure to *Daphnia magna* recorded no observed adverse effects concentration of 3.06 mg/L (J. Jatzek, Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany, unpublished data). This may be attributable to exposure time, because the present study was a 42-d test compared with 21-d exposure for the for *D. magna* study. Longer-term exposure is known to cause a significant effect on survival [35].

At the biochemical level, lipid content was the most sensitive of the energy stores measured, although all 3 types of energy reserves decreased with increasing fungicide concentrations. This suggests that lipids were the primary source of energy to cover for the increased demand incurred from Filan exposure. The present results concur with those of Zubrod et al. [18], who observed that the fungicide tebuconazole significantly reduced the lipid content of the amphipod *Gammarus fossarum* but had no effects on leaf consumption. However, the authors also pointed out that the fungicide tebuconazole could alter the food quality of the amphipod through the effects on the microbial colonization of the leaf material. De Coen et al. [38] also reported that lipid reserves was the most sensitive endpoint among all cellular energy allocation components of *D. magna* exposed to 6 different toxicants. Lipids are often mobilized to meet the increased energy demand associated with toxic stress because lipid is a prominent long-term energy store in most aquatic crustaceans [31], and they provide more than twice as much potential metabolic energy per unit mass as proteins or carbohydrates [39].

Protein and glycogen contents were less sensitive than lipid content; significant effects were only observed at the highest concentration. Proteins are often used by animals during periods of high energy demand [40], and they are the last energy sources to be mobilized in stressed organisms after the metabolization of lipid and carbohydrates [41]. In contrast, glycogen is rapidly metabolized to meet the energy needs of an organism [41,42], and it can be quickly synthesized when carbohydrate supplies are available [39]. Animals exposed to Filan at concentrations of 1 µg a.i./L and 10 µg a.i./L might have a chance to replenish the glycogen they used. The ability of the animal to quickly refill the used glycogen is supported by Hervant et al. [43], who reported a significant overshoot of the glycogen content in the amphipods *Niphargus rhenorhodanensis* and *Niphargus virei* during the first week of recovery from nutritional stress,

reaching 127% and 121% of fed value, respectively, before returning to the prestarvation levels. The results of biochemical biomakers suggest that exposure to 40 µg a.i./L Filan caused serious stress to the amphipods and that lipid content is a sensitive biomarker that could be used to assess the effects of fungicides on amphipods.

The link between biochemical changes and effects at higher levels of organization

Biomarkers have been used increasingly to investigate environmental impacts of pollutants because of a number of advantages compared with conventional toxicity tests, which generally use mortality as an endpoint [44]. Biochemical parameters are very sensitive to sublethal concentrations of many chemicals [40] and are often considered as initial changes caused by toxicants that ultimately lead to adverse effects at higher levels of biological organization [44]. However, currently limited knowledge is available on the ecological relevance of biomarker signals [38], and this could be assessed through investigating their relationship with several life history traits [45].

Our results showed a strong relationship between energy reserves and the growth of female *A. compressa*. Organisms use stored energy for a variety of needs, but most energy is used for growth, reproduction, and basal metabolism [39,40]. Increased energy expenditure in basal metabolism to cope with toxic stress will lead to a reduction in growth and reproduction [38]. Similar observations of reduction of growth with the concurrence of decreased energy reserves were found in *D. magna* exposed to Cd [46] and *Gammarus pseudolimnaeus* exposed to pentachlorophenol [47]. However, the present study further suggests that lipid content is the energy most correlated to the growth of female amphipods because it had the highest standardized coefficients for the predicted model (Supplemental Data Table S1). The relationship of lipid and growth in amphipods has previously been reported in the literature [31,48].

Furthermore, the present results also demonstrated that lipid and protein content are both important in amphipod growth, because they overall significantly increased the coefficient of determination for multiple linear regression analysis (R^2 = 0.724) compared with the simple linear regression with only lipid (R^2 = 0.498) or protein (R^2 = 0.314).

A strong connection also was seen between lipid content and amphipod reproduction. Lipids are prominent storage components in most marine invertebrates [39]. Therefore, not only are they an important energy source for growth they but also play a critical role in amphipod reproductive success, because lipids are used in the development of reproductive tissue and embryos [31,48]. Studies on amphipod reproduction have shown that lipid content correlated to egg production [31] and increased during the reproductive period [49,50]. In the present study, a significant reduction of lipid content and lack of reproductive output in all Filan treatments convincingly demonstrated the important role of lipid reserves in the reproductive success of *A. compressa*.

Finally, protein content could be an explanation for the morphological abnormalities of *A. compressa*. The mechanism underlying the occurrence of deformities in aquatic invertebrates exposed to contaminants and the consequences of deformities to these organisms remain unclear [51,52]. In the present study, we observed antennal deformities in the amphipod *A. compressa* exposed to 40 µg a.i./L of Filan at which a significant reduction of protein content also occurred. We propose the possibility of a relationship between protein content and deformities, because a large proportion of an organism's body is composed of structural proteins [41], and the decrease in protein content might be attributable to a mechanical lipoprotein formation that will be used to repair damaged cells, tissues, and organs [40]. This proposal was supported by David et al. [53], who reported a significant reduction in total, soluble, and structural proteins in deformed

tadpoles of *Duttaphrynus melanostictus* exposed to sublethal concentrations of cypermethrin. On the contrary, Arambourou et al. [54] reported alterations in energy reserves of *C. riparius* exposed to lead-spiked sediment, but no mentum morphological defects were observed.

CONCLUSION

The present study provides the first evidence of the effects of a fungicide on survival, growth, reproduction, and energy reserves on a marine amphipod at environmentally detected concentrations. Although these effects were observed under laboratory conditions, the results suggest that fungicides could affect the viability of amphipod populations in natural ecosystems. There also could be cascading effects on the ecosystem, because semiaquatic amphipods such as *A. compressa* are often a main food source for many fish species and have major influence on detrital turnover in surf-zone environments. Further research needs to address the long-term effects of fungicides on aquatic ecosystems and to assess the condition of *A. compressa* populations in intertidal zones polluted with fungicides.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3247.

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Data availability—Data can be assessed by contacting corresponding author (htvu@student.unimelb.edu.au).

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Figure 1. Percentage of survival (mean ± standard error) of *Allorchestes compressa* in control and Filan® treatments after 6-wk exposure (n = 5). *Significant difference from control ($p \leq 0.05$).

Figure 2. Head length (mean ± standard error) of *Allorchestes compressa* in control and Filan® treatments after 6-wk exposure. Males are dark bars and females are light bars (n = 5). *Significant difference from control ($p \leq 0.05$).

Figure 3. Concentrations of lipid (**A**), glycogen (**B**), protein (**C**), (mean ± standard error) of *Allorchestes compressa* in control and Filan® treatments after 6-wk exposure (n = 5). *Significant difference from control ($p \leq 0.05$).

Figure 4. Relationship between female head length and lipid (**A**), glycogen (**B**), and protein (**C**) content. Lipid content was most positively correlated to female size, then glycogen and protein content.

Figure 5. Normal antennae of *Allorchestes compressa* (**A**) and deformed specimens in Filan® treatment of 40 µg active ingredient/L (**B, C**) after 6-wk exposure. Two antennae were missing; 1 antenna was shortened (**B**); all 4 antennae were shortened (**C**).