Contrasting Effects of Predation Risk and Copper on Copepod Respiration Rates

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Abstract: Natural biotic and anthropogenic stressors can interact to alter contaminant toxicity. Energetic restrictions are potential mechanisms causing this pattern. To identify processes underlying observed effects of predation risk and copper (Cu) on delayed copepod age at maturity, we examined how these 2 stressors affect respiration rates. We tested 2 very different copepod species: the large, pelagic calanoid *Calanus finmarchicus* and the small, semibenthic harpacticoid *Tigriopus brevicornis*. Adult individuals were exposed for 12 h to the treatments: predation risk, Cu (23 µg L⁻¹), combined predation risk and Cu (23 µg L⁻¹), or control. Oxygen concentrations were monitored continuously. The 2 species differed in their responses. We found no clear effects of either stressor in *C. finmarchicus*. In *T. brevicornis*, predation risk increased respiration rates, whereas Cu alone had little impact. In contrast, combined exposure to predation risk and Cu interacted to reduce respiration rates to less than expected. We further observed an effect of sex because female-biased *T. brevicornis* replicates were more sensitive to both predation risk (increased respiration rates) and Cu exposure (reduced respiration rates). The present study provides further evidence that predation risk can interact with copepod responses toward Cu exposure. Interactive effects of biotic stressors ought to be considered to improve future marine environmental monitoring.

Keywords: Stressors; Metal toxicity; Aquatic invertebrates; Aquatic toxicology; Marine toxicity tests; Kairomone

INTRODUCTION

All organisms are continuously exposed to a combination of multiple natural and anthropogenic stressors. Effects of multiple stressors may range from antagonistic to synergistic compared to single-stressor exposure (Holmstrup et al. 2010). Synergistic interactions are generally expected from combined-stressor exposure in marine systems (Crain et al. 2008; Gunderson et al. 2016). Previous multiple-stressor studies of marine systems and species have mainly focused on abiotic factors such as temperature, salinity, and pH (Crain et al. 2008; Gunderson et al. 2016). Comparatively few have looked at combined effects with biotic factors such as predation risk or competition (e.g., Linke-Gamenick et al. 1999; Lode et al. 2018). Empirical evidence from terrestrial and limnic systems suggests that combined-stressor responses with biotic factors range from antagonistic to synergistic (Rose et al. 2001; Relyea and Hoverman 2006; Qin et al. 2011). Failing to consider multiple-stressor effects may cause erroneous estimates of environmental risks from anthropogenic activities (Heugens et al. 2001); thus, more knowledge is needed concerning interactive effects of biotic stressors in marine systems.

Coastal habitats are often characterized by high primary production, structural complexity, and species diversity. Natural abiotic factors such as temperature and pH form the basis of ecological niches, but biotic factors such as predation structure ecological communities (Verity and Smetacek 1996). Predation affects prey populations by direct consumption, but nonconsumptive indirect effects can be equally important for prey population dynamics and distributions (Preisser et al. 2005). Copepods are important energy vectors in marine food webs (Turner 2004) and thus are often used as marine model organisms in ecotoxicology because of their high ecological relevance. Previous studies have shown that perceived predation risk may reduce copepod age at maturity (Bjærke et al. 2014), potentially at the cost of reduced fecundity (Gutiérrez et al. 2010). Predation risk exposure can also reduce...
copepod foraging activity (Bollens and Stearns 1992; Cieri and Stearns 1999). Unless compensated for by reduced metabolic costs, reduced energy income will ultimately affect development, growth, or reproduction (Jager et al. 2014).

Coastal areas are typically subjected to contaminants via industry and agriculture land runoff as well as direct discharges from shipping and aquaculture (Bryan and Langston 1992; Thomas and Brooks 2010). In Norway, copper (Cu) is a widely used antifouling agent in aquaculture (Skarbøvik et al. 2017). Discharges of Cu from aquaculture have increased from an estimated 118 tonnes in 2003 to 1088 tonnes in 2016 and contributed >85% of the total estimated Cu discharge to Norwegian coastal waters in 2016 (Skarbøvik et al. 2017). Copper is a naturally occurring trace metal and essential for normal cell functioning, for example, as a cofactor in enzymes (Bertinato and L’Abbé 2004). However, Cu is toxic at elevated concentrations, potentially causing oxidative stress through reactive oxygen species (ROS) generation and inhibiting enzymes by blocking other cofactor sites (Grosell 2011). Adverse effects of Cu exposure in marine invertebrates include metabolic disruption, cytotoxicity, and neurotoxicity (Brown et al. 2004; Lauer et al. 2012). More specifically in copepods, Cu exposure may delay development and thus increase age at maturity (Sullivan et al. 1983; Kwok et al. 2008), reduce reproductive output (Reeve et al. 1977; Fitzer et al. 2013), and reduce foraging activity (Sharp and Stearns 1997). Few studies have targeted the combined effects of anthropogenic and biotic stressors in marine systems. Lode et al. (2018) found that predation risk doubled the adverse effect of Cu on increased age at maturity in a coastal copepod, despite little effect of predation risk alone. A mechanistic understanding of how natural stressors interact with contaminant toxicity is required to extrapolate from individual- to population-level effects (Jager et al. 2014). Energetic restrictions have been suggested to cause synergistic interactions of predation risk and anthropogenic stressors (Rose et al. 2001; Robison et al. 2018).

Respiration rates and cellular responses are closely linked. Although an extensive literature exists on marine copepod respiration rates (Ikeda 1970; Mcallen et al. 1999; Brun et al. 2017), the effects of predation risk on copepod respiration rates are unknown. In contrast, the effects of Cu are well studied, and Cu exposure generally increases copepod respiration rates (Moraitou-Apostolopoulou and Verriopoulos 1979; Moraitou-Apostolopoulou et al. 1983; but see Reeve et al. 1977). In freshwater, predation risk seems to alter respiration depending on the combination with other stressors (Robison et al. 2018; Van Ginneken et al. 2018). For example, predation risk exposure alone did not affect respiration rate in a freshwater isopod, but respiration rate increased when combined with Cu exposure, whereas Cu alone reduced respiration (Van Ginneken et al. 2018). In another study, a cladoceran exposed to predation risk cues reduced respiration rate, whereas fathead minnow larvae had cue-specific responses ranging from no effect to increased respiration rate (Robison et al. 2018).

In the present study, we tested whether changes in respiration rates can explain an observed interactive effect of predation risk and Cu exposure on copepod age at maturity (Lode et al. 2018). We expected Cu exposure to increase copepod respiration rates (Moraitou-Apostolopoulou and Verriopoulos 1979; Moraitou-Apostolopoulou et al. 1983), with a more than additive interactive effect when combined with predation risk. We also analyzed the temporal dynamics of responses because we expected a more rapid response of predation risk than Cu.

**MATERIALS AND METHODS**

We used the well-established ecotoxicology model copepod *Tigriopus brevicornis* (Raisuddin et al. 2007). This is the same species as used in Lode et al. (2018). We used *T. brevicornis* from a long-standing laboratory culture sampled in June 2016 from Tjärnö, Sweden. Because *T. brevicornis* is
small (0.5 mm prosome length) with a correspondingly low respiration rate per individual, we also included a larger pelagic copepod, *Calanus finmarchicus* (2.4 mm prosome length). We sampled *C. finmarchicus* in October 2018 from the outer parts of Oslofjord, Norway. Copepod cultures were kept at 15 °C and fed ad libitum a mixed diet of the phytoplankton *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Rhodomonas salina*.

We conducted the experiment in December 2018. Adult copepods were exposed to predation risk and waterborne Cu (23 µg L\(^{-1}\)) by the treatment combinations: predation risk, Cu, combined predation risk and Cu, or control. To simulate predation risk, we used three-spined stickleback chemical cues (i.e., kairomones). We kept treatments on separate well plates to avoid cross-contamination, and therefore, we ran the experiment 4 times (i.e., series) with interchanged positions to control for spatial effects (Figure 1). Each series included 1 well plate per treatment with 16 replicate wells, 4 O\(_2\) normal-level blanks, and 4 O\(_2\) undersaturated blanks bubbled with nitrogen. Blanks were subsequently used to correct for oxygen influx rates.

Whereas we incubated *C. finmarchicus* individually, the smaller *T. brevicornis* were incubated in groups of 10 individuals. Copepods were picked and placed in filtered seawater for gut evacuation 24 h prior to incubation. To limit bacteria in the incubations, we rinsed all individuals 3 times in sterile filtered seawater prior to incubation in OxoDish OD24 well plates (Presens Precision Sensing). The experiment was run for 12 h in darkness. A 12-h exposure period kept us within expected half-lives of fish kairomones (Van Buskirk et al. 2014). Oxygen concentrations were measured every 30 s by SensorDish\textsuperscript® Reader units (Presens Precision Sensing) placed in a water bath at 15 °C. To minimize oxygen influx rates, we sealed each well plate using a plastic film (Nunc™ Sealing Tape), removed air bubbles by filling wells with respective exposure solutions using a syringe, and then resealed with an aluminum seal (SilverSeal; Greiner Bio-One). We visually checked for air bubbles before and after incubation. After exposure, we used a Nikon SMZ 745T stereomicroscope to check copepods for physical condition (e.g., survival, damaged antannae) and sex. All individuals were also photographed for future size measurements (ImageJ 1.51j/Java 1.8.0_112).

We prepared all exposure solutions on the day of incubation, as described in Lode et al. (2018), to have similar exposure conditions but with some modifications. In short, water with kairomones was produced by incubating thre спинед stickleback (2 fish L\(^{-1}\)) fed with copepods for 24 h at 15 °C and 29 psu (Atago hand refractometer). After incubation, the water was sterile-filtered (VWR\textsuperscript® Bottle Top Filtration Unit, 0.1 µm polycethersulfone; Sterile). Fish incubation reduced the pH slightly; thus, we adjusted using 0.1 M NaOH to similar levels as water without kairomones (pH 8.04–8.07; Radiometer Copenhagen PHM 92 lab meter). Copper (23 µg L\(^{-1}\)) was added in a 2-step dilution series from a CuSO\(_4\) 0.1 M stock solution.

Statistics were conducted with R, Ver 3.6.0 (R Development Core Team 2019). There was no mortality in controls for either species. Minor mortality effects were observed following exposure to predation risk (0.5 and 1.2%), Cu (0.4 and 0.6%), and combined (0.5 and 3%) in *T. brevicornis* and *C. finmarchicus*, respectively. We excluded dead and nonhealthy individuals from the analysis, and we also excluded replicates with confounding air bubbles. In total, we excluded 18.9% of total replicates, including 19.8% of *C. finmarchicus* replicates and 16.1% of *T. brevicornis* replicates.

Effects of explanatory variables on respiration rates were analyzed using linear mixed-effects models (package nlme; Pinheiro et al. 2017) and stepwise backward model selection using likelihood ratio tests and type II sum of squares analysis of variance (package car; Fox and Weisberg 2011). The full factorial design thus enabled us to test for interactive effects between the 2 explanatory variables predation risk and Cu. We checked the residuals from the final models by visual inspection. For all tests on *T. brevicornis* respiration rates, we had to include weighted variances for the explanatory variables predation risk and Cu.

We excluded the first hour of exposure because of acclimatization and analyzed respiration rates for the following 12 h of exposure. A total of 1440 oxygen concentration measurements were registered per replicate well. We calculated 15-min rolling averages of well oxygen contents (concentrations multiplied by well volumes). Respiration rates (nmol O\(_2\) µg dry wt\(^{-1}\) h\(^{-1}\)) were calculated as successive differences of the rolling averages, normalized by the sampling time interval and the ash-free dry weight of the incubated animals. Ash-free dry weight (µg) was calculated as \(\ln(\text{dry wt}) = 2.74 \times \ln(\text{prosome length} [\mu m]) – 16.41\) (Chisholm and Roff 1990). We corrected for oxygen concentration–dependent influx rates using a nonlinear asymptotic regression model based on all blanks per series and then extracted the median respiration rate per well for the full 12-h exposure period. This conservative approach reduces the data set to one representative respiration rate value per replicate well but is less prone to confounding noise from variation in measured oxygen concentrations stemming, for example, from behavior. This is especially challenging for the active *T. brevicornis* (Mcallen et al. 1999).

For all analyses we identified and removed outliers based on a 3 × standard deviation threshold of the total data per species. We tested the factorial effects of predation risk and Cu as explanatory variables on the response variable respiration rate. *Environmental Toxicology and Chemistry, 2020, 39:1765–1773*
respiration rate for the full 12-h exposure period. Series (i.e., experimental runs) was included as a random effect to control for the experimental block design. To investigate whether respiration rates varied with time, we also extracted median respiration rates for the time intervals 0 to 3, 3 to 6, 6 to 9, and 9 to 12 h and conducted a second test including time as an additional explanatory variable. When testing temporal trends, we included well identity as a random effect nested in series. For T. brevicornis, we did an additional analysis in which we included sex ratio as an explanatory covariate on respiration rates. Sex ratio was calculated as the number of males divided by the total numbers of males and females incubated per well. We did not look into sex differences in C. finmarchicus because close to all incubated individuals were female (>99%). Sex-ratio effects should be interpreted with caution because the experimental design was not balanced according to sex ratios prior to exposure.

RESULTS

The weight-specific respiration rates in control groups were lower for C. finmarchicus (0.054 ± 95% CI 0.007 nmol O2 µg dry wt−1 h−1) than for T. brevicornis (0.099 ± 0.053 nmol O2 µg dry wt−1 h−1), but variation was higher in T. brevicornis (Figure 2).

No effects were found for predation risk or Cu exposure on respiration rates in C. finmarchicus when analyzed for the full 12-h exposure period. Investigating temporal variation in respiration rates, we found that C. finmarchicus respiration rates decreased with time (df = 3, likelihood ratio = 41.294, p = 5.664 × 10−9; Table 1). However, we cannot exclude a potential interaction of predation risk and Cu exposure with time (df = 3, likelihood ratio = 7.251, p = 0.064), suggesting a different response over time in the combined treatment (Figure 3).

In T. brevicornis, predation risk and Cu interacted on respiration rates when analyzed for the 12-h exposure period (df = 1, likelihood ratio = 5.030, p = 0.025). Exposure to predation risk alone increased respiration rates (0.197 ± 0.051 nmol O2 µg dry wt−1 h−1), no effect was observed from Cu exposure alone (0.086 ± 0.051 nmol O2 µg dry wt−1 h−1), whereas combined predation risk and Cu exposure interacted to reduce respiration rates to less than additive (0.042 ± 0.052 nmol O2 µg dry wt−1 h−1; Figure 4). We found no differences in T. brevicornis respiration rates over time.

Including T. brevicornis sex ratio as an explanatory variable for the full 12-h exposure period, we found the same effects of predation risk and Cu; furthermore, we found increasing male proportions to generally increase respiration rates whereas reducing the effect of predation risk (df = 1, likelihood ratio = 3.904, p = 0.048; Figure 5). Investigating temporal variation, we found an interaction of sex ratio and time by which respiration rates were increasingly reduced with time and increasing male proportions (df = 1, likelihood ratio = 4.369, p = 0.037). The reducing effect of male proportions on respiration rates occurred because an additional interaction between Cu and sex

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**TABLE 1: Temporal variation in Calanus finmarchicus respiration rates**

| Time interval (h) | Estimate (nmol O2 µg dry wt−1 h−1) | 95% CI (nmol O2 µg dry wt−1 h−1) |
|------------------|----------------------------------|---------------------------------|
| 0–3              | 0.0615                           | 0.0518–0.0713                   |
| 3–6              | 0.0498                           | 0.0401–0.0596                   |
| 6–9              | 0.0504                           | 0.0406–0.0601                   |
| 9–12             | 0.0557                           | 0.0460–0.0655                   |

*Estimates are calculated from the best model (respiration rate ~ time, random = series).

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**FIGURE 3**: Calanus finmarchicus respiration rates per 3-h time interval. Estimates (mean with 95% CIs) are calculated from the alternative best model (respiration rate ~ time x predation risk x Cu, random = series/well identity).
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FIGURE 4: *Tigriopus brevicornis* respiration rates for the full 12-h exposure period. Estimates (mean with 95% CIs) are calculated from the best model (respiration rate ~ predation risk × Cu, random = series).

ratio reduced respiration rates more in Cu-exposed females than in males (Figure 6).

**DISCUSSION**

Predation risk can potentiate the toxicity of Cu on copepod development time, doubling the increase in age at maturity compared to Cu exposure alone (Lode et al. 2018). To identify the mechanisms of this interaction, we tested combined exposure effects on respiration rates in 2 species of marine copepods and found very different response patterns. Whereas neither stressor affected *C. finmarchicus* respiration rates, compared to Cu exposure alone (Lode et al. 2018). To identify the mechanisms of this interaction, we tested combined exposure effects on respiration rates in 2 species of marine copepods and found very different response patterns. Whereas neither stressor affected *C. finmarchicus* respiration rates,

**FIGURE 5:** *Tigriopus brevicornis* respiration rates for the full 12-h exposure period when accounted for replicate sex-ratio variation. Estimated means (lines) are calculated from the best model (respiration rate ~ predation risk × [Cu + sex ratio], random = series). Median respiration rate per replicate well is shown as points.

**FIGURE 6:** *Tigriopus brevicornis* respiration rates per 3-h time interval when accounted for replicate sex-ratio variation. Estimated means (lines) are calculated by sex ratio from the best model (respiration rate ~ sex-ratio × [predation risk + Cu + time], random = series/ID). Median respiration rate per replicate well is shown as points. The stippled line shows the overall mean of all respiration rates for the given treatment at each time interval.

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predation risk exposure when singly applied increased respiration rates in T. brevicornis. We further found indications that respiration in T. brevicornis was more sensitive to both predation risk and Cu exposure in females than in males.

Mixture toxicity terminology such as “synergistic” and “antagonistic” has been criticized as unsuitable for describing multiple stressor interactions involving natural stressors. The criticism is much due to inconsistencies in interaction type definitions and resulting interpretative ambiguity, particularly when stressors or their interactions have opposing directional effects (Côte et al. 2016). An alternative is to describe stressor interactions simply as greater or lesser than expected, based on the stressors’ effects when singly applied and an additive expectation when combined (Laskowski et al. 2010). In the present study, the 2 stressors when combined interacted to reduce respiration rates in T. brevicornis to less than expected. Thus, we found no support for our hypothesis that energetic restrictions through altered respiration caused the potentiating interactive effect found in Lode et al. (2018). However, given reported increases in copepod respiration rates from Cu exposure (Moraitou-Apostolopoulou and Verriopoulos 1979; Moraitou-Apostolopoulou et al. 1983) and the increased respiration rate following exposure to predation risk observed in the present study, we cannot dismiss the potential importance of altered respiration rates. Interaction types can change with the severity of stressor exposure (Sharma et al. 1999), and we only exposed for a relatively short period. An alternative explanation is that adult copepods are less sensitive to Cu exposure than juveniles (Verriopoulos and Moraitou-Apostolopoulou 1982; Sunda et al. 1987). The metamorphosis from final nauplius to first copepodite stage in copepods is particularly sensitive to combined predation risk and Cu exposure (Heuschele et al. 2019). Energy budgets are governed by foraging as well as respiration. Rose et al. (2001) suggest that a synergistic interaction of predation risk and pesticide exposure on life-history traits in a cladoceran was caused by reduced foraging activity. Both predation risk and Cu exposure can reduce foraging in copepods (Bollens and Stearns 1992; Sharp and Stearns 1997; Cieri and Stearns 1999), and it is likely that combined exposure effects on respiration and foraging act in concert to alter the copepods’ energy budgets.

Metal exposure, in particular by direct water exposure, can disrupt predation risk perception and responses (Lüring and Scheffer 2007). Exposure to Cu was found to impair predation risk responses in Daphnia (Hunter and Pyle 2004; DeMille et al. 2016). Responses to predation risk were found in a marine copepod (Lode et al. 2018) and a freshwater isopod (Van Ginneken et al. 2018) when simultaneously exposed to Cu, but the organisms in these 2 studies were subjected to both waterborne and dietary Cu exposure. The interactive effect found in the present study reduced respiration rates at combined predation risk and Cu exposure, and we cannot rule out that Cu exposure simply inhibited the perception of predation risk cues. Copepods exposed to Cu might thus be relieved of an energetically costly response to perceived predation risk. However, copepods use multisensory input to adjust and enhance their escape responses to potential predation threats (Gutierrez et al. 2011; Fields et al. 2012), and reduced alertness could have lethal consequences in real-life situations.

Respiration rates in C. finmarchicus and T. brevicornis controls were within published ranges for calanoid (Ikeda 1970) and harpacticoid (Mcallen et al. 1999) species. Although no previous study exists of predation risk on marine copepod respiration, studies from freshwater systems have demonstrated varying respiratory responses of predation risk exposure, with reduced respiration rates in daphnids, no effect in isopods, and increased respiration rates in fish larvae (Robison et al. 2018; Van Ginneken et al. 2018). The 2 copepod species tested in the present study differed markedly in their responses to predation risk exposure. We found no respiration response to predation risk exposure in C. finmarchicus. In contrast, predation risk–exposed T. brevicornis increased respiration rates by 98% compared to control respiration rates, with a tendency of female-biased groups being more sensitive. Although this is a substantial increase in energetic requirements, the estimated rate is still within published ranges for harpacticoid copepods (Mcallen et al. 1999).

Variation in predation risk–induced respiration responses might be linked to variation in life-history strategies. Reduced age at maturity and generation time may counter the adverse effects of predation (Reznick et al. 1990). Although experimental studies are few, increased development rates at some ontogenetic stages have been documented in a couple of cases (Gutiérrez et al. 2010; Bjaerke et al. 2014). In contrast, no effect was found of predation risk exposure on age at maturity in Lode et al. (2018). Predation risk may also reduce copepod foraging activity (Bollens and Stearns 1992; Cieri and Stearns 1999). Although an adaptive ontogenetic shift toward reduced age at maturity is still possible if assimilation efficiency is high (Beckerman et al. 2007), a simultaneous increase in respiration rate suggests increased metabolic costs (Jager et al. 2014). Thus, the increased respiration rate response from perceived predation risk in T. brevicornis appears to be merely an adverse stress response. Calanus finmarchicus is a pelagic zooplankton species, and the lack of response to three-spined stickleback chemical cues could be due to a lack of evolutionary experience with this coastal fish species.

Exposure to Cu can cause oxidative stress (ROS) and disrupt metabolism, for example, by affecting mitochondrial membrane potential and thus oxidative phosphorylation (Brown et al. 2004; Grosell 2011; Lauer et al. 2012). Simultaneously, elevated cellular Cu concentrations may entail increased metabolic cost by triggering detoxification processes such as increased production of metallothionein (Barka et al. 2001) and up-regulation of other antioxidant defense mechanisms (Kim et al. 2014). Contrary to our expectations, neither C. finmarchicus nor T. brevicornis responded with altered respiration when exposed to Cu alone. Similarly, Reeve et al. (1977) found no effects of Cu on copepod respiration rates either and concluded that respiration is a poor sublethal endpoint for copepod Cu exposure.

Energetic costs of cellular defense and repair mechanisms following Cu exposure are likely to occur prior to physical damage and adverse effects on life-history traits. Considering
the Cu concentration we used, a 12-h exposure period might have been too short to elicit respiration responses in our 2 copepod species. Copper readily forms organic and inorganic complexes in seawater, which reduces bioavailable cupric ions and thus toxicity (Grosell 2011). In addition, although we exposed the copepods to waterborne Cu only, dietary exposure is another important route of metal uptake in invertebrates (Wang 2002; Rainbow 2007). However, increased respiration in both a calanoid and a harpacticoid copepod species occurred within 24 h of exposure to waterborne Cu concentrations from 1 to 10 µg Cu L−1 (Moraitou-Apostolopoulou and Verriopoulos 1979; Moraitou-Apostolopoulou et al. 1983). Gene expression in the related Tigriopus japonicus was altered after 6-h exposure to waterborne Cu concentrations <10 µg Cu L−1 (Lee et al. 2007; Ki et al. 2009). In the present study, we found tendencies toward reduced respiration rates in female-biased groups exposed to 23 µg Cu L−1.

It is important to consider interactive effects of multiple stressors for successful future environmental management (Heugens et al. 2001). To best extrapolate from individual-level effects to population-level consequences, we must understand the mechanisms underlying stressor interactions (Jager et al. 2014). Most marine multiple stressor studies have focused on anthropogenic stressors combined with abiotic stressors related to climate change, for example, temperature or pH (e.g., Gunderson et al. 2016). There are limited empirical data for marine systems on combined effects with biotic stressors, in particular predation risk.

CONCLUSIONS

We found no evidence in support of our hypothesis that increased metabolic rate caused the reported potentiating interaction of combined predation risk and Cu exposure on delayed copepod development (Lode et al. 2018). On the contrary, we found that predation risk increased respiration rate in T. brevicornis, and when combined with Cu exposure, the stressors interacted to reduce respiration rates to less than expected. We also found indications that female T. brevicornis were more sensitive to both Cu and predation risk exposure than males. These contrasting findings among levels of biological organization, sex, and species emphasize the importance of understanding the underlying mechanisms of multiple stressor interactions.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4804.

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Author Contribution Statement—T. Lode conceived, designed and carried out the experiment, conducted data analysis and interpretation, wrote the manuscript, approved the final draft. J. Heuschele conceived and designed the experiment, conducted data analysis and interpretation, authored or reviewed drafts of the paper, approved the final draft. T. Andersen conceived and designed the experiment, conducted data analysis, authored or reviewed drafts of the paper, approved the final draft. T. J. Titleman conceived and designed the experiment, authored or reviewed drafts of the paper, approved the final draft. K. Borga conceived the experiment, authored or reviewed drafts of the paper, approved the final draft.

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The present article has earned an Open Data/Materials badge for making publicly available the digitally shareable data necessary to reproduce the reported results. The data are available at https://doi.org/10.6084/m9.figshare.12326219. Learn more about the Open Practices badges from the Center for Open Science: https://osf.io/tvyxz/wiki.

Data Availability Statement—Data pertaining to the present study are deposited in figshare (https://doi.org/10.6084/m9.figshare.12326219). This can be used to run the R markdown script found in the Supplemental Data. Data, associated metadata, and calculation tools are available from the corresponding author (torben.lode@bi.uio.no).

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