

Density-Dependent Metabolic Costs of Copper Exposure in a Coastal Copepod

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Abstract: Traditional ecotoxicology methods involving copepods have focused on exposure of pooled individuals and averaged responses, but there is increasing awareness of the importance of individual variation. Many biological traits are density dependent, and decisions to use single-individual or pooled exposure may affect responses to anthropogenic stressors. We investigated how conspecific density as a biotic stressor affects behavioral and respiratory responses to copper (Cu) exposure in the coastal copepod *Tigriopus brevicornis*. Adults were incubated at densities of 1, 2, or 4 individuals per replicate in 3.2 mL of exposure medium (23 $\mu\text{g Cu L}^{-1}$ or control). Our results show an interaction of Cu exposure and density on respiration. The Cu exposure increased respiration, but this effect diminished with increasing density. We also found reduced swimming activity with increasing density. We propose 2 nonexclusive alternative explanations for the density-dependent respiratory increase of Cu exposure: 1) a behavioral stress response to low conspecific density, or 2) increased Cu exposure due to increased swimming activity. We emphasize the importance of considering density-dependency in responses when designing and interpreting ecotoxicology studies. *Environ Toxicol Chem* 2021;40:2538–2546. © 2021 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals LLC on behalf of SETAC.

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INTRODUCTION

Traditional ecotoxicology practices of averaging group responses to stressor exposure neglects the importance of individual heterogeneity and genetic variation (Sih et al. 2004; Fischer et al. 2012; DeMille et al. 2016; Heuschele et al. 2019). Individual variation in responses can provide important information about stressor effects even when mean responses remain unaffected (Nikinmaa and Anttila 2019). Variation in biological traits affects individuals' fitness and populations' susceptibility to environmental change (Stearns 1992; Wolf and Weissing 2012). Failing to recognize consistent individual variation might decrease our ability to detect anthropogenic threats to the environment.

Copper (Cu) is a common environmental pollutant in coastal areas due to its continued use as an antifouling agent on commercial vessels and aquaculture net pens (Flemming and

Trevors 1989; Thomas and Brooks 2010). In Norway, Cu use in the aquaculture industry has increased 10-fold in the last decade, from 118 tonnes in 2003 to 1088 tonnes in 2016 (Skarbøvik et al. 2017). The aquaculture industry contributes >85% of the total estimated release of Cu to Norwegian coastal waters (Skarbøvik et al. 2017). Copper discharges pose an environmental risk for ecologically important nontarget organisms such as copepods.

Copper is an essential micronutrient and is required, for example, as a cofactor in antioxidant and respiration enzymes (Bertinato and L'Abbé 2004). However, Cu is toxic at elevated concentrations, generating reactive oxygen species and displacing other essential micronutrients from their respective cofactor sites (Grosell 2011). Copper toxicity is related to the amount of available free cupric ion (Cu^{2+}), but individual physiological states also modulate biological uptake and effects (Grosell et al. 2007). Adverse effects include neurotoxicity and effects on cellular metabolism and homeostasis (Brown et al. 2004; Lauer et al. 2012). In copepods, Cu exposure can delay ontogenetic development (Kwok et al. 2008; Lode et al. 2018), reduce fecundity (Reeve et al. 1977; Fitzer et al. 2013), decrease foraging activity (Sharp and Stearns 1997), cause hyperactivity (Sullivan et al. 1983), increase respiration (Moraitou-Apostolopoulou and Verriopoulos 1979;

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Moraïtou-Apostolopoulou et al. 1983), and cause increased mortality (Verriopoulos and Moraïtou-Apostolopoulou 1982; Sahlmann et al. 2019).

Conspecific density can alter responses to environmental contaminants in complex ways. For example, increasing density resulted in reduced reproduction in a gastropod that was also exposed to cadmium (Sieratowicz et al. 2013). In contrast, increasing density reduced mortality in a bryozoan that was subjected to several natural stressors in addition to Cu exposure (Lange and Marshall 2017). Interestingly, in a polychaete exposed to the polycyclic aromatic hydrocarbon fluoranthene, density had contrasting effects on population dynamics depending on exposure concentration (Linke-Gamenick et al. 1999).

Copepods are popular marine model species due to their ecological relevance, short generation time, high fecundity, and ease of husbandry (Turner 2004; Kusk and Wollenberger 2007). The number of individuals required for experimental work ranges from a few individuals, to examine life history traits (Jensen et al. 2008), to more than a thousand for certain molecular endpoints (Barka et al. 2001). Conspecific density can affect copepod behavior and physiology. Increasing density reduced copepod activity (Nilsson et al. 2017), whereas effects on respiration are equivocal but suggest potential reduction with increased density (Zeiss 1963; Castellani et al. 2005; Svetlichny et al. 2012). However, we lack knowledge on how conspecific density may modulate responses to toxicant exposure, such as Cu, in copepods.

We examined how conspecific density alters behavior and respiration responses to short-term Cu exposure in the harpacticoid copepod *Tigriopus brevicornis*. We hypothesized that Cu exposure would increase activity and respiration rate, whereas we hypothesized that increasing conspecific density would reduce activity and respiration rate.

MATERIALS AND METHODS

Sampling and experimental setup

We collected *T. brevicornis* in May 2018 from Drøbak, Norway, and kept them in continuous cultures at 18 °C. They are common in northern European tidal zones. Species of the genus *Tigriopus* have emerged as robust models for ecotoxicology studies (Raisuddin et al. 2007). We fed our cultures ad libitum with an equal mixture of the 3 algae *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Rhodomonas salina*.

Exposure solutions were prepared on the day of incubation using sterile filtered seawater (VWR® Bottle Top Filtration Unit, 0.1 µm polyethersulfone) at 33 psu (Atago hand refractometer), and pH 8.10 to 8.15 (Radiometer Copenhagen PHM 92 lab pH meter). We added Cu by a 2-step dilution series of 0.1 M CuSO₄ equivalent to a nominal exposure concentration of 23 µg Cu L⁻¹. Previous studies with *T. brevicornis* have found comparable Cu concentrations to delay development to maturity (Lode et al. 2018) and to interact with respiratory responses to predation risk exposure (Lode et al. 2020). We prepared the control exposure solution similarly but without added Cu.

We exposed adult nonovigerous female *T. brevicornis* at conspecific densities of 1, 2, or 4 individuals per replicate (equivalent to 313–1250 individuals L⁻¹) to Cu or control exposure solutions and tested effects on respiration and behavior in 2 separate experiments. Prior to the experiments (>5 h), we isolated adult copepod individuals from a stock culture into single wells of standard 24-well cell culture plates (polystyrene, Costar; Corning), to allow for gut evacuation, to reduce individual variation due to specific dynamic action (Kiørboe et al. 1985). We grouped copepods according to density treatments and transferred them for incubation into either OxoDish OD24 well plates (for respiration; PreSens Precision Sensing), or standard 24-well cell culture plates (for behavior). Copepods were incubated at 18 °C during the experiments. The respective exposure solution was added to a total of 3.2 mL well⁻¹ before we sealed the plates to reduce gas exchange using a plastic film (Nunc™ Sealing Tape). We removed gas bubbles by filling the exposure solution through the plastic film using a syringe. For the respiration experiment, we finally resealed the plates with an aluminum seal (Silverseal; Greiner Bio-One). For the behavior experiment, we required light for filming and therefore these plates were not aluminum sealed. To avoid cross-contamination we kept Cu and control exposure solutions on different well plates. We ran both experiments twice using 2 well plates with interchanged positions (i.e., series) to control for placement effects.

Respiration experiment

To quantify the metabolic costs of Cu exposure and potential density-dependency in response, we carried out a respiration experiment on 13 and 15 November 2018. The method was similar to that of Lode et al. (2020). We placed readied OxoDish OD24 well plates on SensorDish® Reader units (PreSens Precision Sensing) submerged in a water bath. We incubated in total 80 replicate units spread evenly among Cu treatments and densities. Blanks consisted of the respective treatment exposure solutions. Two normal blanks and 2 undersaturated blanks bubbled with nitrogen were included per well plate for oxygen influx correction. We visually checked for gas bubbles immediately before and after incubation. Oxygen levels were measured every 30 s for the total exposure period of 12 h. At the end of the experiment, we checked for copepod survival and physical condition (e.g., external injuries) with a Nikon SMZ 745T stereo microscope. We then took a picture of all copepods used for subsequent prosome length measurements in ImageJ (Ver 1.51j/Java 1.8.0_112).

We excluded the first hour after sealing the well plates as an acclimation phase. For the following 12-h exposure period, we calculated respiration rates as successive differences of 15-min rolling averages of oxygen measurements per well. We corrected for oxygen concentration-dependent influx using a nonlinear asymptotic regression model based on all blanks per series, and then extracted the median of influx-corrected respiration rates per replicate well. Respiration rates (nmol O₂ µg dry wt⁻¹ h⁻¹) were normalized by ash-free dry weight (in µg) calculated from prosome length (L, µm): ln dry weight = 2.74

$\times \ln L - 16.41$ (Chisholm and Roff 1990). Reducing the data to one representative median value per replicate is a conservative approach and reduces noise from temporal variation in measured oxygen levels, which may be a challenge with *Tigriopus* sp. activity patterns (McCallen et al. 1999).

Behavior experiment

To investigate how conspecific density affects copepod activity and response to Cu, we carried out a behavioral experiment on 24 and 25 July 2018. We incubated in total 48 replicate units spread evenly among Cu treatments and densities. We used a video setup (Heuschele et al. 2019) to repeatedly film each well from below throughout the exposure period. By using an electroluminescent light sheet (36×26 cm, PolyWeld; Technomark) to provide constant light from above (parabolic anodized reflector: $1.5 \mu\text{E m}^{-2} \text{ s}^{-1}$), the image quality was improved compared with that of Heuschele et al. (2019). Each well was filmed at 20 frames s^{-1} for 2 min every 58 min, for an exposure period of 17 h, yielding in total 17 recordings per replicate well. We first subtracted the background (i.e., the average pixel value) from each frame and then determined the position of moving animals using the Trackmate plugin for ImageJ/Fiji (ImageJ Ver 2.0.0 [Schindelin et al. 2012]; Trackmate Ver 3.8.0 [Tinevez et al. 2017]). Finally, we calculated the characteristics swimming speed and gross swimming distance for each track, and summarized all track data per well to determine average speed and gross distance per individual per movie. For each movie, we also described activity switching as the number of changes between resting and moving behavior divided by the number of individuals.

Statistical analysis

All statistics were carried out using R, Ver 3.6.0 (R Core Team 2019). Prior to statistical analyses, we checked for outliers and potential invalid replicates (e.g., mortality, gas bubbles). Respiration and behavioral endpoints were analyzed using linear mixed effects models (package: nlme; Pinheiro et al. 2017). We used logarithmic and square root transformation to correct for non-normality of data, tested for the inclusion of weighted fixed effects to correct for heteroscedasticity, and checked model residuals by visual inspection. We used the dredge function of the MuMIn package (Barton 2019) to test by Akaike information criteria for small sample size (AICc) model selection for the inclusion of, and interactions among, the fixed effects Cu, density and time (model selection criterion: $\Delta\text{AICc} > 2$). If this resulted in multiple alternative models, we conservatively selected the minimum adequate model, but we also report alternative best models based on ΔAICc weight.

Respiration analysis

We tested the effect of the explanatory variables Cu and density on the response variable respiration for the full 12-h exposure period, with series included as random intercept to control for the block design. Prior to analyzing respiration rates, we removed 4 replicates due to handling errors, whereas there

were no invalid replicates due to mortality or gas bubbles. We defined and removed outliers if they exceeded a threshold of $3 \times$ standard deviation of the total data. We also investigated temporal variation in respiration rates by extracting median respiration rates for the time intervals 0 to 3 h, 3 to 6 h, 6 to 9 h, and 9 to 12 h. For this analysis, we included time intervals as levels of a categorical explanatory variable representing time, and we used replicate well identity nested within series as a random intercept to control for temporal pseudoreplication.

To examine the system sensitivity and validity of results, we used the influx-correction model on the normal blanks and extracted the medians of oxygen change over time, similarly as for replicate wells. Overall, these blank median values varied at approximately zero and indicated a good fit of the influx correction model. Variation in total oxygen consumption rates per well ($\text{nmol O}_2 \text{ well}^{-1} \text{ h}^{-1}$) for single-individual control replicates overlapped with the variation for the blank medians. This suggests that single-individual incubation of *T. brevicornis* ($\sim 2 \mu\text{g}$ ash-free dry wt) stretches this system to its limit of detection. Importantly though, estimated respiration rate from single-individual control replicates was similar to those found in the 2- and 4-individual controls, and we have therefore included all data for the statistical analyses. In contrast, total oxygen consumption rates per well were well above detection limits for all tested densities when exposed to Cu. The present study's experimental setup has previously been tested and validated in Lode et al. (2020) for pooled incubation of 10 *T. brevicornis* individuals per replicate and single-individual incubation of the larger copepod species *Calanus finmarchicus*.

We also found indications of a slightly higher O_2 net influx rate in Cu exposure solution blanks than control blanks. This means that we are at risk of underestimating respiration rates in Cu exposure solution. Importantly though, our results suggest increased copepod respiration from Cu exposure, and thus the indicated blank differences make our conclusions conservative.

Behavior analysis

We tested the effect of the explanatory variables Cu, density, and time on the response variables swimming speed, gross distance, and activity switching by analyzing both for tracks and movies (i.e., per individual per movie). To control for the nested design and temporal pseudoreplication, we allowed for a random intercept for each replicate well identity and a random slope for each run (i.e., time). For this, run was centered prior to analysis. We removed 2 replicates due to handling errors, whereas there were no invalid replicates due to mortality. To avoid outliers due to false detections in the behavioral analysis, speed values exceeding the upper 0.001 percentile of total data were set to nonavailable and we removed tracks < 2 mm long, of net distance < 1 mm, or shorter than 2 s. Of the remaining tracks, we tested how many occurred simultaneously. On average, 96% of all frames contained the correct number of tracks (i.e., less than or equal to the number of animals per bottle), whereas animal mirror images occurred in 4% of frames. This occurred in all treatments, and hence we did no further cleaning of the data.

RESULTS

Respiration rates were affected by Cu exposure in a density-dependent manner by which increasing density reduced the respiratory increase caused by Cu ($df=8$, $\Delta AICc$ weight = 0.892; Table 1 and Figure 1). Control respiration rates, when single-individual replicates were excluded, were on average $0.305 \pm 95\%$ confidence interval $0.098 \text{ nmol O}_2 \mu\text{g dry weight}^{-1} \text{ h}^{-1}$. Respiration rates did not vary among densities for control replicates. The Cu exposure increased respiration rates in single-individual density replicates to an average of $0.840 \pm 0.167 \text{ nmol O}_2 \mu\text{g dry weight}^{-1} \text{ h}^{-1}$.

Including the time-variable reduced the effect of density on respiration rate ($df=22$, $\Delta AICc$ weight = 0.420; Table 1 and Figure 2). Model predictions indicate that Cu potentially increased respiration rate at all densities during the first 3 h of exposure.

Swimming speed was not affected by density or Cu when analyzed the movie but decreased with time during the experiment ($df=8$, $\Delta AICc$ wt = 0.244; Table 1 and Figure 3). Gross distance per individual per movie decreased with increasing density and with time ($df=9$, $\Delta AICc$ wt = 0.180; Figure 4), but alternative best models indicated a potential effect of Cu exposure (Table 1 and Figure 5). The best model for gross distance of the tracks suggested a time-dependency by which the activity of copepods leveled off for all densities by the end of exposure ($df=10$, $\Delta AICc$ wt = 0.439; Table 1). Activity switching occurred at a higher rate from 5 h into the

experiment and onward after Cu exposure, but in general decreased with time and increasing density ($df=11$, $\Delta AICc$ wt = 0.237; Table 1 and Figure 6); an alternative best model suggested an inverse density-dependency in this Cu effect (see the Supplemental Data).

DISCUSSION

We investigated effects of conspecific density on responses to Cu exposure in the coastal copepod *Tigriopus brevicornis*. Copper exposure increased respiration with diminishing effect at increasing densities, whereas increasing density by itself also reduced swimming activity. We present 2 alternative, non-exclusive explanations for the interactive effect of density and Cu on respiration: the first related to behavioral stress and the second related to Cu exposure.

Our first explanation is that a behavioral stress response at low conspecific densities in combination with Cu exposure caused elevated metabolic stress in *T. brevicornis*. Combined stressor exposure can increase subtle costs from biological stressors (Lode et al. 2018). Copepods are able to sense conspecifics and predators using mechanical and chemical signals (Heuschele and Selander 2014), and thus should be able to assess their surrounding risk landscape. Interestingly, the pelagic copepod *Acartia tonsa* also responded to increasing conspecific density by reducing activity (Nilsson et al. 2017), but presumably as a stress response to overcrowding. Copepod responses to density probably vary among species

TABLE 1: Output from model selection by AICc using the dredge function in the MuMIn package^a, including minimum adequate best model and alternative best models within $\Delta AICc < 2$

Model	df	Log Lik	AICc	$\Delta AICc$	$\Delta AICc$ weight
Respiration full 12-h exposure period					
= Copper × Density	8	528.160	-1038.171	0.00	0.892
Respiration temporal variation					
= Copper × Density × Time	25	2009.973	-3965.131	0.00	0.573
= (Copper + Density + Time) ²	22	2006.107	-3964.507	0.62	0.420
Swimming speed per track					
= Copper + Time	9	-2122.456	4263.0	0.00	0.180
= Time	8	-2123.585	4263.2	0.24	0.159
= Copper + Density + Time	10	-2121.727	4263.5	0.56	0.136
= Density + Time	9	-2122.988	4264.1	1.06	0.106
= Copper × Density + Time	11	-2121.034	4264.2	1.19	0.099
= Copper × Time	10	-2122.428	4264.9	1.96	0.067
Swimming speed per individual per movie					
= Time	8	-366.590	749.4	0.00	0.244
= Copper + Time	9	-365.725	749.8	0.34	0.206
= Copper × Density + Time	11	-364.211	750.9	1.46	0.117
Gross distance per track					
= Density × Time	10	-3479.338	6978.8	0.00	0.439
= Density × Time + Copper	11	-3479.191	6980.5	1.72	0.185
Gross distance per individual per movie					
= Copper + Density + Time	10	-2865.899	5752.2	0.00	0.184
= Copper × Density + Time	11	-2864.869	5752.2	0.02	0.182
= Density + Time	9	-2866.961	5752.3	0.05	0.180
= Copper × Time + Density	11	-2865.555	5753.6	1.39	0.092
= Copper × (Density + Time)	12	-2864.515	5753.6	1.40	0.091
Activity switching per individual per movie					
= Copper × (Density + Time)	12	-1106.456	2237.5	0.00	0.293
= Copper × Time + Density	11	-1107.714	2237.9	0.43	0.237

^aBarton (2019).

AICc = Akaike information criteria for small samples; Log Lik = log likelihood.

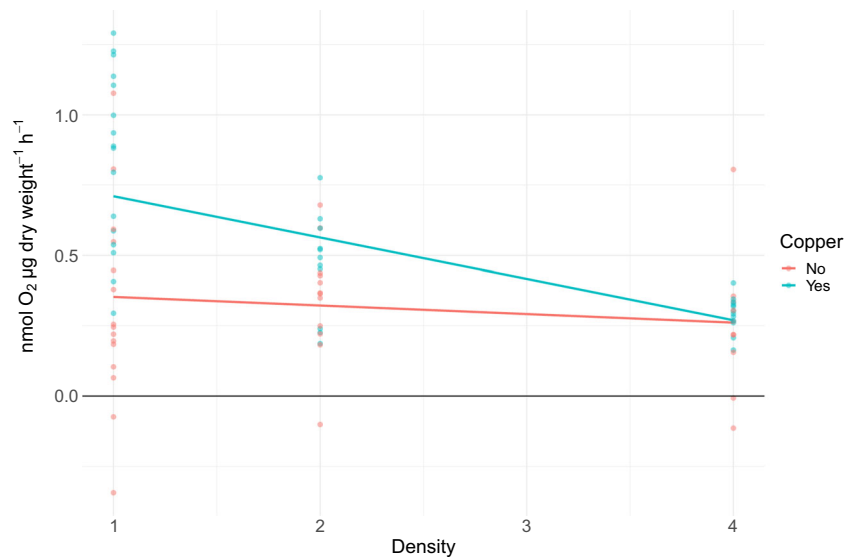


FIGURE 1: *Tigriopus brevicornis* respiration rates ($\text{nmol O}_2 \mu\text{g dry wt}^{-1} \text{h}^{-1}$) for the full 12-h exposure period. Estimated means (lines) are predicted from the minimum adequate model (Respiration rate = Copper \times Density). Median respiration rate per replicate well is shown as points.

according to their natural habitats. *Tigriopus* species are well adapted to high conspecific densities and may reach densities of several thousand individuals/L in tidal pools (Fraser 1936). High conspecific densities can be beneficial in terms of predation risk dilution, mating opportunities, and reproductive success (Ambler 2002; Giske et al. 1997).

Documented respiratory responses to density are ambiguous. Increasing numbers of conspecifics reduced respiration in *C. finmarchicus*, whereas decreasing the confinement volume had no effect (Zeiss 1963). Conspecific density did not affect respiration in the copepods *Oithona similis* (Castellani et al. 2005) or *Arctodiaptomus salinus*, but increasing density possibly reduced respiration in male *Calanipeda aquaedulcis* (Svetlichny et al. 2012). Interestingly, both decreasing

confinement volume and increasing numbers of conspecifics increased ammonium excretion rates in several pelagic copepods (Nakamura et al. 2019), indicating increased metabolic activity. In the present study, we found that density had no apparent effect on *T. brevicornis* respiration in our controls but modulated the response to Cu exposure.

Our findings support previous reports of increased copepod respiration by Cu exposure (Moraitou-Apostolopoulou and Verriopoulos 1979; Moraitou-Apostolopoulou et al. 1983; but see Reeve et al. 1977), but only at low densities. We found no effect of Cu exposure at the highest incubation density when data were analyzed for the full 12-h exposure period. Lode et al. (2020) similarly found no effect of Cu ($23 \mu\text{g L}^{-1}$) on *T. brevicornis* respiration when exposing 10 individuals per

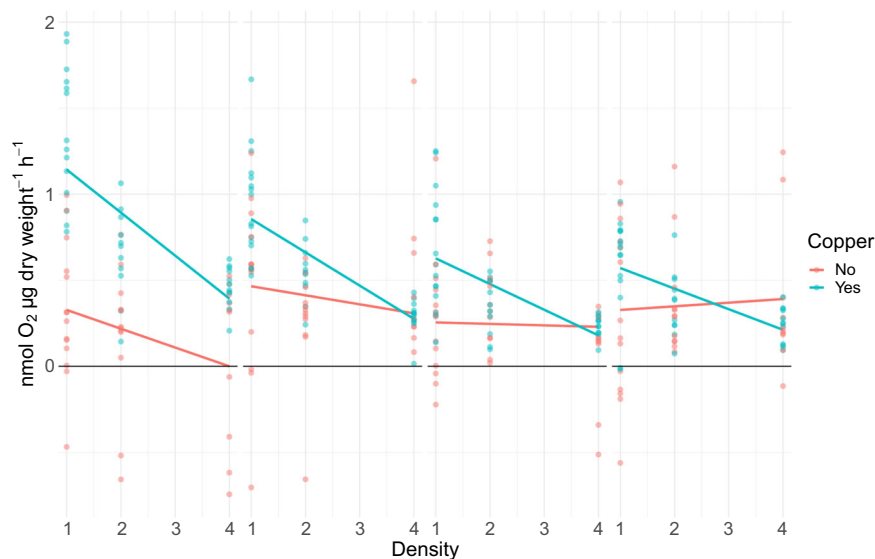


FIGURE 2: *Tigriopus brevicornis* respiration rates ($\text{nmol O}_2 \mu\text{g dry wt}^{-1} \text{h}^{-1}$) per 3-h time interval (temporal variation analysis). Plotted for the 3-h time intervals: 0–3 h, 3–6 h, 6–9 h and 9–12 h. Estimated means (lines) are predicted from the minimum adequate model (Respiration rate = (Copper + Density + Time)²). Median respiration rate per replicate well is shown as points.

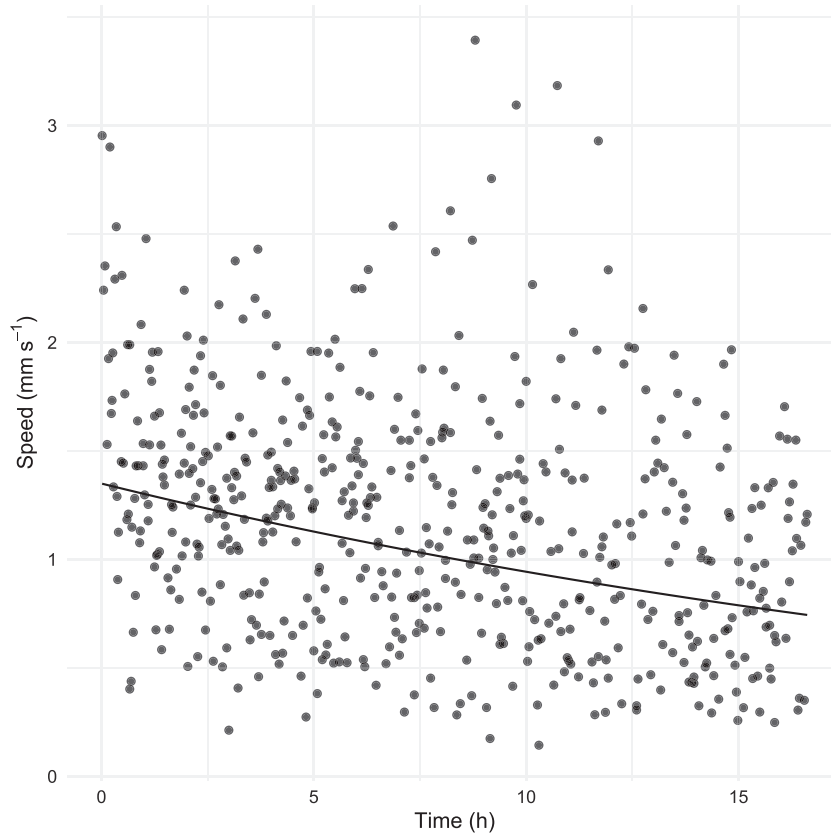


FIGURE 3: Swimming speed (mm s^{-1}) per movie per individual *Tigriopus brevicornis*. Estimated mean (line) is predicted from the best minimum adequate model (Swimming speed = Time). Raw data are shown as points.

replicate (corresponding to $3125 \text{ individuals L}^{-1}$) for 12 h. The density-dependent respiratory response to Cu exposure suggests a behavioral stress response in *T. brevicornis* to low conspecific densities.

Altered behavior is an early response to anthropogenic stressor exposure (Tuomainen and Candolin 2011). Copper exposure can cause avoidance behavior in daphnids (Lopes et al. 2004), increase locomotor activity in shore crabs (Hebel et al. 1997), and alter swimming activity in copepods (Sullivan et al. 1983). Activity switching decreased over time at a slower rate in our Cu-exposed *T. brevicornis* than in controls. Our data further indicated that Cu might have exerted the greatest effect on activity switching in singly incubated individuals. Surprisingly, we found no clear effects of Cu exposure on more traditional behavior endpoints such as swimming speed or gross distance travelled in *T. brevicornis*. Sullivan et al. (1983) reported hyperactivity in copepods exposed to $25 \mu\text{g Cu L}^{-1}$ after 24-h exposure. It is possible our exposure period was too short to induce further behavioral effects with the Cu concentration used; however, the most pronounced respiration responses to Cu were during the initial hours of exposure.

Our second explanation for the respiration rates at low densities is that increased swimming activity at reduced conspecific density caused increased exposure of individuals to Cu. Increased swimming activity could increase cupric ion (Cu^{2+}) encounter rates, analogous to nutrient uptake in motile

phytoplankton (Pasciak and Gavis 1974). A higher uptake of Cu could thus trigger higher metabolic activity to counter the effects of Cu stress, which did not happen in the denser treatments due to lower swimming activity.

Swimming activity in our controls declined over time, but respiration rates were consistent and within reported ranges for harpacticoid copepods (McCallen et al. 1999). We subjected the copepods to a gut evacuation period prior to the experiment and did not feed them during the exposure period. The measured respiration rates thus cover metabolic costs related to basal metabolic rate and motility (Ikeda et al. 2001). Declining energy reserves could have caused reduced copepod swimming activity over time (Holm et al. 2019); however, this should also then have resulted in reduced respiration (Kjørboe et al. 1985). The uncoupling of activity and respiration in our controls support the idea that routine swimming behavior is of marginal importance to copepod energy budgets (Alcaraz and Strickler 1988; Titelman and Kjørboe 2003). Thus, increased swimming activity should not affect copepod respiration response to Cu exposure because of metabolic demands.

There are many advantages to recognizing zooplankton as individuals rather than homogenous groups of similar beings (Wolf and Weissing 2012; Nikinmaa and Anttila 2019). For example, single-individual incubation of *T. brevicornis* was useful for identifying individual effects of predation risk and Cu exposure among different genotypes (Lode et al. 2018). However, it is important to remember, when designing and

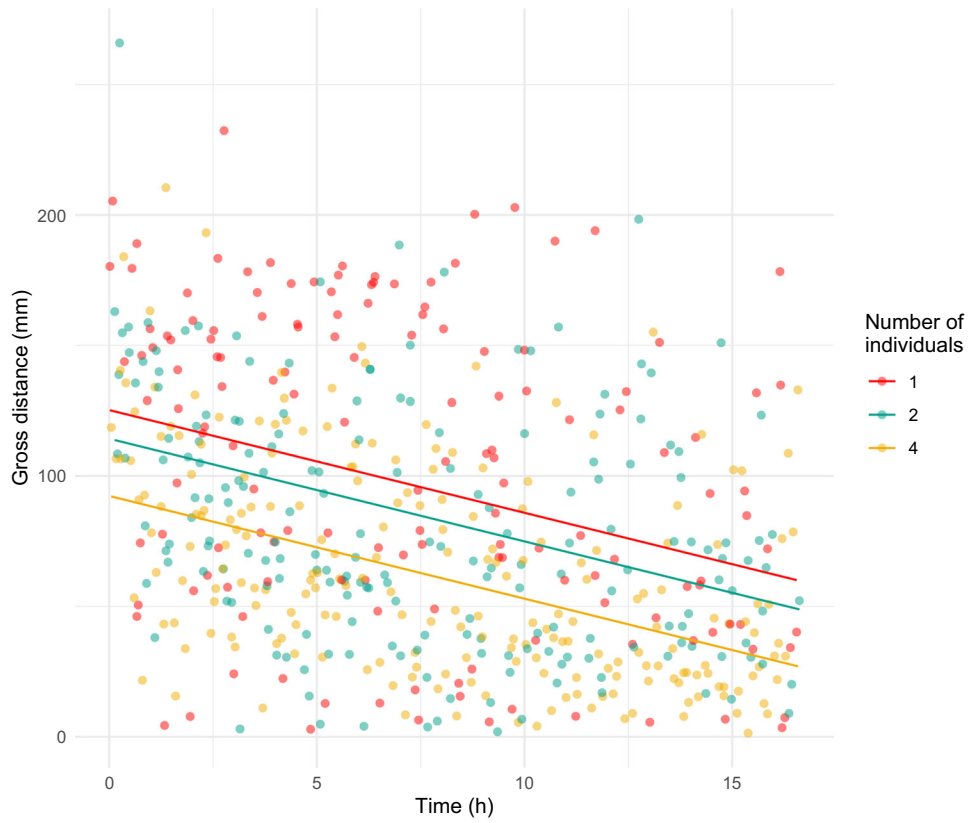


FIGURE 4: Gross distance traveled (mm) per individual *Tigriopus brevicornis* per movie. Estimated means (lines) are predicted from the best minimum adequate model (Gross distance = Density + Time). Raw data are shown as points.

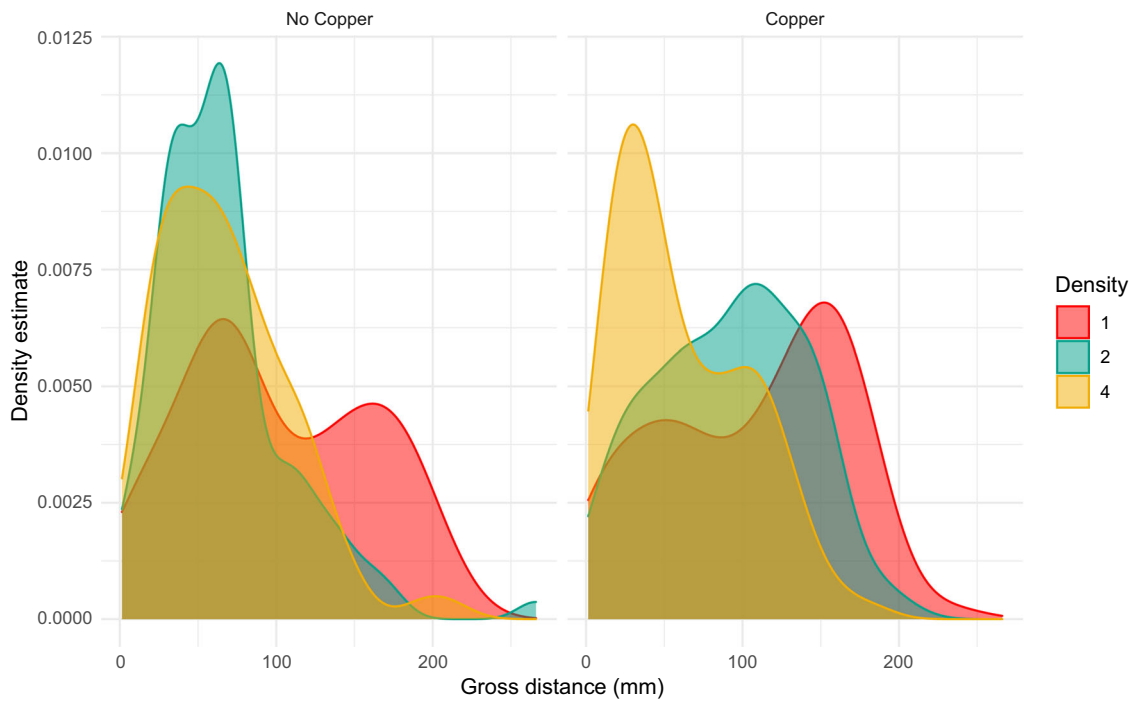


FIGURE 5: Distribution plots of gross distance traveled (mm) per individual *Tigriopus brevicornis* per movie, colored by density and separated by treatment (control/copper). The y-axis represents proportionate kernel density estimates for the registered gross distances traveled (per individual) on the x-axis.

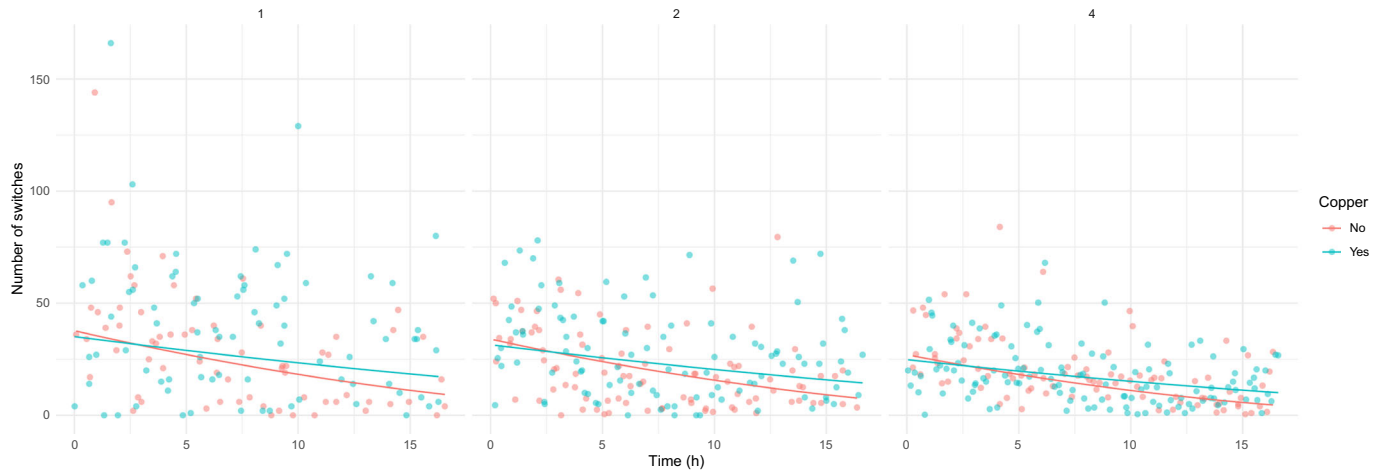


FIGURE 6: Activity switching per individual *Tigriopus brevicornis* per movie (i.e., number of switches between resting and active behavior). Plotted for the incubation densities: 1, 2, and 4 individuals. Estimated means (lines) are predicted from the best minimum adequate model (Activity switching = Copper \times Time + Density). Raw data are shown as points.

interpreting ecotoxicology studies, that conspecific density during exposure can alter responses to anthropogenic stressors.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at <https://doi.org/10.1002/etc.5141>.

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Data Availability Statement—Data, associated metadata, and calculation tools are available from the corresponding author (torben.lode@ibv.uio.no).

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