Evolutionary responses to climate change and contaminants: Evidence and experimental approaches

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Abstract A fundamental objective within ecotoxicology lies in understanding and predicting effects of contaminants. This objective is made more challenging when global climate change is considered as an environmental stress that co-occurs with contaminant exposure. In this multi-stressor context, evolutionary processes are particularly important. In this paper, we consider several non-"omic" approaches wherein evolutionary responses to stress have been studied and discuss those amenable to a multiple stressor context. Specifically, we discuss common-garden designs, artificial and quasi-natural selection, and the estimation of adaptive potential using quantitative genetics as methods for studying evolutionary responses to contaminants and climate change in the absence of expensive molecular tools. While all approaches shed light on potential evolutionary impacts of stress or exposure, they also have limitations. These include logistical constraints, difficulty extrapolating to real systems, and responses tied strongly to specific taxa, populations, and/or testing conditions. The most effective way to lessen these inherent limitations is likely through inclusion of complementary physiological and molecular tools, when available. We believe that an evolutionary context to the study of contaminants and global climate change is a high priority in ecotoxicology and we outline methods that can be implemented by almost any researcher but will also provide valuable insights [Current Zoology 61 (4): 690–701, 2015].

Keywords Climate change, Multiple stressors, Adaptation, Experimental evolution, Quantitative genetics

1 Introduction

The primary goal in ecotoxicology is to understand and ultimately predict effects of contaminant stress on ecological systems (Walker, 2006). A long-standing and important element of this goal lies in understanding and predicting evolutionary effects of contaminants to include changes in gene frequencies (Belfiore and Anderson, 2001), expression (Snape et al., 2004) and traits (Forbes, 1998). Evolutionary responses are important because, in most cases, we are interested in the long-term sustainability of populations despite the fact that the vast majority of ecotoxicity data are based on short-term tests. A further complication is that natural systems are subjected to a myriad of other stressors. Of particular concern is the increasing complexity of environmental stressors associated with global change where an understanding of evolutionary processes is critical to predicting the responses of natural populations and systems.

Global Climate Change (GCC) is a significant ecological driver (Walther et al., 2002; Parmesan, 2006) and a potential evolutionary driver as well (Hendry and Kinnison, 2001; Gienapp et al., 2008). Both environmental contaminants and GCC have received separate, recent scholarly reviews in the context of evolutionary change (e.g., Klerks et al., 2011; Merilä and Hendry, 2013, respectively) and the interaction of GCC and contaminants has been considered but not necessarily in an evolutionary context (Stahl et al., 2013). The combined effects of GCC and contaminants on evolutionary processes, however, have received relatively little attention (Müller et al., 2011; Kimberly and Salice, 2012; Moe et al., 2013). In fact, the interaction between GCC and pollution may demand an increasingly urgent understanding. Concerns include the role of GCC in modifying both exposure and effects of environmental contaminants, each of which has important implications for evolutionary response. GCC is expected to have a substantial effect on release, fate, behavior, and exposure of toxicants (Noyes et al., 2009) thereby influencing environmental concentrations of contaminants (Gouin et al., 2013). From an effects perspective, GCC mediated changes in environmental conditions may interact with contaminants to substantially alter organismal behavior...
and physiology. Since these combined stressors will almost certainly impact adaptive responses of exposed populations, it is crucial that the evolutionary impacts of GCC and environmental contaminants be explored concomitantly. Here, we focus on several approachable methods used to study the evolutionary responses to the combined effects of GCC and environmental contaminants.

Regardless of source, every shift in a species’ environment, such as that mediated by climate change (Houghton et al., 2001) or environmental contaminants (Klerks et al., 2011; Kimberly and Salice, 2014), is a potential cause of new or intensified responses that could be physiological (e.g., acclimation) or evolutionary (e.g., selection). Depending on the intensity, predictability, and recurrence of stress, responses might range from behavioral avoidance, acclimation by phenotypic plasticity, to adaptation by means of genetic change (Klerks and Weis, 1987; Holt, 1990; Davis et al., 2005). Behavioral avoidance might immediate survival but can also promote local extirpation by pushing organisms toward unfavorable habitats and novel risks, so-called evolutionary traps (Schlaepfer et al., 2002). In contrast, physiological acclimation and adaptation prevent local extinction (Gienapp et al., 2008) but can also increase so-called costs of adaptation (Salice et al., 2010). The most likely scenario is that populations rely on a combination of these responses (Davis and Shaw, 2001) for persistence. However, a key challenge to understanding and predicting response is identifying when a population is employing or might employ a specific persistence strategy and how the cost of a particular strategy may manifest.

Behavioral avoidance and physiological acclimation are important survival mechanisms for both GCC and contaminant stress. The evidence for GCC-stressor avoidance, in for the form of range shifts, is substantial (e.g., Walther et al., 2002; Perry et al., 2005; Parmesan, 2006). Similarly, organisms can and will relocate away from localized contaminant exposures (Hansen et al., 1999; Araujo et al., 2012; Salice and Kimberly, 2012), although sometimes actively preferring the contaminated habitat (Tierney et al., 2011). There can also be physiological acclimation and subsequent maternal effects, both of which contribute to phenotypic plasticity in response to climate change stress (Przybylo et al., 2000; Price et al., 2003; Reale et al., 2003) and environmental contaminants (Postma et al., 1995; Marinovic et al., 2012). Importantly, phenotypic plasticity has expression limits and decreases in protectiveness as environmental change progresses on a directional path (DeWitt et al., 1998; de Jong, 2005). Further, plastic genotypes are typically unable to produce phenotypes as extreme as can be developed via microevolutionary adaptive responses (DeWitt, 1998).

Adaptive microevolution is a well-known process that is essential for dealing with long-term exposure to environmental stressors (Hendry and Kinnison, 2001; Stockwell et al., 2003; Merilä and Hendry, 2014) and is characterized by evolution within and among populations in contemporary time frames (Hendry and Kinnison, 2001). Instead of many centuries and generations (evolutionary timescales), genetic responses have been found to occur in as little as years or a few generations (ecological timescales) in some cases (Klerks and Levington, 1989; Theodorakis and Shugart, 1997; Kinnison and Hendry, 2001; Reznick and Ghalambor, 2001; Salice et al., 2010). Moreover, because of the difficult nature of quantifying adaptive evolution in wild populations, adaptive microevolution has been inferred based on changing trait means towards a peak in a theoretical adaptive landscape (Merilä and Hendry, 2013). One caveat of this approach is that genetic sources of trait change are not explicitly identified and therefore, physiological and maternal effects (phenotypic plasticity) cannot be rejected as explanations. In some cases, studies that had originally concluded that phenotypic differences had a genetic basis were subsequently credited to plasticity (Charmantier et al., 2008; Teplitsky et al., 2008). Regardless of the overwhelming qualitative support for microevolution to environmental stress, there remain few explicit, quantitative studies that show a genetic basis for phenotypic change to GCC or environmental contaminants (Gienapp et al., 2008; Klerks et al., 2011; Merilä, 2012; Merilä and Hendry, 2013). Nonetheless, studies identifying changes in traits provide invaluable insight into the potential for evolutionary response even though the exact mechanism of the change may require additional research. In this case, identifying patterns of response across stressors, species and timeframes may lead to novel insights and generalities with regard to evolutionary responses to GCC and contaminant stressors.

Although there have been several recent reviews of evolutionary responses to climate change (Davis et al., 2005; Jump and Penuelas, 2005; Parmesan, 2006; Gienapp et al., 2008; Merilä and Hendry, 2014) and environmental contaminants (Klerks and Weis, 1987; Klerks et al., 2011), no treatment has been given to the interactive impacts of both stressors on evolutionary processes. Moe et al. (2013) highlighted that there could be com-
bined and interactive effects of climate change and contaminants on populations and communities in the context of adaptation. These combined effects, which can be antagonistic, additive, or synergistic, are poorly predicted by single stressor studies (Folt et al., 1999; Moe et al., 2013). Therefore depending on the mechanism of response, adaptation to one stressor may in fact leave populations vulnerable to additional environmental change (Jansen et al., 2011), a so-called “latent cost” because they may only manifest in the presence of a particular environmental change or stressor (Salice et al., 2010; Kimberly and Salice, 2014). However, because little data exists on the effects of combined stressors on adaptive responses, little more than theoretical suggestion could be offered. The field is still very much in need of these data, although a few studies have been recently published (Messiaen et al., 2010; Marinkovic et al., 2012, described below).

In addition to highlighting those few recently published works, our goal here is to describe common experimental methods used for identifying evolutionary responses and emphasize those most amenable to exploring the interactive effects of GCC and contaminants. There are several broad approaches that have been successfully used to explore evolutionary responses: experimental evolution, qualitative genetics and molecular genomics approaches. Experimental evolution focuses on the study of populations over multiple generations and the approach is largely responsible for some of our very first insights into evolutionary processes (Garland and Rose, 2009). Quantitative genetics is a branch of population genetics that uses quantitative trait information to infer the manner of evolutionary response and has been used extensively in agriculture (Falconer and Mackay, 1996; Roff, 1997). Molecular genomics has emerged as a very powerful approach to better understand evolution at the gene level (Meyer et al., 2005) but also necessitates considerable funding and available tools for success. In the past, “omics” approaches have been available for a limited number of model species such as Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster, and Arabidopsis thaliana (van Straalen and Roelofs, 2012). However, ecotoxicology has found recent utility in complete and partial genomes from Danio rerio, Oryzias latipes, Oncorhynchus mykiss, and Xenopus sp (Ankley et al., 2006). While the growing access to partial and complete genomes is promising, there remains a lack of representatives for most ecologically relevant species. Alternatively, experimental evolutionary approaches and quantitative genetics methods do not require costly, sophisticated instrumentation or molecular tools although they lack the precision of molecular techniques in identifying exact differences among populations. Nonetheless, classic papers in showing adaptation to environmental pollutants have involved experimental evolution (Klerks and Levinton, 1989; Klerks and Weis, 1987) and quantitative genetics (Klerks et al., 2011). Because experimental evolution and quantitative genetics methods are more approachable by a wide range of researchers and because they can be implemented with a wide-range of ecologically relevant taxa, this review will focus primarily on these approaches for improving our understanding of GCC and contaminants stressors.

## 2 Multiple-stressor Design Considerations

We argue that the multiple-stressor perspective presented here requires additional considerations for understanding adaptive responses in a complex stressor landscape. Hooper et al. (2013) described two perspectives on the interactions of GCC and contaminants in the context of trade-offs at the individual level: toxicant induced climate sensitivity and climate induced toxicant sensitivity. Toxicant-induced climate sensitivity involves alterations caused by toxicant exposure that impact the ability of an organism to acclimate to a GCC related stressor such as temperature. Alternatively, climate-induced toxicant sensitivity scenarios produce altered or increased toxicity of chemicals as a result of exposure to changes in climate related conditions (Hooper et al., 2013). In both cases, a temporal transition from one stress condition to another is implied. At the population level, if a group has become genetically adapted to long-term contaminant exposure, it might display a diminished ability to adapt to future climate stress, or vice versa (Salice et al., 2010; Kimberly and Salice, 2012). Moe et al. (2013) suggests that depending on the combination of climatic change parameters and contaminants, additive (unaltered tolerance), synergistic (cost-of tolerance), and antagonistic interactions (cotolerance) may result.

As mentioned above, unraveling the evolutionary responses of just one stressor can be difficult, let alone multiple stressors, because of the influence of plasticity (Kawecki and Ebert, 2004) and because many responses are likely to be polygenic (Hoffman and Willi, 2008). Effects from environmental history will be lessened, if not removed, if field caught organisms are acclimated under common experimental conditions for two or three generations before experiments are conducted and end-
points are measured (Kawecki and Ebert, 2004). Moreover, using offspring of laboratory acclimated parents (F1 generation) to initiate experiments may limit the influence of environmental history. This may be desirable for studies conducted in a laboratory in support of regulatory decision making but become less practical when extrapolating laboratory-observed effects to the field. Other designs discussed below can limit but also more clearly define the contribution of different adaptive responses. Lastly, previous considerations of this topic by Klerks et al. (2011) have established resistance as a focal endpoint for quantifying and appropriately describing the genetic basis of an adaptive change. In any scenario where environmental stressors impact fitness, the more resistant individuals will be favored. Because the expression of resistance by a population can be viewed on a numeric scale, it is treated as a quantitative trait (Klerks et al., 2011).

Here we discuss three strategies in experimental evolution that can be employed to understand the effects of GCC and contaminant stressor on evolutionary responses. First, because these stressors vary spatially, populations likely differ spatially in their resistance or stress tolerance, which, when compared to other control/reference sites, might provide evidence for past responses to selective pressure (Klerks and Levinton, 1989, Coors et al., 2009; Jansen et al., 2011). Second, artificial and quasi-natural selection experiments, which seek to re-create a possible selection scenario, can be conducted under controlled conditions. These experiments provide insight into whether a population responds to selection trials and at what rate resistance traits change in experimental populations (Klerks et al., 2011). Lastly, breeding designs can be used to detect changes in genetic variability as a result of adaptive responses to selection (Hoffman and Merilä, 1999; van Straalen and Timmermans, 2002). With decreased variability, the potential to adapt to future stress may be drastically diminished. Therefore, the adaptive potential of an experimental population can be determined after subsequent response to multiple stressors (Kimberly and Salice, 2012).

3 Approaches for Inferring Genetic Responses to Climate Change and Contaminants

3.1 Inferences in spatial contexts and common-garden studies

One way to detect evolutionary responses among spatially varying populations is through the use of common-garden studies. In this study design, many individuals of a given species are sampled from several specified geographic regions or locations and reared in a common laboratory or field environment (Conover and Shultz, 1995). The use of a single common environment minimizes environmentally induced phenotypic differences, allowing for observation and comparison of genetically adapted traits. This design has seen common application with seeds (e.g. Davis and Shaw, 2001; Jimenez-Ambriz et al., 2007; Aitken et al., 2008). For instance, studies with Douglas-fir trees have revealed significant geographic variation in phenotypic traits corresponding to climatic gradients (St. Clair et al., 2005). One classic example of a common garden study in ecotoxicology is from Klerks and Levinton (1989). Briefly, individuals of the oligochaete Limnodrilus hoffmeisteri were collected from three sites that varied in environmental metal concentrations. Those organisms from more heavily contaminated locations displayed greater resistance, in the form of greater time to death, to common garden metal mixtures. A genetic basis for resistance was inferred when organisms that were reared in clean conditions for two generations continued to show differences in resistance.

Another recent common garden study was conducted with the natterjack toad Bufo calamita in Sweden (Rogell et al., 2009). Two populations, a saline and desiccation stressed western population and a less “at risk” southern population, were subjected to a common garden experiment where salinity and temperature were manipulated. Despite being subjected to higher salinity stress in nature, western toads had a poorer performance in high salinity treatments, as indicated by survival, growth rate, and larval period. Furthermore, while high temperature predictably decreased larval period, increased population survival, and increased growth rate, it did not seem to interact adversely with elevated salinity levels (Rogell et al., 2009). A key conclusion of this study was the complexity that multi-stressor environments impart to our understanding of adaptive potential and that correlations among traits can sometimes constrain adaptive responses (Rogell et al., 2009).

In a slight variation of the common garden design, reciprocal transplant experiments transfer individuals among different habitats in the field and subsequently quantify resistance at each location (Kawecki and Ebert, 2004; Blanquart et al., 2013; Merilä and Handry, 2013). Such selection favors the resistant populations that outperform populations from stress-free environments when both are transplanted to stressful habitats. It would
be expected to observe trade-offs of the resistant population in the stress-free treatment. To our knowledge, no common-garden/reciprocal transplant study has been done in the context of climate change and contaminants. However, Altshuler et al. (2011) suggests that because of its life history, genotyping, and abundance of literature, *Daphnia sp.* may be most amenable to multiple stressor, common-garden studies.

### 3.2 Limitations

One main limitation to common-garden studies is the assumption that “control” populations represent the pre-selection or benign environment. This limitation becomes more profound with increasing stressor complexity. Because of the difficulty to confirm this assumption, many contemporary experiments seek instead to understand if resistance in a present population has changed when compared to the same population in the past. In this temporal context, common-garden experiments can be implemented in a number of ways. If past common-garden environments can be accurately replicated in the present, then common-garden studies can be conducted on the same population at multiple time stages. Bradshaw and Holzapfel (2001) used this method to compare the length of the photoperiod that terminates diapause in pitcher-plant mosquitoes *Wyeomyia smithii* populations sampled at different locations and in different years. Additionally, “resurrection ecology” (Klers et al., 2011) utilizes a dormant or resting life stage (e.g., seeds, Franks et al., 2007; *Daphnia ephippia*, Weider et al., 1997) to represent a past environment. The resistance to environmental stressors of those resurrected organisms is then compared with present populations in a common-garden design. An alternative approach, because few taxa are capable of “resurrection”, is to use micro or mesocosm experiments to simulate present and past environments. Higher resistance in present environments implies a trade-off and thus reduced resistance in past environments.

As in all case of inferential experimental evolution studies, showing differences in stress response and resistance between two populations is not always indicative of a genetic basis for observed differences. Acclimation-based stress resistance can be verified by examining descendants of exposed individuals, generally at least one or two generations removed from the stressor environment (Lonsdale and Levinton, 1985). If instead, resistance is a consequence of acclimation, resistance will likely disappear in subsequent generations reared in stress-free conditions (Watson and Hoffman, 1996; Klerks and Lentz, 1998). Admittedly, this caveat does not completely rule out maternal effects as offspring could still be influenced a generation removed (Kimberly and Salice, 2014). A technique gaining popularity that gets to the specific source of variation, and can be incorporated into a common-garden design, is to compare the differentiation of the quantitative genetic trait with a neutral genetic marker. The concept here is that selection will cause quantitative trait variation ($Q_{ST}$) to be more prominent than that of neutral marker variation ($F_{ST}$) (Lande, 1992; McKay and Latta, 2002; Leinonen et al., 2008). Therefore, selection is inferred when quantitative trait variance for a population exceeds values for neutral marker differentiation.

### 3.3 Artificial and Quasi-natural selection experiments

The premise for selection experiments within the context of stress ecology and ecotoxicology is fairly straightforward: establish populations exposed to specific suites of stressors for a long enough period to cause adaptive change. While molecular tools can be employed to explore specific segments of a genome, selection experiments generally affect multiple regions within the genome at the same time. The rate of change in resistance under controlled conditions can also be observed while still simulating natural selection (Falcoher, 1981; Rose et al., 1990; Huey and Bennett, 1990). Because the selection process requires multiple generations, those taxa with short generation times (e.g., *Daphnia*) have the most utility, although vertebrates do have a significant history from which to draw methodological considerations (Meyer and Di Giulio, 2003). There are two types of selection designs described below: Artificial and Quasi-natural selection (Schneiner, 2002); each with its strengths and limitations, depending on the question and study system at hand.

The obvious difference between these methods is whether the researcher actually selects the particular trait for propagation or whether, in the case of quasi-natural selection, the researcher allows the subjects within experimental populations to mate freely, where reproductive success is influenced by environmental conditions. Consider a scenario for artificial selection of cadmium resistance in the least killifish *Heterandria formosa* conducted by Xie and Klerks (2003), where time-to-death (TTD) was measured as resistance. In each generation, fish were exposed to a lethal concentration of cadmium until the population reached 50% mortality. Surviving members of the cull were transferred to clean water and served as parents for the next generation. Lethal cadmium exposure and subsequent
reproduction was repeated for six generations. As a result of the selection for cadmium resistance, TTD showed a significant increase over six generations. Similarly, Kelly et al. (2012) conducted selection experiments in multiple geographically differing populations of the copepod Tigriopus californicus on heat tolerance. Those populations at high-altitude could not achieve heat tolerance, neither by acclimation nor 10 generations of strong selection. The authors suggest that in some isolated populations, plasticity and adaptation will have a very limited capacity to protect against temperature change. Additionally, a long history of selection experiments exist using the model vertebrate species Fundulus heteroclitus where some of the more recent studies have dealt with combined exposures of multiple contaminants such as PCBs (Nacci et al., 1999), methyl mercury (Weis et al., 2001), and PAHs (Ownby et al., 2002).

In quasi-natural selection scenarios, resistance traits are evaluated over time, but organisms within the experimental population are free to mate with other individuals regardless of their trait values (e.g., chemical resistance). After multiple generations under stressor conditions, differentially exposed populations can be compared under common conditions to quantify any genetically based phenotypic change (Rose et al., 1990; Kawecki et al., 2012; Salice et al., 2010). Similarly to Fundulus, a long history of natural selection study exists for Drosophila melanogaster. Huey et al. (1991) exposed Drosophila populations to two temperature regimes for four years before conducting a heat shock assay. Heat tolerance was greater from the high temperature selected line, but significant variability existed within replicates. In a very rigorous example of a quasi-natural selection experiment, Van Doorslaer et al. (2007) exposed zooplankton to different global warming scenarios for one year. After the 1-year exposure duration, a life table experiment was conducted at three temperatures to determine any potential adaptive responses. It was found that zooplankton exposed to a warmer scenario longitudinally for 1-year had significantly greater resistance (survival) to high temperature, compared to control lines, during the life-table experiment. Additionally, because the zooplankton used in the life-table phase came from longitudinally exposed parents that had been transferred to clean water for two generations, resistance can be inferred to have a genetic basis.

In the multiple stressor context, quasi-natural selection is likely the most informative design. For example, Daphnia were exposed to sublethal low and high concentrations of cadmium at two different temperatures (22 and 28°C) for 66 days in a quasi-natural selection design (Kimberly and Salice, unpublished data). A cadmium and temperature challenge on the offspring of Daphnia one generation removed from longitudinal exposure conditions was then conducted. Daphnia exposed to high cadmium longitudinally displayed increased cadmium resistance during the cadmium and temperature challenge than did low cadmium exposed animals, signaling the selection for high cadmium resistant phenotypes. However, all treatments experiencing novel high temperature in addition to cadmium seemed overwhelmed, exhibiting decreased resistance in all treatments. In a similar example of potential trade-offs among stress resistance traits, the freshwater snail Biomphalaria glabrata was exposed to three different concentrations of cadmium for three generations and then removed from cadmium exposures and tested for cadmium and temperature tolerance a generations later (Salice et al., 2010). These examples of potential trade-offs may constrain adaptive responses – this is particularly important when considering adaptation to multiple stressors as in the case of GCC and contaminant-related stress.

### 3.4 Limitations

The first limitation of artificial and quasi-natural selection is that logistical constraints (generations/time, number of treatments, replicates, and individuals per replicate) often restricts the design to organisms with short generation times such as Daphnia, Drosophila, and Chironimids (Merilä and Hendry, 2013). While these are certainly model organisms used across fields and within ecological risk assessments, they are truly only representative of a small proportion of organisms experiencing environmental change. Another limitation is that because selection experiments are often performed in the laboratory, insight into natural selection/adaptation is limited. These experiments become solely proof-of-concept as selection in experiments occurs with a specific experimental population under very specific environmental conditions (Klerks et al., 2011; Merilä and Hendry, 2013). By increasing the variety of stressors included, like concomitant exposures to climate change and contaminants, the strength of inference regarding responses to multiple stressor increases but still is limited to the suite of stressors evaluated. Further, laboratory selection experiments conducted in the present will not be able to fully reconstruct past natural
selection scenarios. Regardless of these obvious limitations, this approach can indeed measure the response to actual selection (Fuller et al., 2005) and perhaps more importantly, can provide insight into the adaptive potential in multi-stressor environments (Rogell et al., 2009; Salice et al., 2010; Kimberly and Salice, unpublished data).

### 3.5 Breeding designs and adaptive potential

The response to selection (\( R \)) can be better understood using the heritability of a trait (\( h^2 \) or \( H^2 \); resistance) and the selection differential (\( S \)), which indicates intensity and direction of selection (Falconer and Mackay, 1996). The approximation of \( R \) depends on many assumptions including, the focal trait having no correlations with other genetic traits, the environment that influences the trait remains constant over many generations, and that heritability and \( S \) are estimated accurately (Falconer and Mackay, 1996; Kruuk et al., 2003). In addition to determining the response to selection of resistance (to contaminants, for example), heritability can also be used to help predict the ability of a population to adapt to future stressors, such as climate change (Kimberly and Salice, 2012).

More specifically, heritability is a quantitative estimate of the amount of genetic variation contributing to the total variation displayed by a particular trait and, hence, estimates the degree to which the trait can be passed to the next generation (scale, 0–1; Hoffman and Merilä, 1999; Klerks et al., 2011). Since a response, such as resistance, requires the presence of genetic variation (Lynch and Walsh 1998), the heritability indicates the potential a population has to adapt to a specific stressor or suite of stressors (Hoffmann and Merilä, 1999). As such heritability is often thought of in terms of adaptive potential (Houle, 1992). “Narrow-sense heritability” (\( h^2 \)) expresses the proportion of total phenotypic variation that is additive genetic variation. Narrow-sense heritability is defined as \( h^2 = V_a/V_p \) where \( V_a \) is additive genetic variance and \( V_p \) is the phenotypic variance (Falconer and Mackay, 1996). Additive effects describe alleles whose contributions to the trait are independent of other genes or the environment. “Broad-sense heritability” (\( H^2 \)) is another common estimate, which is defined as \( H^2 = V_G/V_p \) where \( V_G \) is the total genetic variance, which includes additive as well as non-additive (e.g., epistasis, dominance, etc.) genetic effects. Quantifying additive genetic variance is most useful for understanding adaptive response because any allele that is advantageous will likely only remain if it confers an advantage without influence or interaction with other genes, or other non-genetic factors. Therefore, broad sense heritability is not as useful a concept as narrow sense heritability but is frequently easier to estimate and does provide some insight into the potential for evolutionary change.

While heritability can be estimated from different experimental designs and equations, here we highlight heritability estimates from observed resemblance between relatives. More specifically heritability is estimated from the correlation of offspring and parental phenotypes or the correlation of full or half siblings. While half-sib designs are more complicated, they reduce or eliminate bias from maternal effects (e.g., Offspring-mother design) or non-additive effects (e.g., Full-sib design) (Falconer and Mackay, 1996). With regards to the effects of environmental stress, studies have shown that exposure to environmental stressors decreases genetic variability (Hoffmann and Merilä, 1999; Klerks and Moreau, 2001; van Straalen and Timmermans, 2002; Müller et al., 2011). Conversely, populations of *Fundulus* under strong selection were not found to have decreased genetic diversity compared to that of reference populations (McMillan et al., 2006). Therefore, considerable uncertainty remains regarding the generality of effect of environmental stress on genetic variability, and therefore heritability. Additionally, alternative hypotheses have been suggested and supported (heritability increases as a result of stress; Hoffman and Parsons, 1991; Barker and Krebs, 1995; Imasheva et al., 1998), where stressor type may be of particular importance (Kimberly and Salice, 2012).

In one of the few, but very robust examples that investigated multiple stressor influence on heritability estimates, *Daphnia magna* was exposed to cadmium stress at two different temperatures (Messiaen et al., 2012). In this study, both additive and non-additive components of genetic variability of net reproductive rate were estimated. Life-table experiments were conducted with 20 parental and 39 offspring clonal lineages with two cadmium treatments (control and one cadmium concentration) and two temperature treatments (low and high) as factors. Offspring lineages were obtained by multiple crossings of the parental stocks. Broad and narrow sense heritability was then calculated using maximum likelihood estimation. Predictably, net reproductive rate decreased in increasingly stressful treatments, with the most stressful treatment comprised of cadmium and high temperature. However, it was also found that there was a considerable degree of genetic determination as indicated by an increase in broad sense...
heritability in more stressful treatments (0.392 to 0.563). Additionally, narrow sense heritability also increased in the most stressful environment (0.06 to 0.23). This suggests that resistance to stressful environments, in the form of increased reproduction, may be inherited.

Similarly, Klerks and Moreau (2001) investigated multiple stressors in the form on contaminant mixtures. Although, they did not include a climate change parameter, their methodology serves as a useful case study in the inclusion of more than one source of potential selection. Heritability was quantified in the sheepshead minnow *Cyprinodon variegatus* for resistance (in the form of time to death) to individual and mixtures of contaminants. Estimates were obtained from various parent-offspring regressions, and families of sibs and half-sibs. The parent-offspring and the half-sib regressions yielded very low heritability (0.08 and -0.01, respectively), while the full-sib design yielded average heritability of 0.85. The full-sib resemblances are most likely due to non-genetic factors. Moreover, heritability estimates in the parent-offspring regressions decreased as contaminant complexity increased. Therefore, because there is a small additive-genetic basis for resistance to the contaminants used in this study, rapid resistance is not likely to occur. Likewise, as stressor complexity increases, especially with the inclusion of climate change projections, heritability and thus adaptive potential may decrease.

### 3.6 Limitations

A major limitation to heritability estimates is that they require relatedness data, which is usually available only in long-term studies of individually marked or carefully followed organisms (Charmantier et al., 2008). Additionally, the larger the sample size, but also the more time and labor intensive, the more precise the estimates can be. Unfortunately, many heritability studies suffer from low sample size and thus low precision, as seen by having large standard errors (Klerks et al., 2011).

Another shortcoming stems from the difficulty in determining if the experimental results are meaningful or a methodological determinant (Postma, 2006; Hadfield et al., 2010). In particular, a trait value measured multiple times on the same individual may not remain consistent through time. Falconer and Mackay (1996) describe this phenomenon as “trait repeatability” and a repeatability of less than 100% inherently reduces estimates of heritability.

One of the biggest limitations to heritability estimates is that a single trait is unlikely to evolve independently of other traits (Houle, 1991). Genetic correlations may exist between resistance related traits and fitness related traits (e.g., Rogell et al., 2009). For example, the selection for resistance traits may have a negative correlation with fitness traits (Shirley and Shiple, 1999). In such a case, selection would be slowed or prevented altogether, depending on the intensity of the correlation. This has broad implications when considering selection for resistance traits in multiple stressor environments. Unless the stressors share resistance traits, as seen in the co-tolerance of multiple contaminants (Xie and Klerks, 2003), multiple, genetically correlated traits may be under selection. Whether this trade-off vs co-tolerance emerges as a general rule requires further study but could provide valuable insight into understanding the fate of populations in multi-stressor landscapes.

### 4 Combining Experimental Evolution and other Methods

Experimental evolution and quantitative genetic approaches provide important insights into how populations may respond to selection in novel- and multi-stressor environments. However, there are important, and many times unavoidable limitations to those practices. The most effective way to answer the inherently complex and difficult questions outlined above is through interdisciplinary research. Utilizing complementary physiological and molecular tools will further confirm, reject, or refine the results produced from quantitative genetic practices.

The goal when using molecular tools to confirm adaptation is to demonstrate shifts in allele frequency in relation to changing environments. A key challenge is to determine the specific shifts that are relevant, because some shifting is inevitable under all circumstances (Merrilä and Hendry, 2013). As a result of this challenge, research tends to focus on loci known to respond to selection, or on closely linked neutral loci (Hoffmann and Daborn, 2007). Müller et al. (2011) utilized molecular tools to understand the effects of climate change and a fungicide on genetic variability over multiple generations. Microsatellite fragments were amplified and alleles scored in each treatment. They found that genetic diversity was significantly decreased in chironomids exposed to the fungicide at high temperatures, which represent future climate change projections. In addition to changes in allele frequencies, gene expression can also be used to describe evidence of genetic change (Roberge et al., 2007). These ‘omics’ techniques, which
include genomics, proteomics, and metabolomics hold potential for inferring and identifying resistant genetic changes driven by climate change and contaminants (Meyer et al., 2005; Franks and Hoffmann, 2012).

Lastly, Adverse Outcome Pathways (AOPs) can be used as a framework to link mechanism-based molecular events and the impacts on individuals to populations (Ankley et al., 2010). Hooper et al. (2013) constructed a specific AOP for the interdisciplinary understanding of the effects of climate change and contaminants on natural populations, where adaptation is one cog in the overall response machine. These AOPs are designed to work in either direction along the hierarchy of biological organization where each entry helps to inform the level above and below. Therefore, if only molecular and physiological data are known, the AOP can be used to predict effects at the individual and population level, before those levels are filled. Conversely, understanding population level effects, like the change in quantitative genetic traits, will provide context to predict causation at the lower levels (Hooper et al., 2013).

If we take the perspective that an ecotoxicologists’ role is to understand and predict effects of contaminant stress on populations through time, then identifying adaptive responses, in the form of increased resistance or trade-offs among different resistance traits, would be worthy goals. We have indicated several cases where the inclusion of multiple stressors provides valued (and different) insights compared to considering stressors in isolation. Therefore, framing the question of adaptation in a multiple stressor context, where the multiple stressors are climate change related stressors and environmental contaminants, likely provide the most salient insights. We highlighted three ways to experimentally test for adaptation and/or adaptive potential, including common-garden studies, artificial/quasi-natural selection, and breeding designs and quantitative genetics. While experimental evolution and quantitative genetics approaches provide powerful tools for studying evolutionary processes the main limitation is that studies are almost exclusively performed in a laboratory setting, which differs from and limit predictions about natural conditions. These limitations (and others) can, however, be improved upon by interdisciplinary frameworks and approaches including adverse outcome pathways and molecular tools. Nonetheless, the approaches in experimental evolution we highlight will continue to provide much needed insight into the potential for adaptive responses to stressors in a complex, human-dominated landscape.

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