

Effect of habitat quality and phenotypic variation on abundance- and trait-based early warning signals of population collapses

Keywords: *EWS, stability, population decline, body size, phenotypic variation, habitat quality*

Abstract

Loss of resilience in population numbers in response to environmental perturbations may be predicted with statistical metrics called early warning signals (EWS) that are derived from abundance time series. These signals, however, have been shown to have limited success, leading to the development of trait-based EWS that are based on information collected from phenotypic traits such as body size. Experimental work assessing the efficacy of EWS under varying ecological and environmental factors are rare. In addition, disentangling how such warning signals are affected under varying ecological and environmental factors is key to their application in biological conservation. Here, we experimentally test how different rates of environmental forcing (i.e., warming) and varying ecological factors (i.e., habitat quality and phenotypic diversity) affected population stability and predictive power of early warning signals of population collapse. We analyzed population density and body size time series data from three phenotypically different populations of a protozoan ciliate *Askenasia volvox* in two levels of habitat quality subjected to three different treatments of warming (i.e., no warming, fast warming, and slow warming). We then evaluated how well abundance- and trait-based EWS predicted population collapses under different levels of phenotypic diversity, habitat quality, and warming treatments. Our results suggest that habitat quality and warming treatments had more profound effects than phenotypic diversity had on both population stability and on the performance of abundance-based signals of population collapse. In addition, trait-based EWS generally performed well, were reliable, and more robust in forecasting population collapse than abundance-based EWS, regardless of variation in environmental and ecological factors. Our study points towards the development of a predictive framework that includes information from phenotypic traits such as body size as an indicator of loss of resilience of ecological systems in response to environmental perturbations.

This document is the accepted manuscript version of the following article:
Baruah, G., Clements, C. F., & Ozgul, A. (2021). Effect of habitat quality and phenotypic variation on abundance- and trait-based early warning signals of population collapses. *Oikos*. <https://doi.org/10.1111/oik.07925>

Introduction

Ecological systems are inherently complex due to their stochastic, multi-dimensional, and nonlinear nature (Hastings et al. 2018). In the face of anthropogenic climate change, predicting such complex dynamics has thus become increasingly important ((Maris et al. 2018, Clements and Ozgul 2018, Pennekamp et al. 2019). One approach for predicting future ecological states in response to environmental change is to search for phenomenological signals in data which can act as predictors of the future state of that system. Such signals, also known as early warning signals (EWS), have been demonstrated to be useful in predicting abrupt shifts in algal blooms in lake ecosystems (Wilkinson et al. 2018), predicting collapse in experimental microcosms (Drake and Griffen 2010, Dai et al. 2012, Clements and Ozgul 2016a), and also been useful in detecting historical collapses of whale populations (Clements et al. 2017a). The effectiveness of these signals, however, (van Nes and Scheffer 2007, Dakos et al. 2008, 2012, Clements and Ozgul 2018), in forecasting collapses have not been yet been tested in differing ecological and environmental conditions.

Broadly EWS approaches can be broken down into abundance-based (aEWS) (van Nes and Scheffer 2007, Drake and Griffen 2010, Dutta et al. 2018) and trait-based (tEWS) (Baruah, Clements, & Ozgul, 2020; Clements, Blanchard, Nash, Hindell, & Ozgul, 2017a; Clements & Ozgul, 2016). aEWS are a suite of statistical signatures that can be derived from abundance (or biomass as a proxy for abundance) time series data of dynamical systems undergoing rapid changes in response to gradual changes in environmental pressures (Kéfi et al. 2013). Suggested statistical signatures estimated from abundance, that could measure whether a dynamical system is near a transition, are variance and autocorrelation, although various other metrics have also been developed (Boettiger and Hastings 2012). As environmental perturbation on a system keeps increasing, both variance and autocorrelation in abundance time series, quantified using a moving time window, are expected to increase over time (Kéfi et al. 2013). aEWS are applicable to dynamical systems that displays both catastrophic (abrupt collapse observed in systems with strong positive feedback loops) and non-catastrophic (gradual declines as observed in our population collapse experiment) transitions. According to Kefi et al., 2013, aEWS are expected to be observed when a dynamical system becomes increasingly sensitive to continuous environmental perturbation and exhibits a non-linear response to a linear forcing, even in the absence of a catastrophic bifurcation point.

Previous theoretical findings on robustness of aEWS suggested that performance of aEWS are related to the slope of the stability landscape curve (also known as ‘ball-in-well’ curve; see Nolting & Abbott, 2015 for details) which quantifies stability (Dai et al. 2015, Baruah et al. 2020). Stability landscape curves provides a visual

representation of how resilience and stability of a dynamical system are correlated with the state of the system (Nolting and Abbott 2015). For instance, a sharp slope in the stability-landscape curve means that the population is inherently stable to environmental perturbations. The steeper the slope of the stability-landscape curve, the more stable the population is, and stronger the aEWS are when a population is forced to collapse (Baruah et al. 2020). This is due to the fact that populations in higher quality habitats have higher growth rates and are thus inherently more stable (able to recover faster after perturbation) and the time series are thus less variable. Consequently, when highly stable populations are forced to collapse, the change in metrics such as standard deviation and autocorrelation are more easily observed as the baseline values for these metrics are more dissimilar to the values in the vicinity of a bifurcation or tipping point, thus making them easier to detect. Hence, any ecological (here phenotypic variation) or environmental variable (habitat quality, rate of forcing) that influences the inherent baseline stability of populations, should have an effect on the performance of aEWS (table 1).

In addition, aEWS require only state-based data such as abundance or biomass, but they have had limited success in forecasting population collapses (Boerlijst et al. 2013, Baruah et al. 2020). As a consequence, parallel time series data collected from phenotypic traits such as body size, used together with variance or autocorrelation estimated from abundance time series data, has been shown to predict collapses reliably and accurately (Clements and Ozgul 2016b, Clements et al. 2017b, Baruah et al. 2019, Arkilanian et al. 2020). EWS that focus on shifts in phenotypic traits such as body size or standard deviation of body size fall into the category of tEWS. Such tEWS have been demonstrated to reduce the rate of false positives; it also allowed true positives to appear earlier (Baruah et al., 2020; Clements et al., 2017b; Clements & Ozgul, 2016). The speed of environmental change could be put in terms of generation time of an observed organism. Baruah et al. 2019 suggested that medium to slow rate of environmental change in terms of generation time of an organism is needed for body size or fitness related trait shift to be useful in forecasting population collapses. Under these conditions, tEWS can outperform abundance-based metrics in predicting population collapses in response to environmental forcing. However, experimental evaluation of whether EWS (aEWS and tEWS) can reliably forecast population collapses under differing environmental conditions is still unknown.

One environmental forcing that poses a threat to biodiversity is anthropogenic warming. Warming could expose natural populations to new climatic niches that could induce shifts in ecological processes and destabilize populations (Walther et al. 2002, Clements et al. 2013, Baruah et al. 2018). Such changes in temperature could not only influence a species' survival in a habitat, but could also alter dynamics of a population, and in turn could

induce a cascading collapse at the community level (Kratina et al. 2012, DeLong et al. 2015, Svensson et al. 2017, Rudolf and Roman 2018). In addition, ecological responses to climate warming could also range from shifts in species distribution ranges to shifts in phenology, and to declines in mean body size (Auth et al. 2018, Jara et al. 2019). In fact, declines in mean body size has been suggested to be the third universal response to global warming (Van Buskirk et al. 2010, Gardner et al. 2011, Baudron et al. 2014).

Concurrent with anthropogenic warming, habitat degradation poses another major threat to global biodiversity (Griffen and Drake 2008). Decreases in habitat quality can be driven by increases in habitat degradation, consequently causing a decline in resources available for a species (Franken and Hik 2004, Griffen and Drake 2008). Specifically, low habitat quality due to low number of resources could cause populations to have a low growth rate (Griffen & Drake, 2008). Consequently, abundances remain low, populations have low resilience to environmental change, and are at increased risk of collapse due to demographic stochasticity (Lande 1993, Willi and Hoffmann 2009, Fraser et al. 2014, Sæther et al. 2016). Theoretical work has suggested that our ability to predict population declines decreases as population growth rate decreases (Baruah et al. 2020), implying that populations in poor quality habitats are at risk of collapsing unpredictably. Unravelling how EWS are affected by varying drivers of collapse is thus key to their application to real-world conservation issues. In this study, we changed the quality of habitat by manipulating the per capita resource available for the microcosm populations.

To deal with changing environmental conditions, natural populations persisting in different habitats could be phenotypically diverse (Violle et al. 2012). For instance, a population could consist of different phenotypes that respond differently, in terms of intrinsic growth rates, to changes in temperature (different temperature reaction norms). Variation in growth rates in response to different temperature regimes could be particularly important from a demographic viewpoint. In general, variation in demographic rates negatively affects long-term growth rate (Tuljapurkar and Orzack 1980) and in turn the stability of populations. Whether the basis of phenotypic variation is genetic, environmental, or demographic, trait variation could potentially decrease extinction risk (Doebeli 1996, Bolnick et al. 2011). If phenotypic variation in growth rates in the population is high, the risk of a population collapse due to extreme environmental stochasticity is expected to be low (Schindler et al. 2010). This is because, some phenotypes, which would otherwise have low fitness in a stable environment, could now have high fitness as the environment fluctuates and matches their optimum conditions.

Theoretical predictions from previous studies had indicated that higher phenotypic variation would negatively influence the performance of aEWS (table 1). This is because high phenotypic variation leads to low average

population growth rate, which then leads to low asymptotic stability (Baruah et al. 2020), and consequently negatively influences aEWS (table 1). On the contrary, habitat quality is predicted to increase the carrying capacity and growth rate of a population (Griffen and Drake 2008), which then increases population stability and resilience (Baruah et al. 2020). An increase in stability leads to increase in the effectiveness of aEWS in forecasting population declines (table 1). In terms of tEWS, we hypothesize that strength in tEWS (e.g., average body size) would be higher in a good quality habitat in comparison to a poor quality habitat. This is because in poor quality habitats, mean body size would already be low, and shifts in average body size or tEWS would be weak when populations are forced to collapse. We also hypothesize that strength of shifts in mean body size would be higher in populations with high phenotypic variation in comparison to populations with low phenotypic variation (table 1). This is because high phenotypic variation, either arising due to environmental conditions (plasticity) or genetic variation, should lead to a faster shift in mean body size in response to changes in the environment (Baruah et al. 2019) (table 1).

Here, we assess the effects of warming (slow, fast, and constant), habitat quality manipulated by the amount of food resources made available (good and poor), and phenotypic diversity (three different populations) on early warning signals of population collapse. We analyzed population abundance and body size time series data from three phenotypically diverse populations of the protozoan ciliate *Askenasia volvox* in two levels of habitat quality (low and high quality) subjected to three different rates of warming (no warming, fast warming, and slow warming). We then investigated whether habitat quality, phenotypic diversity and rate of warming altered the performance of abundance- and trait-based warning signals of population collapse. Our study highlights the effectiveness of EWS under varying ecological and environmental conditions and sheds lights to their overall applicability to real-world scenarios.

2. Methods and Materials:

2.1 Experiment 1: Thermal performance curves

We collected individuals of the freshwater protozoan ciliate *Askenasia volvox* along an elevational gradient (405 m, 1000 m, 1500 m, 2100 m) from four freshwater ponds in several different regions in Switzerland. From each of the four populations, a single individual was selected to produce clonal populations. *A. volvox* can readily grow in filtered mineral water (Volvic mineral water, see appendix 1 for mineral concentrations). Each of the four

populations were fed 0.7 ml of the Cryptophyte, *Cryptomonas* (kept at a density of 1.02×10^5 individuals/ml) every three days to keep the population growing.

To investigate for differences in thermal performances across the four clonal populations, we conducted a thermal performance experiment. The four populations were grown at 8 different constant temperature treatments ranging from 15^o C to 28^o C with four replicates each in 20 ml plastic cell culture flasks. The replicate populations had the same initial population density at the start of the experiment (70 individuals/ml). Each of the replicates in all the temperature treatments were provided with 0.5 ml of *Cryptomonas* (at a density of $\sim 35 \times 10^3$ individuals/ml) every three days of the experiment to keep the populations growing (Fig. S1B for food levels on population density). Sampling for population density were carried out every day for the next 14 days. A logistic growth model was fitted to population density over time for all the replicates of the four *A.volvox* populations to estimate r_{max} across the temperature range (Fig. S1A). r_{max} is the per capita growth rate which could be used as a proxy for population fitness. Thermal response curves suggested that, out of the four *A.volvox* populations, one from (405m altitude) and one from (1500m) had similar thermal reaction norms. In other words, r_{max} curves of two populations (405m and 1500m) overlapped substantially. However, the two other clonal populations (1000m and 2100m) had different response curves indicating that there were at least three distinct phenotypes of *A.volvox*. We only show the thermal response curves of the three distinct clonal populations in fig. S1A. Henceforth, we took three of the four *A.volvox* populations, named as C1 (405 m), C2 (1000 m), and C3 (2100 m) and used them for our next population collapse experiment.

2.2 Experiment 2: Population Collapse

With three populations of *A. volvox* that had three different optimum temperatures (Fig. S1A), we assessed whether phenotypic diversity and habitat quality affected our ability to predict population collapses. First, to create a gradient of phenotypic diversity, we assembled different populations with three levels of phenotypic variation that comprised of combinations of three clones of *A.volvox* together (high diversity) (see Fig. 1 for experimental design); combinations of two clonal populations together (medium diversity), and single clonal populations (low diversity). Thus, the high diversity population was assembled by having all three phenotypes (C1, C2 and C3) in the same culture flask, with a total of four replicates. The medium phenotypic diversity population was assembled by combining two of the three phenotypically different clones (C1 and C2, C2 and C3, C3 and C1, each of the combinations having 4 replicates) in the same culture flask with a total of twelve replicates; and finally low phenotypic diversity level was assembled with single clonal populations (C1, C2, C3 each with 4 replicates) in a

given culture flask, with a total of twelve replicate populations. The replicate populations in different diversity levels were subjected to three different experimental warming treatments: a) a control treatment of 18⁰ C where the temperature remained constant throughout the experiment, b) a slow warming treatment of +0.2⁰ C per day, and c) a fast warming of + 0.4⁰ C per day.

To assess the effect of habitat quality on the ability to predict collapses with EWS, the above setup was divided into habitats with two different food quality: a) a high-quality habitat (~35 x 10³ ml⁻¹ of *Cryptomonas* fed every three days to the replicate populations) and (b) a poor-quality habitat (~12 x 10³ ml⁻¹ of *Cryptomonas* fed every three days to the populations). The amount of food given to *A. volvox* populations in the high quality habitat treatment led to a higher carrying capacity in comparison to the populations in the poor quality habitat (Fig. S1B). In total 168 microcosms (7 groups of clones x 3 warming treatments x 2 habitat quality x 4 replicates) were established for the experiment.

The starting density for all populations was 70 individuals/ml, divided equally between the number of phenotypes within the population (i.e. when phenotypic diversity is one, 70 individuals/ml were added; when phenotypic diversity was two, 35 individuals/ml were added of each phenotype; and when phenotypic diversity was three, 23 individuals/ml were added of each of the phenotype). Warming treatments were started on day 2 of the experiment to ensure that none of the clones were outcompeted before the warming treatments started and impacted population dynamics. Each replicate microcosm was sampled with a cell counter under a light microscope to assess the number of individuals in 1 ml of culture medium, and sampling continued until the extinction of the population. Extinction was assumed if 3 ml of the sampled microcosm had zero individuals. The warming treatments started from 18⁰ C. Of 168 total microcosms, 4 in control, 2 in fast, and 1 in slow warming treatment failed to establish within five days and were discarded.

For estimating mean body size and standard deviation of body size, 1 ml of culture medium from each of the replicates was pipetted into a counting chamber and was then covered with a glass slide. Next, 5 to 10 second videos of each of the replicates were taken under the microscope every day until populations were inferred to be extinct. Note that in our study we define population decline as decreases in population numbers and collapse as local extinction of the population. We took one video per sample. However, when the number of individuals were less than 5 in a given frame, we took multiple (n = 3) videos. Later, these videos were analysed with the help of ImageJ and R package BEMOVI to estimate mean and standard deviation of body size (details in Pennekamp et al. 2015). We used BEMOVI solely to estimate body size (mean width). Furthermore, the threshold values used

in ImageJ to define the masks of individuals were 55 and a maximum value of 255 (see example Fig. S8-11). However, it is possible that misidentification due to strict threshold values could lead to underestimation of body size.

2.3 Stability, mean body size and average population size

From the control warming treatment, we estimated average population size, mean body size and temporal variability for each habitat quality and phenotypic diversity treatment. One measure of stability is temporal variability (Donohue et al. 2016), which can be estimated from coefficient of variation (CV) of population . Next, we assessed whether mean body size, population size, and CV, were affected by habitat quality and phenotypic diversity in the control warming treatment.

2.4 Abundance-based early warning signals of population collapse

Nonetheless, we estimated the timing of the bifurcation point for each replicate's abundance time series independently from the population collapse experiment. For a population with density-dependent growth, a bifurcation point occurs when the growth rate of the population shifts and remains below one (Drake and Griffen 2010). We estimated the bifurcation time point by fitting *loess* to realised growth rates (N_{t+1}/N_t) over time t , where N_t is the population density at day t . The timing of the bifurcation point was established by recording the first time point at which the realized growth rate fell and remained below 1 prior to the collapse of the population (i.e. the timepoint t when $N_{t+1}/N_t < 1$). To note that the timing of the bifurcation points was estimated on population time series data generated from the population collapse experiment and not from the temperature performance curves. Due to variation in the timing of bifurcation point in replicate populations, we took the mean of the bifurcation time points across the replicates in each of the diversity levels. aEWS analyses were done on abundance time series data up to the bifurcation time point. Doing this ensured standardization of the length of the time series (Arkilanian et al. 2020). We then quantified two aEWS namely autocorrelation at first-lag (*ARI*) and standard deviation (*SD*) - most other aEWS can be mathematically derived from *ARI* and *SD*. The two statistical metrics *ARI* and *SD* were calculated with a predefined sliding window which is 50% of the length of the time series analyzed (Dakos et al. 2012). We used Gaussian detrending to discard any trend in the abundance time series data. Next, we z-standardized both the aEWS metrics so that it would be easier to compare with trends of tEWS metrics (see section 2.5). We quantified the strength of aEWS by estimating Kendall's tau rank correlation coefficient.

Higher positive value of Kendall's tau correlation coefficient with time would indicate an approaching population collapse.

2.5 Trait-based early warning signals of population collapse

We used body size based information to create trait-based warning signals (tEWS) (Baruah et al., 2019; Clements et al., 2017b; Clements & Ozgul, 2016). Mean body size (*mean.size*) and standard deviation of body size (*SDsize*) time series data were z-standardized. The length of body size time series that were analyzed were of the same length as its corresponding abundance time series. Before an imminent population collapse, aEWS such as *SD* and *ARI* are expected to increase over time (Kéfi et al. 2013). However, mean body size is expected to decrease over time in response to the different warming treatments (Brown et al. 2004). Hence, the standardized mean body size (*mean.size*) time series was subsequently multiplied by -1 so that it could be compared alongside aEWS. Hence, increases in mean body size would now indicate an approaching collapse. Standard deviation of body size, suggested to be a descriptor of variation in size, does not have an expected trend, although size-dependent competition can cause a rise in variance of body size (Clements & Ozgul, 2016).

We then evaluated and compared the performance of *ARI*, *SD*, *mean.size*, *SDsize* as the four unique indicators of collapse in populations. Specifically, we compared how shifts in body size-based indicators performed in comparison to abundance-based metrics alone.

2.6 Statistical analysis of abundance- and trait-based early warning signals

To assess the effects of habitat quality, rate of warming, and phenotypic variation on performance of aEWS and tEWS we used linear mixed-effects models (Bates et al. 2012). Kendall's tau correlation coefficient of *ARI*, *SD*, mean body size (*mean.size*), and standard deviation of body size (*SDsize*) were the response variables, and predictor variables were categorical factors of warming (constant, slow and fast), habitat quality (good and poor), and phenotypic diversity (one, two and three). The categorical predictor variables were included in the model as three-way and two-way interactive fixed effects and replicates nested within phenotypic diversity were included as random effects. Model selection was done using AICc for small sample sizes. Significance of the fixed effects were assessed based on their standardized coefficients and their corresponding F-values. Post-hoc analysis on interactive fixed effects were done using Tukey's linear contrasts using the *multcomp* R package.

2.7 Performance of abundance- and trait-based warning signals: ROC curves

In addition, we evaluated the overall performance of tEWS and aEWS in predicting collapses with receiver-operating characteristics (ROC) curves. ROC is a comparison of sensitivity (true positives) and 1- specificity (false positives) of an evaluated metric. ROC curve evaluates whether a metric can distinguish between two events - population collapse (increasing temperature treatments) and population persistence (constant temperature treatment). Next, we calculated ROC curves with a series of different threshold values of Kendall's Tau and plotted true positive rate (TPR i.e. ability to predict collapses) (also known as sensitivity) and false positive rate (also known as 1-specificity) with the help of R library *plotROC*.

3. RESULTS

Populations of *A.volvox* collapsed through transcritical bifurcation. Such protozoan populations reproduce asexually and consequently are unlikely to exhibit fold bifurcations, a fact supported by the lack of sudden collapses in our experimental systems (Fig. 2). The timing of population collapses was longer in the slow warming treatment than in the fast warming treatment (Fig. S12), however, there were not substantial differences in collapse points across the phenotypic diversity or habitat quality treatments (see Fig. S12).

3.1 Phenotypic diversity and habitat quality on population dynamics in the control temperature treatment

In the control temperature treatment, CV was higher in the poor quality habitat than in the good quality habitat signifying lower stability in the populations in poor quality habitats than in the good quality habitats (est = 0.087, s.e = 0.02, $P = 0.0001$) (Fig. 3A). In the good quality habitat, high phenotypic diversity (three clones together) led to high stability in comparison to low phenotypic diversity (one clone) (linear contrasts, $P = 0.03$). However, in the poor quality habitat, phenotypic diversity did not have an impact on CV (Fig. 3A).

Poor quality habitat had a greater negative effect on average population size than high quality habitat in the control temperature treatment (est = -11.03, s. e. = 1.52, $P < 0.00001$) (Fig. 3B). Within the habitat quality treatment, however, phenotypic diversity did not have any effect on average population size.

Mean body size was on average smaller in the poor quality habitat than in the good quality habitat for the control treatment (est = -3.27, s.e = 0.843, $P = 0.0005$). Phenotypic diversity did not have an effect on mean body size in either of the habitats (Fig. 3C).

3.2 Phenotypic diversity, habitat quality, and warming on abundance-based EWS of population collapse

In the population collapse experiment (experiment 2), the final temperatures experienced by replicate populations in slow warming treatment was 29°C and for fast warming treatment it was 38°C.

Strength of AR1 (Kendall's tau value of AR1) in predicting population collapse was unaffected by phenotypic diversity. AR1 performed slightly worse in the poor quality habitat (est = -0.08, s.e = 0.07) than in the good quality habitat (Fig. 4). However, Kendall's tau value of AR1 was on average below zero suggesting that shifts in AR1 did not occur before population collapse.

In the good quality habitat, *SD* performed better in the slow warming treatment than in the fast warming treatment (est = 0.40, s.e = 0.10, P = 0.002). However, even though Kendall's tau value of *SD* was higher in the slow warming treatment than in the fast warming, the overall strength was below zero, suggesting that *SD* was unable to predict collapses in the good-quality habitat. In the poor-quality habitat, regardless of different rates of warming, *SD* was unable to predict population collapse; there was no rise in *SD* before an approaching collapse, indicated by negative Kendall's tau values (Fig. 4).

3.3 Phenotypic diversity, habitat quality and warming on trait-based EWS of population collapse

Shifts in mean body size (*mean.size*) were affected by habitat quality and warming treatment (table 2, table S5). For the slow warming treatment, shifts in body size were larger (denoted by higher Kendall's tau value) in the good quality habitat than in the poor quality habitat (est = -0.42, s.e = 0.12, P = 0.005, Fig. 4). However, in the fast warming treatment, shifts in mean body size were larger in the poor quality than in the good quality habitat (est = 0.43, s.e = 0.11, P = 0.002, Fig. 4). On average, when compared within the poor habitat quality, shifts in mean body size were observed more in the slow warming treatment than in the fast warming treatment (est = -0.378, s.e = 0.12, P = 0.021). However, in the good quality habitat, shifts in mean body size were observed more in the fast warming treatment than in the slow warming treatment (est = 0.48, s.e = 0.11, P = 0.002).

Warming influenced shifts in standard deviation of body size, i.e., *SDsize*. Shifts in *SDsize* was more evident in the poor quality habitat and slow warming treatment, then in the fast warming treatment (est = 0.43, s.e = 0.13, P = 0.008) (Fig. 4). In addition, in the slow warming treatment, shifts in *SDsize* was more prominent in the poor quality habitat than in the <good quality habitat (est = 0.40, s.e = 0.14, P = 0.032).

3.4 Performance of abundance- and trait-based EWS: ROC analysis

ROC analysis suggested that *SDsize* was the best performing metric in predicting population collapse as well as in predicting population persistence with an AUC (area under the curve) of 0.95. This meant that the rate of true positives in predicting whether a population is persisting or is declining are high. In case of *mean.size*, AUC was around 0.90 (Fig. 5). *mean.size* metric performed slightly worse than *SDsize* particularly in predicting population persistence, where false positive fraction was a bit higher than *SDsize*. In the case of abundance-based signals, *SD* and *ARI* were very sensitive and less specific in predicting either population persistence or population decline (Fig. 5).

4. Discussion

While research on EWS have grown recently, their efficacy in predicting population collapses has rarely been tested under different ecological and environmental factors. Here, we experimentally tested the utility of abundance and trait-based EWS in predicting population collapses under changes in ecological (habitat quality and phenotypic diversity) and environmental factors (rate of warming). Overall, aEWS performed poorly across all the treatments (Fig. 4). On the other hand, tEWS generally performed better and were robust to changes in different ecological and environmental factors (Fig. 4-5).

Any ecological (here phenotypic variation) or environmental variable (habitat quality, rate of warming) that influences inherent stability of populations, should have an effect on the performance of aEWS (table 1). To determine, whether a population was inherently stable, one could estimate CV of population size (Donohue et al. 2016). If CV was large, population would be less stable to environmental perturbations. Indeed, we found that replicate populations in good quality habitat on average were inherently less variable (low CV) in population size and thus more stable (Fig. 3), than populations in low quality habitat (high CV). Such a result indicated that populations in good quality are predicted to exhibit stronger aEWS than populations in low quality habitat (table 1) (note that our control treatment timeseries were not long transients as they did not show any sudden shifts to another alternative state). In principle, we did find that Kendall's tau value of *SD* to be higher in good quality habitat than in poor quality habitat, however, *SD* still performed poorly in predicting population collapses in all experimental treatments (Fig. 4C, table S4).

Rate of increase in temperature in the fast warming treatment was twice that of the slow warming treatment. Hence, populations exposed to the fast warming treatment experienced higher temperatures and consequently

collapsed earlier than those exposed to the slow warming treatments (Fig. S12). In addition, in the fast warming treatment, *SD* was worse in predicting population collapses even when quality of habitat was high, indicating that higher amount of food availability was not enough to offset the negative effect of fast warming treatment (Fig. 4C, table S4). Earlier theoretical studies have suggested that rate of environmental forcing could influence the trends of aEWS (Clements & Ozgul, 2016), as we also experimentally observe here.

Contrary to theoretical expectations (table 1), our experimental results showed that the effects of phenotypic variation on the performance of aEWS were largely negligible in the warming treatments, regardless of the quality of the habitat. In the control treatment, however, when equilibrium population sizes were tracked, the effects of phenotypic variation on CV was noticeable in good quality habitat, with higher stability observed when phenotypic variation was higher, particularly in high phenotypic diversity (Fig. 3). This would suggest that populations with high phenotypic variation should exhibit stronger aEWS when forced to collapse than populations with low phenotypic variation (Baruah et al. 2020). However, we did not observe any such result. The lack of noticeable effect of phenotypic variation on performance of aEWS could probably be attributed to relatively stronger effects of both environmental forcing and habitat quality that might have swamped the effects of phenotypic variation. Moreover, the failure of aEWS in forecasting population collapses could also be due to noise in the experimental data and the length of timeseries used for EWS analysis, both of which have been previously studied in detail (Dutta et al. 2018, Arkilanian et al. 2020).

Shifts in body size were influenced both by the quality of habitat and the rate of warming. Specifically, for slow rate of warming, larger body size shifts were observed in the good quality habitat than in the poor quality habitat. We lacked a reasonable explanation, but we speculated that due to higher amount of food availability in the good quality habitat, decreases in mean body size were solely because of warming as followed from the metabolic theory of ecology (Brown et al. 2004). However, in the poor quality habitat and slow rate of warming treatment, lack of food availability already caused mean body size to be small (Fig. 2, table 1) before slow increases in temperature crossed the optimum and affected body size. As such, further decreases in body size due to warming was not possible, owing to the limits of plastic capacity of body size of *A. volvox*. Hence, we observed less stronger trends in shifts in mean body size in poor quality habitat than in good quality habitat in the slow warming treatment, which was in line with our hypothesis. However, a different response was observed in the fast warming treatment. Body size shifts were stronger in the poor quality habitat for the fast warming scenario than in the slow warming scenario (Fig. 4A). Because the rate of warming was faster, increases in temperature crossed

the optimum of the different clonal populations faster, and hence shifts in mean body size was relatively steeper than in slow warming treatment, which was compounded by the lack of food availability in the poor quality habitat. Consequently, stronger trends in shifts in body size were observed in poor quality habitat when compared with good quality habitat, where high amount of food availability possibly compensated the decrease in body size. In addition to this, shifts in body size produced fewer false positives and its performance, based on ROC analysis, was better than the other abundance-based metrics.

Our experimental populations also showed shifts in standard deviation of body size before population collapses (Fig. 4B). In harvested ecological systems, decline in variance in body size distribution had been shown to occur before collapse of populations (Clements et al., 2017). However, it was also possible for variance in trait distribution to increase when size dependent competition for resources was predominant, and where competitive ability of individuals depend on their trait value (Clements & Ozgul, 2016). In our experiment, variance in mean body size increased following different warming treatments before collapses of populations. Further, based on ROC analysis, variance in body size produced the fewest false positives and had the highest accuracy in predicting whether a population is persisting or declining (Fig. 5). To this end, we were unsure about the mechanisms behind the increases in standard deviation in body size before a population collapse in response to warming. Body size based signals are thus obvious candidates to include within the suite of generic early warning signals. This is because of consistent predictions of population collapse by shifts in body size in response to different environmental scenarios ranging from population harvests, decline in food availability, and now warming in this study.

Critical transitions such as collapses of populations (Wissel 1984) or transition to an alternative state as observed in freshwater lakes, where a clear-water macrophyte dominated state can transition to a turbid-water dominated by plankton due to eutrophication (Scheffer et al. 1993) are examples where phenomenological signals could be useful in forecasting early transitions. These transitions are driven by external forcing such as increasing nutrient load, or temperature that pushes the system away from equilibrium and thereby degrading the ecosystem functions (Jeppesen et al. 2010). With acute increases in global temperature, shifts in ecosystem functions, functional traits, or body size are expected to increase along with shifts in abundances and biomass. Such shifts in traits, ecosystem functions, or biomass could be indicative of an ecological system moving away from its equilibrium, and consequently resilience of the system could decline. EWS are thus a tool to predict these changes beforehand.

However, our results point towards the fact where aEWS fail in forecasting future changes, tEWS can greatly improve forecasting ability of future population collapses.

In conclusion, our experimental results demonstrated that aEWS were unreliable in forecasting population declines regardless in the presence of phenotypic variation or higher habitat quality, results that were quite contrary to theoretical predictions. However, tEWS were robust in forecasting population declines across all different treatments. In particular, shifts in body size was influenced by quality of habitat and rate of warming (table 1). Mean body size shifts were prominent in good quality habitat when rate of warming was slow in line with our predictions (table 1). However, combined effect of fast rate of warming and poor quality habitat resulted in stronger shifts in body size, in comparison to body size shifts in good quality habitat. Nevertheless, tEWS outcompeted aEWS in forecasting population declines. Thus, we advocate a predictive framework where both aEWS and tEWS are combined to inform management decision. Because EWS (aEWS and tEWS) do not make mechanistic assumptions, these signals could be used as a first-pass tools to identify populations or communities at risk of collapse, before a more detailed investigation is carried out. Such a framework then plays to the strength of EWS as a predictive tool.

Data availability statement: Data uploaded at Zenodo doi: 10.5281/zenodo.4564955.

Link: <https://zenodo.org/record/4564955#.YDkx2JNKjlw>

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Table 1: Predicted theoretical outcomes of aEWS and tEWS of population collapse associated with different levels of treatments used in the experiment.

Treatments	Treatment Levels	EWS	Predicted or hypothesized outcome of aEWS and tEWS*
Phenotypic variation	High	aEWS	Low strength in SD and AR1[1]
		tEWS	High strength in mean body size shift [1]
	Medium	aEWS	Medium strength in SD and AR1[1]
		tEWS	Medium strength [1]
	Low	aEWS	Highest strength in SD and AR1[1]
		tEWS	Low strength [1]
Warming	Fast	tEWS	Low strength in mean body size shift [4]
		aEWS	High strength in both SD and AR1[2,3]
	Slow	tEWS	High strength in mean body size shift [4]
		aEWS	High strength in both SD and AR1[1]
Habitat quality	High	tEWS	High strength in mean body size shift [4]
		aEWS	Low strength in both SD and AR1[1]
	Low	tEWS	Low strength in mean body size shift [4]

*(hypothesized for only mean body size)

[1] (Baruah et al. 2020); [2](Clements & Ozgul, 2016) ; [3] (Arkilanian et al., 2020); [4] this study

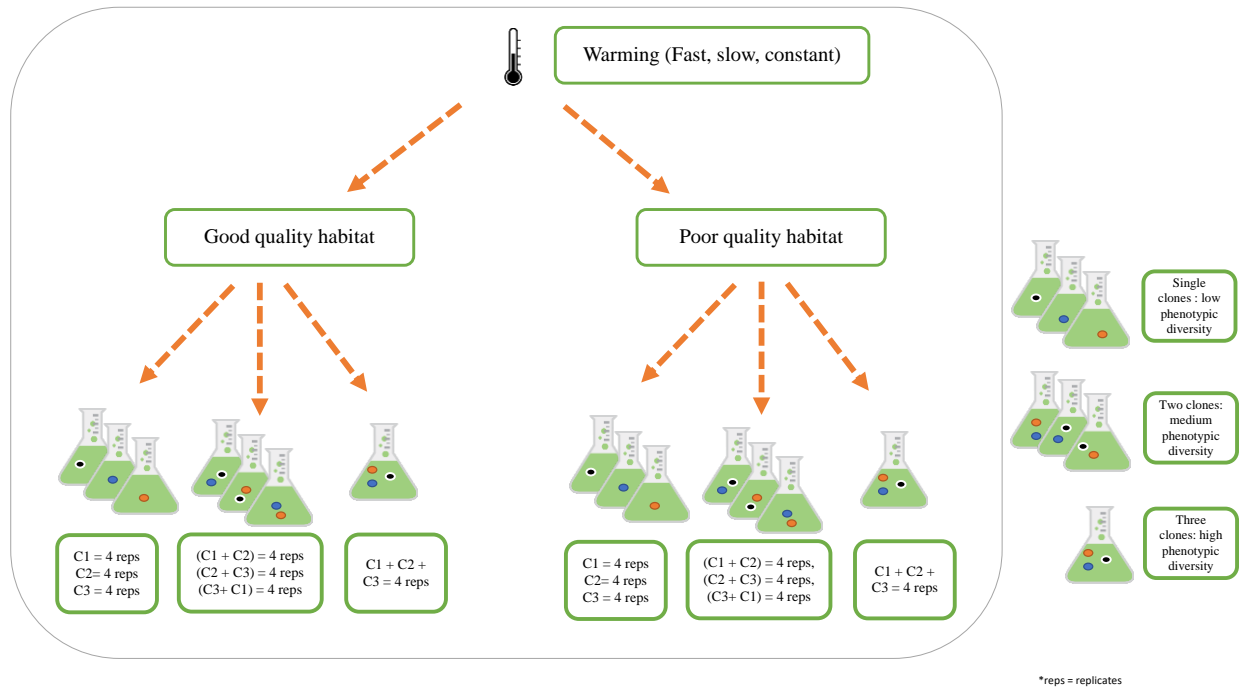
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528 **Table 2:** Models indicating the relationships between the response variables (*SD*, *ARI*, *mean.size*, *SDsize*) and
 529 predictor variables of habitat quality (Habitat), warming (Warming), and phenotypic diversity (Diversity). The
 530 best model was determined by the lowest AICc value. $\Delta AICc$ in the table gives the difference between the best
 531 model (in bold) and the next best one. Note that phenotypic diversity treatment did not appear as an important
 532 predictor in any of the aEWS and tEWS signals and hence not listed in the table. (DF= degrees of freedom of the
 533 model).

Response variable	Fixed effects	DF	AICc	$\Delta AICc$
(Kendall's tau value)				
<i>SD</i>	Habitat + Warming + Habitat:Warming	9	162	0
	Warming	5	170.2	8.2
<i>ARI</i>	Habitat	4	100.2	0
	Warming	6	101.7	1.5
<i>mean.size</i>	Habitat + Warming + Habitat: Warming	9	216.7	0
	Habitat + Warming	7	242.1	25.4
<i>SDsize</i>	Warming	6	195.7	0
	Habitat + Warming + Habitat:Warming	9	197.7	2

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537 **Figure 1:** Experimental design with three warming treatments (fast, slow, and constant) for two different levels
 538 of habitat quality (good and poor) and for three different levels of phenotypic variation (low, medium, and
 539 high). Each replicate populations both in good quality and poor quality habitat collapsed either through fast or
 540 slow warming treatment or did not collapse (constant temperature treatment). Total replicate populations used in
 541 the experiment was 168.

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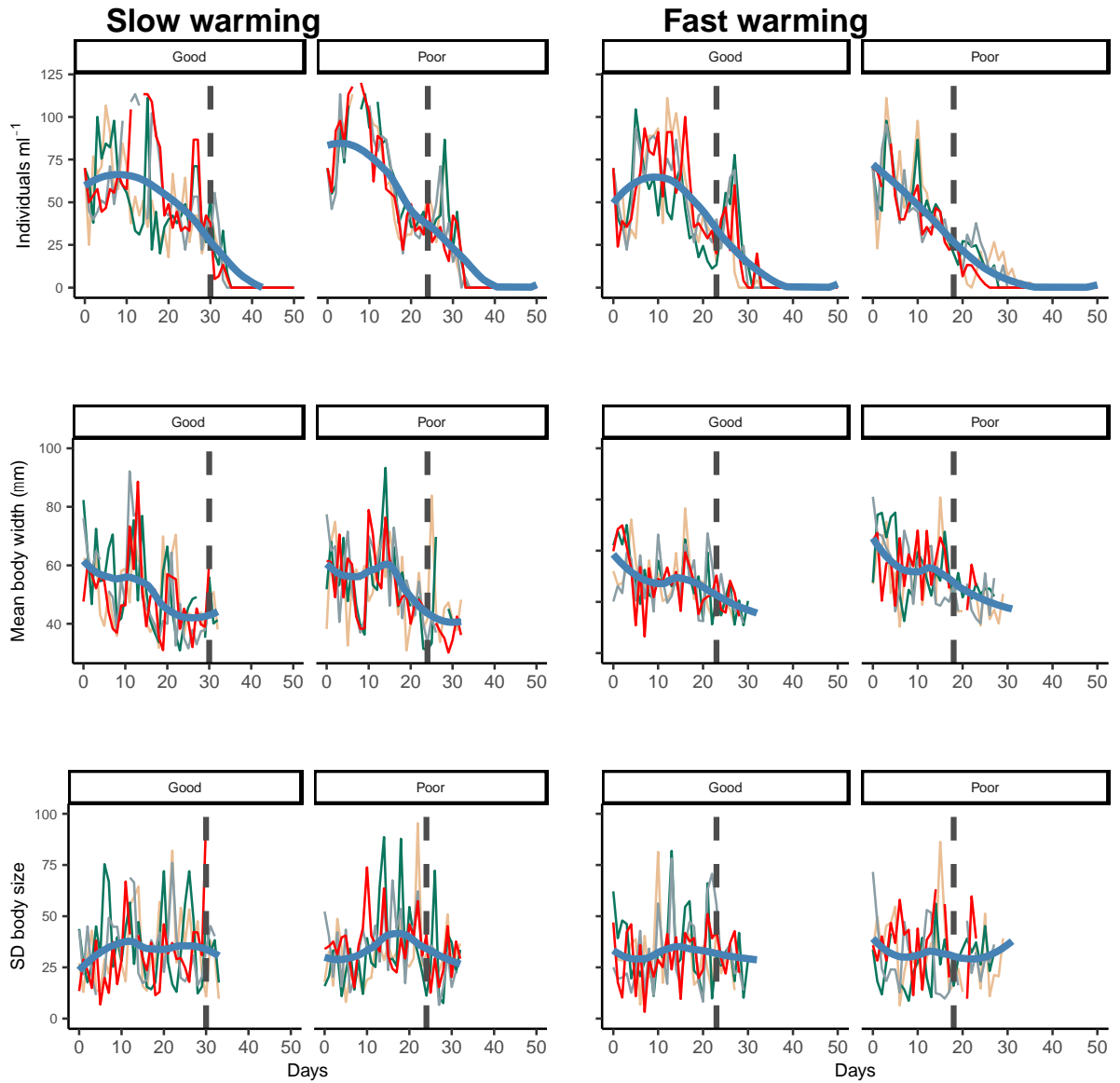
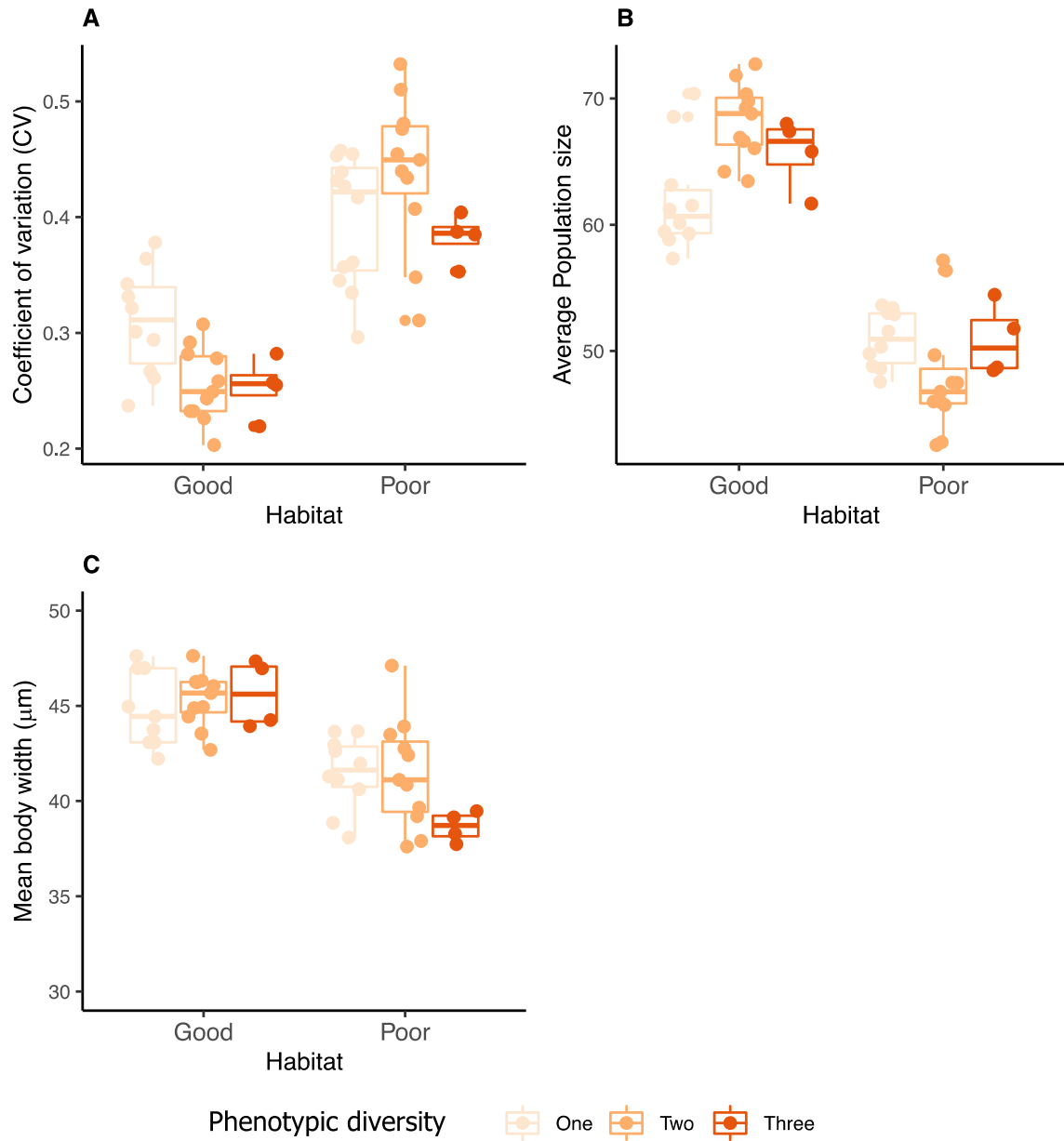


Figure 2: Density (Individuals per ml), mean body size (in μm) and standard deviation of body size over time till population collapse for all the four replicates for the high level of phenotypic diversity treatment across two qualities of habitat (Good and Poor) and across two rates of warming –Slow warming and Fast warming. The thick blue lines are *loess* smoothing across replicates; dashed black line indicates the bifurcation point across replicates.



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551 **Figure 3:** Coefficient of variation (CV), average population size, and mean body size for control temperature
 552 treatment (18⁰ C) for different levels of phenotypic diversity and habitat quality. Note that high value of CV results
 553 in unstable population dynamics.

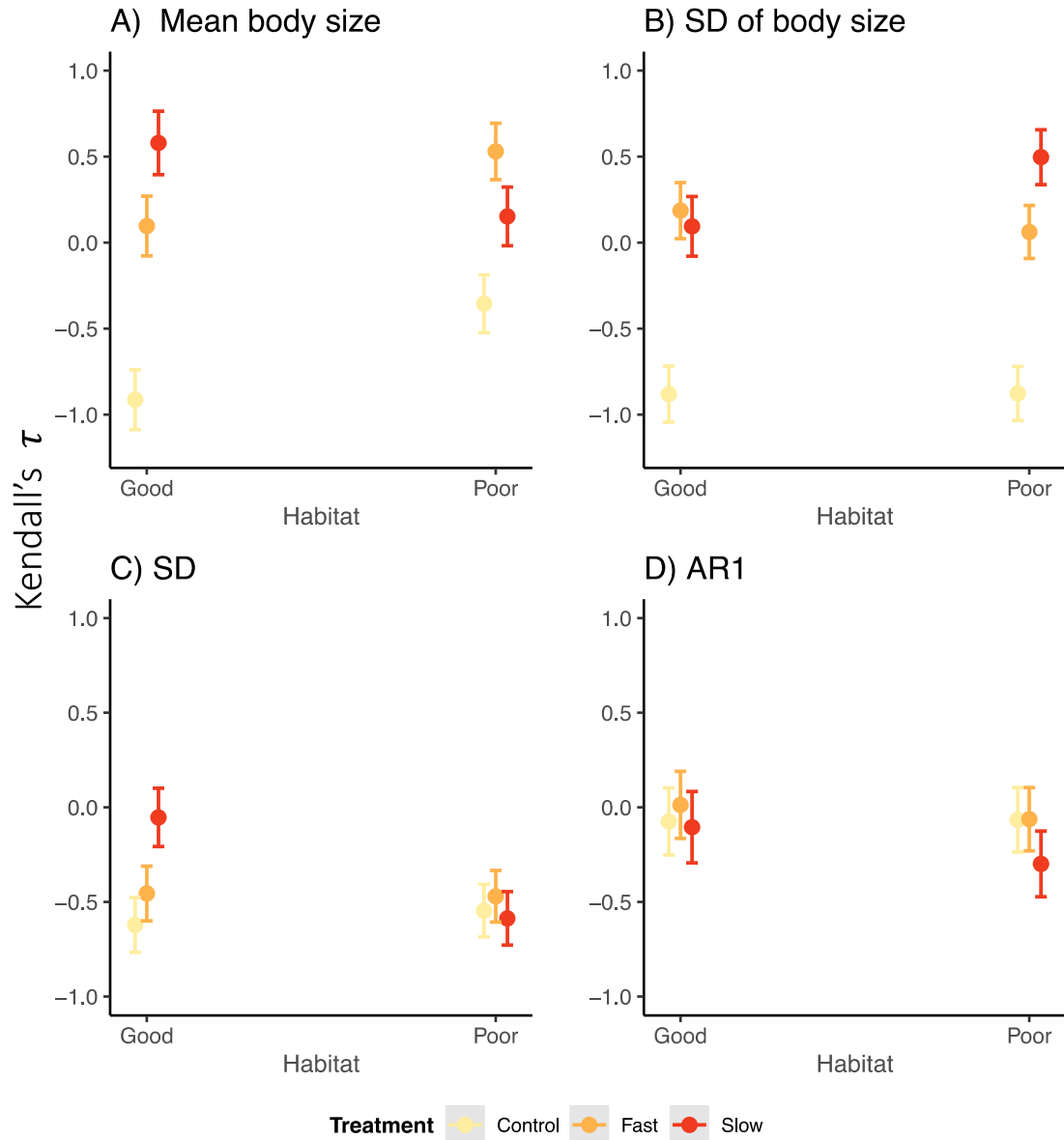


Figure 4: Model predicted values of Kendall's tau correlation coefficient with standard error bars on Y-axis for the tEWS i.e., mean body size (*mean.size*), SD of body size (*SDsize*) and for aEWS i.e., *SD* and *ARI*, with habitat quality and warming treatment as interactive factors on X-axis. Note that Kendall's tau coefficient below zero would indicate that the EWS did not shift before an approaching population collapse. Also note that we did not include 'phenotypic diversity' treatment in the above figure as it was not an important factor in any of the best models (see table 2). aEWS metrics such as *SD* and *ARI* were unable to predict population collapse across all treatments; tEWS metrics, such as 'Mean body size' (*mean.size*) and 'SD of body size' (*SDsize*), had Kendall's tau values above zero indicating reliable prediction of population collapses across treatments. For control

temperature treatment, both aEWS and tEWS metrics did not shift i.e., Kendall's tau were below zero, accurately predicting no population collapse.

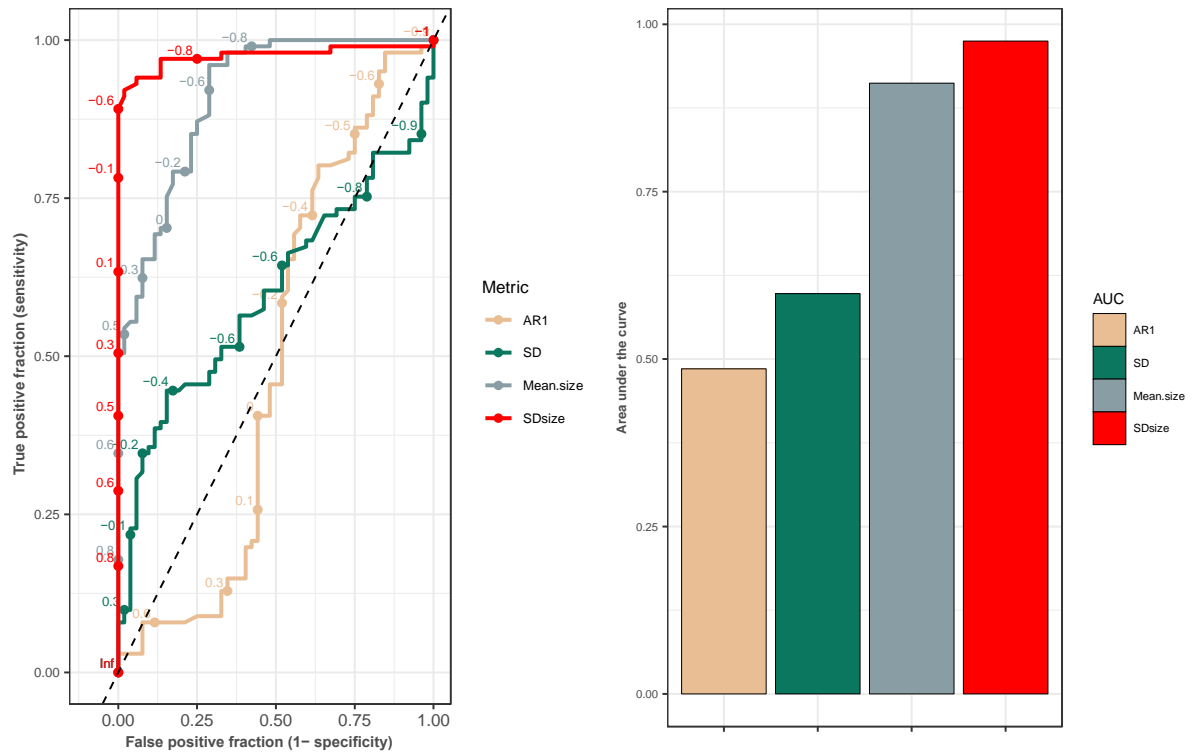


Figure 5: ROC curves comparing the performances of the four early warning metrics, for a range of threshold Kendall's tau values from -1 to 1 (left). ROC curves above 1:1 line denote a higher probability of true positives than by chance alone. The area under the curve (AUC) quantifies the probability of true detection of population collapse versus the false positives of collapse. Higher the value of AUC, better the EWS (right).