The Hidden Dimension: Context-Dependent Expression of Repeatable Behavior in Copepods

Jan Heuschele,* Torben Lode, Tom Andersen, and Josefin Titelman
Section for Aquatic Biology and Toxicology, Department of Biosciences, University of Oslo, Oslo, Norway

Abstract: In ecotoxicology and aquatic ecology, we often ignore responses of individuals and focus on average responses. However, both terrestrial and aquatic animals display consistent behavioral differences between individuals. The distribution of behavioral differences within a population contains vital information for predicting population responses to novel environmental challenges. Currently, individual data for behavioral and physiological traits of small marine invertebrates are few, partly because such variation is lost within published group means and assumed normality. We tested the combined effects of an inorganic contaminant (copper) and a biological stressor (i.e., chemical cues of a fish predator) on activity in a marine copepod. Although direct stress effects were weak, individuals behaved consistently differently, depending on the context. Individual differences in behavior were only expressed under the influence of kairomones, but not by copper exposure alone. This finding indicates that copepods express repeatable and context-dependent behavior. We also demonstrate how large variations in behavioral data can hide consistent differences between individuals. Environ Toxicol Chem 2020;39:1017–1026. © 2020 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals LLC on behalf of SETAC.

Keywords: Behavior; Zooplankton; Individuality; Predator-prey; Multiple stressors; Copper

INTRODUCTION

Behavioral modifications are generally the first response to novel environmental conditions (Tuomainen and Candolin 2011), for example, when individuals modify motility patterns, actively hide from perceived risk, or migrate to more suitable places. Behavior thus plays a key role in the interaction between an organism and its environment (Sih et al. 2004). Regardless of their perceived simplicity, the motility patterns of zooplankton and other marine invertebrates determine their exposure to predators, their success in finding mates, and their foraging success (see examples in Kiorboe 2008). Copepods form the crucial link between primary production and higher trophic levels in the oceans (Turner 2004) and are one of most numerous animal groups on the earth (Humes 1994). Due to their importance, they are also increasingly used in ecotoxicological experiments (Raisuddin et al. 2007; Kulkarni et al. 2013).

Although behavior is a plastic trait, individuals also exhibit consistent differences in behavior across and within situations (Sih et al. 2004), which are sometimes referred to as “animal personality” (Carter et al. 2013). We refrain from discussing the phenomena in an anthropomorphic tone, but we are interested in consistent behavioral differences between individual aquatic invertebrates. Consistent differences in behavior can be quantified using the concept of repeatability. Repeatability is defined as the proportion of total variance in a behavioral trait that can be explained by the variance between individuals; it is sometimes also referred to as the intraclass correlation coefficient (Nakagawa and Schielzeth 2010).

Behavioral dissimilarities between individuals can be due to differences in internal states (e.g., physiology, gender, age, satiation), current and past context (e.g., feeding, predator avoidance), and genetics (Wolf and Weissing 2010). Given that behavior is heritable (Stirling et al. 2002), a novel selection pressure acting on the variation in behavior can lead to rapid adaptation of other advantageous traits (Wolf and Weissing 2012). Novel selection pressures typically result from changes in the chemical environment or the biotic community, for example, those caused by anthropogenic toxicants (Coutellec and Barata 2011). Studying interindividual differences in behavior when assessing the toxicity of a pollutant would allow us to quantify the proportion of the population...
that could potentially swim to safer grounds and form the basis of a more resistant population. The existence of consistent differences in behavior is well established in vertebrates but such differences not well documented in marine invertebrates, perhaps reflecting a discrepancy in research efforts (Kralj-Fiser and Schuett 2014). The call for making better use of individual variation information in ecotoxicology studies has recently also become louder (Nikinmaa and Anttila 2019).

Even clonal cladocerans display consistent behavioral differences between individuals (Heuschele et al. 2017). However, one would expect larger differences in sexually reproducing species, like copepods. One would also expect larger and persistent individual differences in environments with a trade-off between risk exposure and resource availability (Luttbeg and Sih 2010). For example, if food cues are absent, but predator cues are present, all prey should behave cautiously (Brown and Kotler 2004) because there is less “chemically” apparent benefit of searching for food (Fechter et al. 2004). However, when both cues are present (or absent), innate risk-taking behavior of individuals might trigger differences in exploratory behavior (e.g., Vainikka et al. 2011). Balanced conditions of risk and resources should be abundant in both the benthic and pelagic aquatic environment, given the patchy nature of the marine environment, with its varying levels of risk and resources. These conditions give us an opportunity to develop and establish consistent differences in behavior.

Coping with combinations of stressors such as predation risk and food limitation is also expected to manifest in direct behavioral changes. Effects of combined exposure to multiple stressors can range from antagonistic to synergistic when compared with effects of single stressors (Holmstrup et al. 2010; Laskowski et al. 2010; Fischer et al. 2012). Even so, the effects of environmental stressors are often tested separately, using laboratory populations of limited genetic diversity and with an unlimited food supply (e.g., Organisation for Economic Co-operation and Development 2012).

In the present study our aim was 2-fold: 1) to test how multiple stressors affect motility patterns in copepods, and 2) to quantify context-dependent behavioral consistency (i.e., repeatability in behavior).

We tested this by exposing a temperate harpacticoid copepod (Tigriopus brevicornis) to copper (CuSO₄) and perceived predation risk (fish chemical cues, i.e., kairomones) while repeatedly recording individual behavior.

We chose Cu for its long history as a biocide in agricultural and aquaculture (Treves-Brown 2000), its use in antifouling paints (Burridge et al. 2010), and its potential toxicity to nontarget organisms (Engle and Spears 2000; Van-Zwieten et al. 2004; Rábago-Castro et al. 2006). Copper is an essential element and a cofactor for enzymes (Bertinato and L’Abbé 2004), for example, cytochrome c oxidase. However, elevated Cu concentrations induce the formation of reactive oxygen species (Grosell 2011), which act as a general metabolic stressor and damage lipids, proteins, and DNA (Flemming and Trevors 1989; Lewis et al. 2016). We anticipated that Cu would modify behavior by inducing initial hyperactivity (Sullivan et al. 1983), thereby increasing metabolic activity and depleting energy resources. Kairomones induce behavioral changes that reduce the detectability of individuals under predation risk (Kats and Dill 1998). The kairomones were sourced from three-spined stickleback (Gasterosteus aculeatus), a fish common in marine and limnic systems of the northern hemisphere; kairomones are known to trigger responses in copepods (Bjærke et al. 2016; Lode et al. 2018). We predicted that kairomones would reduce swimming and exploration activities to reduce encounters with predators, and that their combined effects on activity measures would be stronger than the additive effects of the stressors, because synergistic effects seem to be common with respect to toxicant–biotic stressor interactions (reviewed in Holmstrup 2010). We further anticipated consistent differences in swimming activity between individuals throughout the duration of the experiment, especially in the control treatment.

**METHODS**

**Experimental organism**

*Tigriopus brevicornis* inhabits tidal zones and splash pools, and is tolerant of salinity and temperature fluctuations (Damgaard and Davenport 1994). A population of approximately 200 *T. brevicornis* individuals was collected from a splash pool (32 PSU) near Drebak Biological station, University of Oslo, Oslo, Norway (59°39′48.2″N, 10°37′32.7″E) 1 mo prior to the experiments. The culture was kept in filtered seawater at 30 PSU, 18°C, and a 12:12-h light:dark cycle. Copepods were fed ad libitum an equally mixed diet of *Isochrysis galbana*, *Dunaliella tertiolecta*, and *Rhodomonas salina*.

**Experimental procedure**

We tested the behavioral effects of Cu and kairomones using a 2 × 2 factorial design. We quantified the motility pattern of individual copepods every 22 min for a 1-min period over a time period of 13.2 h during exposure to one of 4 treatment combinations (control, Cu, kairomone, and Cu + kairomone).

To obtain water with kairomones, we incubated acclimatized field-caught three-spined sticklebacks at a concentration of 2 fish l⁻¹ for 48 h in an acid-washed (0.1 M HCl) glass tank filled with filtered and continuously aerated seawater (13°C, 30 PSU, 12:12-h light:dark cycle). Three-spined sticklebacks and our model organism *T. brevicornis* co-occur in the Oslofjord (Norway) area. During incubation, fish were fed with *T. brevicornis* at initiation and again after 24 h. After 48 h, we removed the fish with a clean fishnet. The water was filtered (1.2 μm, GF/C filter) and frozen in glass bottles for subsequent use in the experiments. Water for the treatments without kairomones was prepared similarly, but without addition of fish or copepods. The preparation was previously tested and applied in an experiment targeting the development of *T. brevicornis* (Lode et al. 2018).

Approximately 5 h prior to each experimental round, the frozen water with and without kairomones was thawed in...
separate temperate water baths (18°C). Then CuSO₄ was added to the relevant treatments in a 2-step dilution process to a final concentration of 6 µg CuL⁻¹, which is in the sublethal range for copepods (Reeve et al. 1977a, 1977b; Kwok et al. 2008) and has been measured in busy harbor areas (Wang et al. 2002). Water for the non-Cu treatments (control and kairomone) was prepared through the same 2-step dilution process, but distilled water was added instead. Once at the experimental temperature (20°C), we filled 70-mL cell culture flasks (TC Flask T25; Sarstedt) with 40 mL of the respective treatment water.

Three hours prior to the start of each round, egg-bearing copepod females were picked from the stock culture. Within 15 min prior to filming we checked females for intact egg sacs under a dissecting microscope and transferred them to individual flasks without food. We chose to only include egg-bearing females to focus on behavioral variability within a single sex and reproduction state. None of the females dropped egg sacs during the experimental period.

**Filming**

We filmed the swimming behavior of the copepods using an automated setup that allows for the unsupervised recording of 10 flasks at a time (Wolf and Heuschele 2018). In the setup, a row of flasks is filmed one flask at a time using a camera (Raspberry Pi NoIR Camera with CS mount, 6 mm lens) mounted on a movable computer-controlled platform (Figure 1). An LED strip mounted 4 cm above the bottles provided white light with a photosynthetically active radiation of 25.1 µEm⁻²s⁻¹. The exact light spectrum is provided in Supplemental Data 1 and Figure EA1.

For practical reasons associated with the filming setup (Figure 1), the experiment was conducted in 8 rounds. In each round, we tested 10 new individual egg-bearing females, each of which was randomly assigned to 1 of the 4 treatments. Each treatment was represented with at least 2 randomly placed replicates during each round. In each experimental round, 10 individuals were filmed sequentially, each for a 1-min period every 22 min. This was repeated 36 times, resulting in an experimental period of 13.2 h. Filming in each round started at the same time of day (~14:00). In total, we tested activity and behavior of 80 individuals, that is, 20 individuals/treatment, with 36 measurements/individual.

The video sequences were recorded at 30 frames s⁻¹, and the field of view included the whole waterbody (44 × 35 × 25 mm, w × h × d), including the bottom of each flask. The filmed animal was in focus at all times. We used a custom python script to control the camera and the connected stepper motor (Phidgets Bipolar Stepper) that moved the camera (see Supplemental Data 2). The treatment was randomly assigned to each bottle for each experimental round.

At the end of each round, the copepods were preserved individually in Lugol’s solution. One week after the end of all rounds, we photographed the preserved individuals using a digital camera mounted on a binocular microscope and measured individual body length as distance from the tip of the cephalosome to the...
caudal ramus using the image analysis software Fiji (Schindelin et al. 2012). We corrected the reported body sizes for shrinkage by 17% (Jaspers and Carstensen 2009). The females had an average total body length of 1.069 mm ± 0.071 standard deviation (SD).

Movie analysis

The recorded video sequences were analyzed using custom tracking software based on the Open Source Computer Vision Library (OpenCV, Ver 3.3.0; Bradski 2000). The software identifies the position of the individual copepod in the flask (XY coordinates). Each sequence of continuous position data is assigned a unique track identifier, if the particle is within a certain threshold distance from the previous one. Because we had only one individual in each bottle, mostly one track was recorded at any given time.

Individuals were not tracked when they remained stationary for longer than 10 s due to the use of a rolling average in the feature extraction process, and thus missing position data were classified as resting behavior. We excluded tracks shorter than 1 s in duration from the final data set, to prevent false positives from sensor flickering or reflections in the flask wall. Occasionally a mirror image in the bottle caused a second longer track to appear, which we sorted out by comparing the time and length of 2 tracks with an R script prior to the statistical analysis (see Supplemental Data 3). We also removed sudden speed increases caused due to flickering or false track assignment during the particle detection process, by setting speed and position measurements exceeding the 95 quantile of all data as nonavailable. Thereby gross distance, area, and speed measurements were not influenced by these outliers. Tracks were also visually inspected prior to analysis to ensure that they represented natural movement patterns (see Supplemental Data 3).

As a first measure of general activity, we determined whether movement was detected at all during one filming event. If movement was not detected in a specific 1-min filming sequence ("run"), the animal was considered to be resting. As a second proxy for the general activity of the animals, we measured the duration an individual was detected swimming during each run. From the position data of each run we then computed 3 behavioral traits, each of which captured different movement characteristics (see Supplemental Data 3 and 4). First, as a measure of nontargeted exploration behavior, we determined the explored area (mm²) calculated as the area enclosed by the convex hull of the coordinates in the track. Second, we calculated the average swimming speed (mm s⁻¹) excluding periods of rest, and last the travelled gross distance (mm).

Statistical analysis

Statistical analyses were done using statistical software R (Ver 3.5.1; R Core Development Team 2018). We always started out with a mixed effect model allowing for all fixed factor interactions (~Cu x kairomone). To control for the nested design and the repeated measurement of individuals, we included individual ID nested within experimental round as random factors in all models. All models also allowed for random slopes for time (~1 + run | round/ID). We further centered time and included it in all our analysis to avoid strong correlations between intercept and slope in the random factor. The best minimal adequate models were selected using stepwise backward elimination. As long as an interaction was statistically significant, all included main effects and lower order interactions were retained in the model. We also tested the best minimal adequate model against the next simpler one with a likelihood ratio test. If the models were similar, we chose the simpler model. The distribution of the residuals of the final model was checked visually (normal quantile–quantile plots, histogram of residuals) to confirm the adequacy of the chosen test. For transparency, we included the complete analysis in the Supplemental Data (3 and 4).

Direct effects. To examine whether Cu and kairomones affected the overall activity of the copepods, we first tested whether the animals moved or remained still in the video sequences using generalized linear mixed effect models of the binomial family (lme4 package; Bates et al. 2015). We then tested whether the time spent swimming depended on treatment and time using a linear mixed effect model in the nlme package (Pinheiro et al. 2017).

The general influence of body size on the behavioral responses was tested using a linear mixed effect model with size and time as fixed variables. Although body size seemed to influence the behavioral trait expressions, the results depended on 2 large outliers (see complete analysis in the Supplemental Data for details). We therefore omitted the factor body size in the subsequent analyses of direct effects on traits related to swimming behavior.

The direct effects of the treatments on each of explored area, average swimming speed, and gross distance were also analyzed using linear mixed models, with kairomones and Cu and time as fixed independent variables. Each response variable was power-transformed prior to analysis to meet normality assumptions (Box and Cox 1964). In these analyses, we only included video sequences in which the animals moved.

Repeatability analysis. Consistent behavioral differences between individuals were calculated using repeatability (R), which is simply the variance between individuals divided by the total phenotypic variance R = V_C/(V_C + V_e), with V_C being the variation between individuals and V_e being the variance within repeated measures of one individual (Boake 1989). We used the package rptR (Stoffel et al. 2017) to calculate repeatability for Gaussian models. Each model included the respective fixed factors and individual ID and experiment round as grouping factors. Confidence intervals for the repeatability estimates were estimated using parametric bootstrapping and significance tested using likelihood ratio tests (Stoffel et al. 2017).

We ran 2 conceptually different repeatability analyses for each of the 3 behavioral traits describing the individuals’ movement pattern (explored area, swimming speed, gross
distance). In the first analysis, we tested how the number of recordings and thus time influenced repeatability estimates. We did this by varying the number of included measurements for each individual starting from the first 5 runs up to all measurements. In the second analysis, we tested how sensitive repeatability estimates were to varying starting points. In this analysis, we only included 5 consecutive recordings in the analysis, and continuously moved the starting point forward in time from start to end of exposure (see Figure 1B for a conceptual overview). To test whether body length explained part of the consistent individual differences in behavior, we repeated both analyses while including body length as a covariate.

**RESULTS**

In total, we obtained 2853 1-min video sequences, and detected movement in 2072 of them. Twenty-seven video sequences had to be excluded because of a malfunctioning of the automated filming platform during 3 rounds that affected recordings during later runs.

**Direct effects on the general activity pattern**

The presence and absence of movement was only influenced by time (Table 1 and Figure 2A). The duration spent swimming in each run was only influenced by time and declined as the experiment progressed (Table 1). The area explored during each sequence was influenced by an interaction of Cu and time (Table 1 and Figure 2B). Whereas the explored area was initially bigger in the presence of Cu, over time it was reduced to slightly below the levels of the treatments without Cu. Kairomones did not affect the explored area. Swimming speed was influenced by an interaction between kairomones and time. In the presence of kairomones, animals were slower in the beginning of the experiment. Without kairomones, swimming speeds declined more rapidly over time (Figure 2C and Table 1). The travelled gross distances in each sequence were not influenced by kairomone or Cu, but became shorter over time (Figure 2D and Table 1).

**Repeatability analysis**

We tested whether the variability in motility traits was random or whether it could be attributed to consistent differences between individuals. Repeatability estimates depended on the studied traits and the treatments (Figure 3). Under the influence of kairomones, repeatability estimates were up to 0.4 for swimming speed, explored area, and gross distance travelled (Figure 3). This means that up to 40% of the behavioral variability could be attributed to consistent differences between individuals. Repeatability estimates were slightly reduced when Cu was also present with the kairomones, but still, for large parts of the data, variation could be attributed to consistent differences between individuals. In contrast, in control and in the Cu treatment none of the behavioral traits were repeatable (Figure 3). Overall, repeatability estimates also decreased with the number of included measurements we included to calculate the repeatability (Figure 4). The repeatability values for the kairomeone treatment approached those measured in the control treatment with increasing number of measurements. When body size was included in the analysis,

| Treatment | No. of individuals | No. of observations | z value | p value | Estimate | SE |
| --- | --- | --- | --- | --- | --- | --- |
| General activity pattern (movement or not) | Intercept | 80 | 2853 | 9.721 | <0.001 | 1.918 | 0.197 |
| | Time | | | -6.339 | <0.001 | -0.183 | 0.0289 |

**TABLE 1:** Analysis of variance tables of the final models examining the direct effects of copper and kairomone on the general swimming pattern, on time spent swimming, and on swimming characteristics

| Treatment | numDF | denDF | F value | p value | Estimate | SE | R² cond | R² marg |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Time spent swimming (s) during each run | Intercept | 1 | 1990 | 1428.6 | <0.001 | 27.92 | 0.739 | 0.31 | 0.10 |
| | Time | 1 | 1990 | 64.590 | <0.001 | -0.495 | 0.0616 |
| Motility characteristics | Intercept | 1 | 1989 | 1403.2 | <0.001 | 3.889 | 0.103 | 0.283 | 0.129 |
| | Copper (-) | 1 | 71 | 1.801 | 0.184 | -0.198 | 0.147 |
| | Time | 1 | 1989 | 70.986 | <0.001 | -0.0685 | 0.00813 |
| | Copper (-):Time | 1 | 1989 | 3.999 | 0.0457 | 0.0164 | 0.00822 |
| Explored area | Intercept | 1 | 1989 | 11510 | <0.001 | 1.805 | 0.0168 | 0.32 | 0.17 |
| | Kairomone (+) | 1 | 71 | 0.032 | 0.857 | -0.00416 | 0.0230 |
| | Time | 1 | 1989 | 37.66 | <0.001 | -0.00915 | 0.00149 |
| | Kairomone (+):Time | 1 | 1989 | 8.746 | 0.0031 | -0.00435 | 0.00147 |
| Swimming speed | Intercept | 1 | 1990 | 1727.07 | <0.001 | 17.76 | 0.428 | 0.37 | 0.15 |
| | Time | 1 | 1990 | 79.446 | <0.001 | -0.34 | 0.0383 |

*Estimates are for transformed data. When possible, conditional and marginal R-squared values were extracted from the models with the function rsquared of the piecewiseSEM package (Lefcheck 2015). Estimates and standard errors (SEs) are for reported for transformed data. numDF = numerator degrees of freedom; denDF = denominator degrees of freedom.
The general pattern was retained, but confidence intervals became wider (Supplemental Data 1 and Figure EA2).

**DISCUSSION**

The direct effects of Cu and kairomones on behavioral patterns were small at the stressor concentrations used in the test. However, up to 40% of the behavioral variability in our experiments could be explained by underlying consistent differences between individuals. Interestingly, these differences were triggered by the presence of kairomones. A similar pattern was reported for three-spined stickleback responding to predator exposure (Bell and Sih 2007). In contrast to kairomones, Cu did not prompt the expression of consistent differences between individuals but reduced such differences in our experiments. This result also accords with the lack of influence of Cu exposure on repeatability in a hermit crab (*Pagurus bernhardus*; White and Briffa 2017).

Consistent behaviors are caused by several factors. Stable and labile states, different developmental trajectories, genetics, and previous experiences all correlate with repeatable behaviors (Koolhaas et al. 1999; Careau et al. 2008; Wolf and Weissing 2010). Although the direct effects of treatment on swimming behavior were small, they indicate potential state changes. Copper increased activity measured as explored area at the beginning of the experiment, whereas swimming speeds in the kairomone treatment were slightly reduced. Although the sublethal Cu concentration used in our experiments had limited effects on direct behavioral traits, in the long run such concentrations can negatively affect development and reproduction in harpacticoid copepods (*Tigriopus japonicus*; Kwok et al. 2008). Copper may also elevate physiological stress and in turn initiate escape attempts from the contaminated...
waterbody (Lopes et al. 2004), which could explain the increased speeds in the Cu treatment. Copper might thus impose an equal physiological stress in all individuals and thus over-ride any consistent behavioral differences between individuals. Such different initial states of stress can cause consistent short-term differences in behavior (Luttbeg and Sih 2010).

In contrast, the presence of a natural stressor in the form of predation risk (kairomones) induced consistent differences in swimming behavior between individuals. However, we cannot exclude the possibility that remnants of conspecific infochemicals also influenced the behavior. A model by Luttbeg and Sih (2010) predicts that when risk balances
resource availability, different behavioral strategies can emerge and persist in a population. A high-risk situation with no potential benefits should produce similar behavioral types in the population (Luttbeg and Sih 2010). This would apply to all treatments with kairomones in our experiment. Contrary to the model, we only found repeatability in behavior in this potentially risky environment, but not in the control treatment, a situation with a seemingly balanced risk–benefit ratio. This might be explained by a state-dependent safety mechanism, whereby individuals with higher initial reserves would “dare” to explore the environment for food, assuming better escape abilities than those with less reserves (Luttbeg and Sih 2010). Another possibility is that our experiments, which lasted for half a day, were too short to converge to the predicted distribution of behavioral types, especially in the absence of food.

Repeatability values in the kairomone treatment were in the range of activity measures in other organisms for the initial hours of exposure, but decreased when we increased the duration and number of measurements (see Figure 2A in Bell et al. 2009). Our data indicate that the number of measurements considered in the analysis strongly influences the magnitude of repeatability estimates, as also shown in the meta-analyses by Bell et al. (2009; Figure 4). This is not surprising given that the animals’ state changes over time and that animals may habituate to external stimuli. The observed reduction of swimming activity over time is anticipated given the lack of stimuli from other individuals or resources, such as food or environmental structure. Reduced effects of kairomones over time may be caused by degrading predator stimuli (Van Buskirk et al. 2014) or by animals habituating to the uniform predation risk landscape (Laundré et al. 2001; Van Buskirk et al. 2014; Hintz and Relyea 2017). Assuming that fish kairomones have an average half-life of 77 h (reviewed in Van Buskirk et al. 2014), habituation seems like the more plausible explanation.

The high number of consecutive measurements also allowed us to explore whether the point in time of the analysis influences repeatability. When we shifted the starting point of a repeatability analysis including 5 consecutive measurements, the data revealed that the estimates seem to follow approximately 5-h cycles (Figure 5). This finding suggests some innate activity patterns, but the cycles could also reflect some potential unaccounted fluctuation in the laboratory environment. Overall, our experiment shows that it might be difficult to detect consistent differences in behavior when measurements are either too few or when the included period is too long.

Comparing repeatability estimates using models with and without body size as a fixed factor revealed only weak, if any, effects of body size on repeatability. If body size was important, including it should decrease repeatability estimates, because body size would explain some of the variation in the data. In our case, size mostly increased the confidence intervals of the predictions (Supplemental Data, Figures EA1 and EA2). This finding indicates that the consistent differences in behavior in the kairomone treatment were not caused by different swimming speeds by differently sized animals but were instead due to intrinsically different motility behavior.
In the aquatic environment in general, resources are patchy (Omori and Hammer 1982; Folt and Burns 1999; Martin 2003), and strong spatiotemporal dynamics exist for food availability, predation risk, and access to mates, with all degrees of risk–benefit conditions being likely to occur. Therefore, consistent differences in behavior are likely to emerge and be maintained in the aquatic environment. Just like morphological and physiological traits, behavioral traits are heritable (Mousseau and Roff 1987). With knowledge about the duration of consistent behavior, and the target and degree of selection, one could determine whether individuals are sufficiently different for selection to act (Boake 1989). When selection acts on the available behavioral types in a population, adaptation of advantageous traits in novel environments may be accelerated (Wolf and Weissing 2012). Anthropogenic toxicants are potent selective agents. Behavioral traits are also heritable and can be under selection (Stirling et al. 2002). Although in our experiment, we could not find any strong induced repeatability in behavior with regard to Cu exposure, this might be different for other toxicants, or toxicants might also affect the repeatability to natural stressors, such as kairomone. Hence any toxicant-driven selection on repeatable behaviors could influence population genetics and its ability to cope with future environmental changes.

Consistent differences in behavior in coping with novel environmental conditions have been extensively documented in higher aquatic and terrestrial animals (Bell et al. 2009). In this light and considering the vast literature on plankton behavior (e.g., Kiørboe 2008), it is surprising how little we know about variability and consistency in the behavior of plankton, especially when considering their central role in pelagic food webs. The context-dependent expression of repeatability and its dependency on the number and timing of measurements indicate that consistent behavioral differences could often go undetected. Such consistent behavioral differences matter in the context of escape behavior, diel vertical migration, and other behaviors that influence lifetime reproductive success (e.g., Kaartvedt et al. 2011). Quantifying such behavioral differences also allows us to detect potentially underlying physiological differences within populations (Wolf and Weissing 2010). Studies focused on individual and context-dependent behavioral responses in marine planktonic organisms paired with heritability studies could increase our knowledge of the occurrence and potential implications for the adaptive potential of populations, and could improve our predictions about the fate of populations facing environmental changes.

**Supplemental Data**—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4688.

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**This 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://figshare.com/s/4bc40d6df863439c80f1](https://figshare.com/s/4bc40d6df863439c80f1). Learn more about the Open Practices badges from the Center for Open Science: [https://osf.io/tvyyz/wiki](https://osf.io/tvyyz/wiki).**

**REFERENCES**

Bates D, Maechler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using lme4. J Stat Softw 67:1–48.

Bell AM, Hankison SJ, Laskowski KL. 2009. The repeatability of behavior: A meta-analysis. Anim Behav 77:771–783.

Bell AM, Sih A. 2007. Exposure to predation generates personality in threespined sticklebacks (Gasterosteus aculeatus). Ecol Lett 10: 828–834.

Bertinato J, L’Abbé MR. 2004. Maintaining copper homeostasis: Regulation of copper-trafficking proteins in response to copper deficiency or overload. J Nutr Biochem 15:316–322.

Bjærke O, Andersen T, Baekkedal KS, Nordbotten M, Skau LF, Titelman J. 2016. Paternal energetic investments in copepods. Limnol Oceanogr 61:508–517.

Boake CR. 1989. Repeatability: Its role in evolutionary studies of mating behavior. Evol Ecol 3:173–182.

Box GEP, Cox DR. 1964. An analysis of transformations. J R Stat Soc B Stat Methodol 26:211–252.

Brandi G. 2000. The OpenCV Library. Dr. Dobb’s Journal of Software Tools. [cited 2018 April 5]. Available from: http://opencv.org

Brown JS, Kotler BP. 2004. Hazardous duty pay and the foraging cost of predation: Foraging cost of predation. Ecol Lett 7:999–1014.

Burdidge L, Weis JS, Cabello F, Pizarro J, Bostick K. 2010. Chemical use in salmon aquaculture: A review of current practices and possible environmental effects. Aquaculture 306:7–23.

Careau V, Thomas D, Humphries MM, Réale D. 2008. Energy metabolism and animal personality. Oikos 117:641–653.

Carter AJ, Feeney WE, Marshall HH, Cowlishaw G, Heinsohn R. 2013. Animal personality: What are behavioral ecologists measuring? Biol Rev 88:465–475.

Coutellec M-A, Barata C. 2011. An introduction to evolutionary processes in ecotoxicology. Ecotoxicology 20:493–496.

Damgaard RM, Davenport J. 1994. Salinity tolerance, salinity preference and temperature tolerance in the high-shore harpacticoid copepod Tigniporus brevicornis. Mar Biol 118:443–449.

Engle TE, Spears JW. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J Animal Sci 78:2446–2451.

Fechter A, Thistle D, Ailt G, Sudeman K, Vopel K. 2004. Do harpacticoids (Copepoda) use water-borne cues to aid in locating food parcels? Mar Ecol 25:217–223.

Fischer BB, Roffler S, Eggen RI. 2012. Multiple stressor effects of predation by rotifers and herbicide pollution on different Chlamydomonas strains and potential impacts on population dynamics. Environ Toxicol Chem 31:2832–2840.

Flemming CA, Trevors JT. 1989. Copper toxicity and chemistry in the environment: A review. Water Air Soil Pollut 44:134–158.

Folt CL, Burns CW. 1999. Biological divers of zooplankton patchiness. Trends Ecol Evol 14:300–305.

Grosell M. 2011. Copper. In Wood CM, Farrel AP, Brauner CJ, eds, Fish Physiology: Homeostasis and Toxicology of Essential Metals, Vol 31A, 1st ed. Elsevier, New York, NY, USA, pp 53–133.
Heuschele J, Ekvall MT, Bianco G, Hylander S, Hansson LA. 2017. Context-dependent individual behavioral consistency in Daphnia. Ecosphere 8:e01679.

Hintz WD, Relyea RA. 2017. A salty landscape of fear: Responses of fish and zooplankton to freshwater salinization and predatory stress. Oecologia 185:147–156.

Holmstrup M, Bindesbøl AM, Oostingh GJ, Ferreira AL, Kienle C, Gerhardt A. 2010. Interactions between effects of environmental chemicals and natural stressors: A review. Sci Total Environ 408:3746–3762.

Humes AG. 1994. How many copepods? Hydrobiologia 292/293:1–7.

Jaspers C, Carstensen J. 2009. Effect of acid Lugol solution as preservative on two representative chitineous and gelatinous zooplankton groups. Limnol Oceanogr Methods 7:430–435.

Kaartvedt S, Titelman J, Røstad A, Klevjer TA. 2011. Beyond the average: Diverse individual migration patterns in a population of mesopelagic jellyfish. Limnol Oceanogr 56:2189–2199.

Kats LB, Dill LM. 1998. The scent of death: Chemosensory assessment of actions between effects of environmental chemicals and natural stressors. Proc Natl Acad Sci USA 95:1116–1120.

Kwok KW, Leung KM, Bao VW, Lee JS. 2008. Copper toxicity in the native crustacean Eurytemora affinis. J Evol Biol 21:1477–1483.

Kulkarni D, Gergs A, Hommen U, Ratte HT, Preuss TG. 2013. A plea for lethal toxic stress on marine zooplankton (feeding, fecundity, respiration, and excretion): Controlled ecosystem pollution experiment. Bull Mar Sci 79:271–282.

Laundré JW, Hernández L, Altendorf KB. 2001. Wolves, elk and bison: Reestablishing the “landscape of fear” in Yellowstone National Park, USA. Can J Zool 79:1401–1409.

Lefcheck JS. 2015. piecewiseSEM: Piecewise structural equation modeling in R for ecology, evolution, and systematics. Methods Ecol Evol 7:573–579.

Lewis C, Ellis RP, Vernon E, Elliot K, Newbatt S, Wilson RW. 2016. Ocean acidification increases copper toxicity differentially in two key marine invertebrates with distinct acid-base responses. Sci Rep 6:21554.

Lodé T, Heuschele J, Andersen T, Titelman J, Hylland K, BORGÀ K. 2018. Predation risk potentiates toxicity of a common metal contaminant in a coastal copepod. Environ Sci Technol 52:15353–15352.

Lopes I, Baird DJ, Ribeiro R. 2004. Avoidance of copper contamination by invertebrates with distinct acid-base responses. Sci Rep 6:21554.

Omori M, Hammer WM. 1982. Patchy distribution of zooplankton: Behavior, population assessment and sampling problems. Mar Biol 72:193–200.

Organisation for Economic Co-operation and Development. 2012. Test No. 211: Daphnia magna reproduction test. OECD Guidelines for the Testing of Chemicals. Paris, France.

Pinheiro JC, Bates D, DebRoy S, Sarkar D, R Core Team. 2017. nlme: Linear and Nonlinear Mixed Effects Models. R package Version 3.1-131. [cited 2018 April 5]. Available from: https://CRAN.R-project.org/package=nlme.

R Core Development Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Rábago-Castro JL, Sanchez JG, PÉREZ-Castañeda R, GONZÁLEZ-González A. 2006. Effects of the prophylactic use of Romet®-30 and copper sulfate on growth, condition and feeding indices in Channel catfish (Ictalurus punctatus). Aquaculture 253:343–349.

Raisuddin S, Kwon KWH, Leung KMY, Schlenk D, Lee J-S. 2007. The copepod Tigriopus: A promising marine model organism for ecotoxicology and environmental genomics. Aquat Toxicol 83:161–173.

Reeve MR, Gamble JC, Walter MA. 1977a. Experimental observations on the effects of copper on copepods and other zooplankton: Controlled ecosystem pollution experiment. Bull Mar Sci 27:92–104.

Reeve MR, Walter MA, Darcy K, Ikeda T. 1977b. Evaluation of potential indicators of sub-lethal toxic stress on marine zooplankton (feeding, fecundity, respiration, and excretion): Controlled ecosystem pollution experiment. Bull Mar Sci 27:105–113.

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A. 2012. Fiji: An open-source platform for biological-image analysis. Nat Methods 9:66–682.

Sih A, Bell A, Johnson JC. 2004. Behavioral syndromes: An ecological and evolutionary overview. Trends Ecol Evol 19:372–378.

Stirling DG, Réale D, Roff DA. 2002. Selection, structure and the heritability of behavior. J Evol Biol 15:277–289.

Stoffel MA, Nakagawa S, Schielzeth H. 2017. rpltR: Repeatability estimation and variance decomposition by generalized linear mixed-effects models. Methods Ecol Evol 8:1639–1644.

Sullivan BK, Buskey E, Miller DC, Ritacco PJ. 1983. Effects of copper and cadmium on growth, swimming and predator avoidance in Eurytemora affinis (Copepoda). Mar Biol 77:299–306.

Treves-Brown KM. 2000. Applied Fish Pharmacology. Klwer Academic, Dordrecht, The Netherlands.

Tuomainen U, Candolin U. 2011. Behavioural responses to human-induced environmental change. Biol Rev 86:640–657.

Turner JT. 2004. The importance of small planktonic copepods and their roles in pelagic marine food webs. Zool Stud 43:255–266.

Vainikka A, Rantalai MJ, Niemelä P, Hirvonen H, Kortet R. 2011. Boldness as a consistent personality trait in the noble crayfish, Astacus Astacus. Acta Ethol 14:17–25.

Van Buskirk J, Krügel A, Kunz J, Miss F, Stam F. 2014. The rate of degradation of chemical cues indicating predation risk: An experiment and review. Ethol 20:942–949.

Van-Zwieten L, Merrington G, Van-Zwieten M. 2004. Review of impacts on soil biota caused by copper residues from fungicide application. Proceedings, SuperSoil 2004: 3rd Australian New Zealand Soils Conference, Sydney, Australia, December 5–9, 2004.

Wang DC, Li XD, Wang CX, Wai OWH, Li YS. 2002. Heavy metals in the coastal water of Hong Kong. In Cai Y, Braids OC, eds, Biogeochemistry of Environmentally Important Trace Elements. American Chemical Society, Washington, DC, USA, pp 404–419.

White SJ, Briffa M. 2017. How do anthropogenic contaminants (ACs. affect behavior? Multi-level analysis of the effects of copper on boldness in hermit crabs. Oecologia 183:391–400.

Wolf M, Weissing FJ. 2010. An explanatory framework for adaptive personality differences. Philos Trans R Soc Lond B Biol Sci 365:3959–3968.

Wolf M, Weissing FJ. 2012. Animal personalities: Consequences for ecology and evolution. Trends Ecol Evol 27:452–461.

Wolf R, Heuschele J. 2018. Water browning influences the behavioral effects of ultraviolet radiation on zooplankton. Front Ecol Evol 6:26.