Title

Disturbance decreases genotypic diversity by reducing colonization: Implications for disturbance–diversity feedbacks

Journal

Ecology, 103(7)

ISSN

0012-9658

Authors

Kollars, Nicole M
Stachowicz, John J

Publication Date

2022-07-01

DOI

10.1002/ecy.3710

Peer reviewed

Kollars Nicole M. (Orcid ID: 0000-0002-4513-1659)

Kollars and Stachowicz -
Journal: Ecology

Manuscript type: Article

Disturbance decreases genotypic diversity by reducing colonization: Implications for disturbance–diversity feedbacks

Nicole M. Kollars[1,2*] and John J. Stachowicz[1]

1. Center for Population Biology and the Department of Evolution and Ecology, University of California Davis, One Shields Ave, Davis, CA 95616, USA.
2. Present address: Department of Marine and Environmental Sciences, Northeastern University Marine Science Center, 430 Nahant Road, Nahant, MA 01908, USA.

*Corresponding author: nmkollars@gmail.com

OPEN RESEARCH: Data and code (Kollars, 2022) are available in Zenodo at https://doi.org/10.5281/zenodo.6011003

ABSTRACT: One objective of eco-evolutionary dynamics is to understand how the interplay between ecology and evolution on contemporary timescales contributes to the maintenance of biodiversity. Disturbance is an ecological process that can alter species diversity through both ecological and evolutionary effects on colonization and extinction dynamics. While analogous mechanisms likely operate among genotypes within a population, empirical evidence demonstrating the relationship between disturbance and genotypic diversity remains limited. We experimentally tested how disturbance altered the colonization (gain) and extinction (loss) of genets within a population of the marine angiosperm *Zostera marina* (eelgrass). In a 2-year field experiment conducted in northern California, we mimicked grazing disturbance by migratory geese by clipping leaves at varying frequencies during the winter months. Surprisingly, we found the greatest rates of new colonization in the absence of disturbance and that clipping had negligible effects on extinction. We hypothesize that genet extinction was not driven by selective mortality from clipping or from any stochastic loss resulting from the reduced shoot densities in clipped plots. We also hypothesize that increased flowering effort and facilitation within and among clones drove the increased colonization of new genets in the undisturbed treatment. This balance between colonization and extinction resulted in a negative relationship between clipping frequency and net changes in genotypic richness. We interpret our results in light of prior work showing that genotypic diversity increased resistance to grazing disturbance. We suggest that both directions of a feedback between disturbance and diversity occur in this system with consequences for the maintenance of eelgrass genotypic diversity.

KEYWORDS: disturbance, eelgrass, feedback, genotypic diversity, grazing, colonization

INTRODUCTION: The field of eco-evolutionary dynamics asks how the "evolutionary play acts within the ecological theater" (Hutchinson 1965) on contemporary timescales (Schoener 2011). Current work largely focuses on how natural selection drives phenotypic change that then simultaneously feeds back to alter the selection agent (reviewed in Hendry 2017). However, this framework may be limiting our understanding of feedbacks given that relationships between particular trait states and ecological function can be complex, indirect, and contingent on other traits. Furthermore, selection is not the only mechanism of evolution in natural populations. Rapid changes in allele frequencies can also occur through ecological processes driving stochastic loss (e.g., over-hunting increased genetic drift in Northern elephant seals; Bonnell and Selander 1974), gene flow (e.g., a hurricane caused admixture of a brooding isopod; Pagán et al. 2020), mutation (e.g., starvation led to mutagenesis in *E. coli* bacteria; Bjedov et al. 2003), and non-random mating (e.g., parasite infection influenced mate choice in red jungle fowl; Zuk et al. 1990). Broadening the framework beyond studies with eco-to-evo pathways centered on selection, or systems where trait-function relationships are well-defined, will expand our understanding of how the interplay between ecology and evolution on contemporary timescales operates in natural systems.

In addition to influencing the allelic composition of a population, ecological processes, such as disturbance (*sensu* Sousa 1979), can alter evolutionary processes that influence the diversity of genotypes in a population. Community ecology provides multiple hypotheses for predicting how disturbance should affect biodiversity. For example, the intermediate disturbance hypothesis suggests that moderate levels of disturbance increase richness by delaying extinction from competitive exclusion and facilitating colonization (*sensu* Connell 1978, Sousa 1979, Chesson and Huntly 1997). However, disturbance can reduce diversity under conditions of

limited colonization where disturbance-driven extinctions dominate, such that only species tolerant of, or resistant to, the disturbance persist (e.g., Tilman and El Haddi 1992). Disturbance can also decrease diversity if it reduces habitat complexity or otherwise weakens facilitative interactions within or among species (Bruno et al. 2003). Conversely, disturbance can enhance diversity if successful colonization is limited by resource availability and disturbance opens space for the successful establishment of migrants (e.g., Goodsell and Connell 2005 and references therein). Finally, multiple studies in community ecology reveal no effect of disturbance on richness (reviewed in Mackey and Currie 2001, Hughes et al. 2007), especially in systems not structured by competitive hierarchies (Chesson and Huntly 1997) or where several of the above mechanisms counteract each other.

The shape of the relationship between disturbance and *genotypic* diversity also varies considerably among systems. For example, correlative studies show that sites with intensive disturbance histories can have higher (Hunter 1993, McMahon et al. 2017, Foster et al. 2021), lower (Hangelbroek et al. 2002, Rusterholz et al. 2009) or indistinguishable (Diaz-Almela et al. 2007) genotypic richness values relative to undisturbed sites. Manipulative experiments also show mixed results in that while some studies demonstrate that variation in the strength of selection drives diversity differences between disturbed and undisturbed plots (Herrera and Bazaga 2011, Whitney et al. 2019), other studies show no effect (Reusch 2006, Larkin et al. 2010, Hidding et al. 2014) or increased richness under moderately disturbed regimes (Peng et al. 2015). The variable nature of disturbance may be better explained by focusing on how it separately affects colonization (the gain of new genets) and extinction (the loss of established genets). Just as for species diversity, disturbance can have variable effects on genotypic colonization and extinction beyond net changes in richness alone.

Here, we used a manipulative field experiment to test the effects of a simulated disturbance (grazing by migratory waterfowl) on genotypic diversity in a wild population of eelgrass, *Zostera marina* (hereafter *Zostera*). *Zostera* is a marine flowering plant that reproduces sexually via seeds and asexually via the vegetative propagation of shoots (i.e., clonal ramets) along a rhizome. *Zostera* forms monospecific stands with patchy distributions of genetic diversity both within and among populations (Ruckelshaus 1998, Reusch et al. 1999a, Olsen et al. 2004, Hughes and Stachowicz 2009, Kamel et al. 2012, Furman et al. 2015). The maintenance of variation in genetic diversity is, at least in part, driven by its interaction with seagrass life history characteristics (Kendrick et al. 2012). In perennial meadows, the clonal propagation of competitively dominant genotypes can result in long-lived genets spread across large spatial scales and low local diversity (Reusch et al. 1999b). Within a localized area in the meadow, the colonization of new genets may arise from seedling recruitment (reviewed in Kendrick et al. 2012), clonal encroachment from genets outside the patch, the re-establishment of vegetative ramets dislodged from other locations (*present study*), and potentially somatic mutations (Yu et al. 2020). Previous studies predict that disturbance, such as grazing by waterfowl, can alter *Zostera* genetic diversity via effects on sexual reproduction (Kollars et al. 2017, Shaughnessy et al. 2021), but the magnitude of this influence depends on disturbance intensity. For example, clipping vegetative shoots to a height of 45 cm in the late winter increased the production of flowering shoots in the following spring (Shaughnessy et al. 2021), but severe disturbances that dislodge rhizomes, such as grazing by Canada geese (*Branta canadensis*), can drive perennial populations to extinction in the absence of adequate seedling recruitment (Rivers and Short 2007).

Over two years, we simulated grazing by migratory waterfowl via a field-based clipping manipulation and monitored net change in genotypic richness over time. We further separated net change in richness into colonization and extinction events to deduce how different clipping scenarios affected genet loss (e.g., because of selection and/or stochasticity) or gain (e.g., resulting from clonal propagation or sexual recruitment). We recognize that the combined effects of multiple, and potentially contrasting, processes operating in our experiment would ultimately determine the net change in genotypic richness over time. We first considered that grazing is most intense in the intertidal zone (Moore and Black 2006) and genotypic richness within populations is often highest in these areas (Kamel et al. 2012). This observation, coupled with research showing increased sexual recruitment of *Zostera* in areas of reduced standing biomass (Robertson and Mann 1984, Reusch 2006, Johnson et al. 2020), suggests that clipping disturbance may enhance genotypic diversity. However, clipping could reduce diversity if it creates population bottlenecks that drive the stochastic loss of genets or if clipping acts as a selective agent by favoring more tolerant genotypes (Kollars et al. 2021). Therefore, we expected that clipping disturbance would increase both the colonization of new genets and the extinction of established genets, but we did not have an *a priori* prediction for how the combined effects of these two processes would drive net change in richness. We interpret our results in light of previous research establishing that genotypic diversity positively influences assemblage resistance to and resilience from grazing (Hughes and Stachowicz 2004) in order to assess the potential for a disturbance–diversity feedback in this system that is driven by both ecological and evolutionary processes.

METHODS: *Study System*: We conducted our experiment within a perennial, natural assemblage of intertidal *Zostera* in Bodega Harbor, Bodega Bay, CA, USA. Within Bodega Harbor, the number of genotypes in a $1m^2$ area can range from one to more than 15 (Hughes and Stachowicz 2009) and diversity and differentiation among sites have been stable for at least a decade (Reynolds et al. 2017). Genotypes collected from the harbor also show variation in functionally important traits (Hughes et al. 2009, Abbott et al. 2018, DuBois et al. 2019), including tolerance to simulated waterfowl grazing (Kollars et al. 2021). Waterfowl known to graze eelgrass in northern California include the Pacific Black Brant goose (*Branta bernicla nigricans*), the American coot (*Fulica americana*), and Canada geese (*Branta canadensis*) (Kollars et al. 2017). These birds are seasonal migrants and are only present in Bodega Harbor from November through May of each year, with peak abundances occurring in February (C. Dunford, *unpublished data* from 1986-2015). We choose to focus our efforts on simulating Brant grazing because they are the dominant grazers of *Zostera* along the western coast of North America (Kollars et al. 2017), grazing by Canada geese in Bodega Harbor is rare (*personal observation*), and the resilience of eelgrass plots to grazing by Brant is demonstrably increased by genotypic diversity (Hughes and Stachowicz 2004). Grazing by Brant does not damage the basal meristem (and so does not directly cause shoot mortality) and we mimicked grazing by using scissors to remove all leaf tissue above the sheath for any individual shoot. We manipulated within-season clipping frequency to mimic varying grazing scenarios, accounting for the observation that Brant will visit previously grazed meadows to target nutritionally rich regrowth (Ganter 2000, Moore and Black 2006). The density of *Zostera* in Bodega Harbor typically peaks between May and August (Olyarnik and Stachowicz 2012) and flowering peaks toward the latter half of this period (*personal observations*).

Experimental design: In fall of 2016, we set up a manipulative field experiment along a ~ 50 m continuous stretch of intertidal *Zostera* meadow on the west side of Bodega Harbor (tidal height: ~ -0.1 m Mean Low Low water; GPS: 38.318222 ºN and -123.0536 ºE to 38.317432 ºN and -123.05308 ºE). Along this transect, we delineated 9 experimental blocks parallel to the shore with a 3 m distance between each block. A block consisted of four 1 m x 1m plots positioned in a square layout separated by 1m in all directions (Figure 1). At the beginning of the experiment, we used a spade to sever the rhizomes along the perimeter of each plot so that connections to ramets outside of the plot boundary would not affect the initial performance of ramets inside the plot. We did not continue to sever rhizome connections throughout the experiment to allow for the vegetative propagation of neighboring genets into the plots.

We controlled the amount of disturbance imposed in each treatment by designing detachable exclosures to prevent natural grazing by waterfowl in any of the experimental plots (Figure 1). We constructed exclosures by using PVC (diameter: 2.54 cm) to create posts consisting of two parts: a 1.5 m high aboveground portion which connected to a 1 m belowground portion pounded into the sediment. We attached exclosures in October prior to bird arrival and removed them after the birds migrated the following spring to avoid entanglement of the exclosures with macroalgae that bloom over the summer (primarily *Ulva* spp. and *Agarophyton vermiculophllum*). The PVC posts alone are adequate to prevent grazing by Brant, but not coots (*personal observation*). Therefore, we strung orange nylon line (commonly used in landscaping as trimmer line) around the perimeter of the posts every ~ 40 cm to discourage coots from swimming into the plot area. Shore observations confirmed the effectiveness of the exclosures. We removed detritus from the nylon lines at least once a month.

We implemented four levels of disturbance: clipped zero times per season (0X, a control treatment representing the absence of grazing), clipped once per season (1X), clipped twice per season (2X), and clipped four times per season (4X). We clipped the 1X treatment in February, the 2X treatment in both February and March, and the 4X treatment in January, February, March, and April. We repeated the 1X and 2X treatments for two grazing seasons (2017 and 2018) but applied the 4X treatment only in 2017. We did not repeat 4X for a second grazing season because this clipping frequency severely reduced shoot densities (see Results) and we wanted to document recovery from such a severe disturbance regime. We randomly assigned one replicate of each treatment to one of the four plots within each experimental block (n = 9 plots per clipping frequency).

We genotyped samples collected from each plot at the beginning and end of the two-year experimental period (December 2016 and October 2018, respectively) using 9 microsatellite loci we have previously used at this location (Abbott et al. 2018; see Appendices S2 and S3 for full genotyping and DNA extraction methods, respectively). We collected tissue using a 0.6 x 0.6 m quadrat of 36 evenly spaced points (see Appendix S1: Figure S1) and we removed the inner leaf of the shoot closest to each point. We did not collect a sample at a grid position if there was not a shoot present within a ~4 cm radius of the point. We recorded the location of the collected tissue along the grid and transported all samples to the lab where we dissected ~ 5 cm of the greenest tissue of the leaf, rinsed it with Ultrapure water, placed the sample in a 1.7 ml tube, and stored the sample at -80^{o}C until extraction.

We counted vegetative and flowering shoots in a 20 cm x 20 cm quadrat placed in the center of each plot at approximately monthly intervals. We counted a shoot as “flowering” if it exhibited characteristics of any flowering stage (*sensu* von Staats et al. 2021). We also quantified

seedling recruitment at the block-level using containers of unvegetated sediment (seedling traps) placed in the center of each block (Figure 1). Traps consisted of plastic containers (19.5 cm long × 14.5 cm wide × 12.5 cm high) lined with 2 mm diameter mesh, filled with bare sediment, and placed flush with the sediment surface. When we noticed shoots in the trap, we removed the shoots and genotyped tissue from each putative genet.

Analysis: We separated our analysis into two parts to avoid confusing the effects of clipping frequency within versus among years: we compared the control (0X) to treatments clipped in 1) both years of the experiment (1X and 2X treatments) or 2) only in the first year (4X). For both sets of analyses, we assessed the effect of clipping treatment, initial richness, and the interaction between treatment and initial richness on each response variable using generalized linear models (hereafter glm; calculated in the *lme4* package [Bates et al. 2007, *public communication*]; we conducted this and all subsequent analyses in R version 4.0.0 [R Core Team 2013]). We chose to include initial richness in the model *in lieu* of experimental block because of the unexpectedly high variation in the initial number of genotypes among plots within each block (Appendix S1: Figure S2), which rendered the "block" designation less meaningful. For count data that merited a Poisson family distribution, we tested for overdispersion using the "dispersiontest" function in the *AER* package (Kleiber et al. 2020, *public communication*). We followed the glm with an Analysis of Deviance (Chi-squared test and F test for Poisson and Gaussian distributions, respectively) using the "Anova" function in the *car* package (Fox and Weisberg 2018) to assess the importance of each factor in the model. When the model showed a treatment effect with a p-value < 0.05, we performed among level comparisons using a post-hoc Tukey's test with the *multcomp* package (Hothorn et al. 2016, *public communication*).

We first assessed the response of genotypic richness to clipping treatments. Though genotypic richness in clonal populations is often calculated as G-1/N-1 (where G = the number of genotypes and N = the number of samples; Dorken and Eckert 2001), we considered G alone to be an appropriate metric of richness given that our sampling effort was consistent across plots. Furthermore, any reduction in sample size due to treatment could affect colonization and extinction dynamics, which may be lost by standardization. We focused our analysis on net change in richness over time (final number of genotypes – initial number of genotypes) using the methods described above because of high variability in initial richness among plots and a strong effect of initial richness on final richness (see Results and Appendix S1: Figure S2),

Next, we decomposed net richness changes into the number of colonization and extinction events that occurred in each plot during the experiment. Our sampling design of collecting shoots in approximately the same position across time allowed us to classify the appearance of a genotype as a colonization event when a genotype was absent within a plot at the beginning of the experiment but present at the end. Similarly, we classified an extinction event as a genotype that was present within a plot at the beginning of the experiment but absent at the final timepoint.

Finally, we asked how clipping treatment affected shoot density, flowering effort, and site-level sexual recruitment through time. We qualitatively assessed changes in shoot densities and results from the seedling traps. We analyzed flowering effort by considering the proportion of flowering shoots relative to the total number of shoots in each plot, resulting in a proportional variable bounded between 0 and 1. Instead of using linear models that require a specified error distribution, we used non-parametric permutation tests with 1000 permutations within an Analysis of Variance framework (*sensu* Anderson 2001) with initial richness, clipping treatment,

and their interaction as the main factors of interest and time as a blocking factor to account for repeated measures. We permuted the *F*-statistic and calculated a *p*-value using one-tailed tests of the null hypothesis that the observed and simulated *F*-statistic come from the same distribution.

RESULTS: Clipping acted as a disturbance agent by reducing canopy height (Figure 1) and shoot density (Figure 2). During the first year, all clipped plots had fewer shoots than unclipped plots. The 2X treatment appeared to recover by the end of the growing season and did not appear affected by clipping during the second year. In contrast the 1X treatment recovered more slowly and did not reach similar densities to the control treatment until the summer after the second clipping season. On average, the 4X treatment, which was only clipped in the first year, recovered to close to control densities during the second year of the experiment, but with high variation among plots.

Contrary to predictions that disturbance would increase genotypic richness by increasing colonization, two consecutive seasons of clipping (1X and 2X treatments) resulted in a net loss of genotypes, whereas the absence of disturbance (0X treatment) resulted in a net increase in genotypes (Figure 3, Table 1, Appendix S1: Table S1). Separate analysis of the 4X treatment showed that despite severe biomass loss during the first year (Figure 2), shoot density mostly recovered in the second year and there was no residual effect of clipping frequency (0X versus 4X) on the net change in genotypic richness by the experiment's end (Figure 3, Table 1).

Separating net richness change into colonization and extinction showed that differences among clipping treatments were primarily driven by treatment effects on colonization rather than extinction. Clipping reduced colonization in the 1X and 2X treatments (Figure 4A, Table 1, Appendix S1: Table S1) such that the mean number of new genets gained in the 0X treatment

was greater than in the 2X treatment (mean +/- se: 0X = 1.22 +/- 0.36; versus 2X = 0.11 +/- 0.33). Clipping did not result in differences in the number of genotypes gained between the 0X and 4X treatment by the experiment's end (Figure 4A, Table 1). Overall, we observed a total of 36 colonization events (i.e., unique genotypes that were present across the plots at the end of the experiment but not at the beginning) and colonization occurred in a higher number of replicates in the 0X treatment relative to the 1X and 2X clipped treatments ($X^2 = 8.31$, $d.f. = 2$, $p = 0.02$; Appendix S1: Table S2), but not the 4X treatment ($X^2 = 0.4$, $d.f. = 1$, $p = 0.53$; Appendix S1: Table S2).

In contrast to colonization, clipping frequency did not alter the number of genotypes lost from a plot (Figure 4B; Table 1). We observed a total of 41 extinction events during the experiment and extinctions occurred in a similar number of plots across the 0X, 1X, 2X, and 4X treatments ($X^2 = 4.0$, $d.f. = 3$, $p = 0.26$; Appendix S1: Table S2).

We next examined whether reduced colonization in the clipped plots was connected to the effects of clipping on flowering effort. Clipping decreased the proportion of flowering shoots produced in the spring of 2018 (see inset on Figure 2, Appendix S1: Table S3). In May of 2018, the mean proportion of flowering shoots in the 0X treatment was three times higher than in the 1X or 2X treatments. However, by June of 2018, the mean proportion of flowering shoots for both the 0X and 1X plots were similar (~ 2% of all shoots and three times higher than the 2X plots), further suggesting that clipping delayed flowering phenology. Strikingly, the density of flowering shoots in the recovering 4X plots (Spring 2018) was still lower than in the undisturbed (0X) plots despite a year passing since the last application of the 4X treatment (Figure 2; Appendix S1: Table S3). Overall, the percentage of plots in which we observed flowering was similar across all treatments ($X^2 = 1.33$, $d.f. = 3$, $p = 0.72$; Appendix S1: Table S2).

Some of the colonization events were due to vegetative spread or rafting of detached shoots into plots. Seven of the 36 colonization events (n = 2 plots in 0X, n = 2 plots in 1X, and n = 3 plots in 4X), were genetically identical to a rooted shoot that we genotyped in a neighboring plot located no further than 3 m away. Data from seed traps also provided evidence that the establishment of rafting ramets occurred. We observed three cases of shoots being present in the seedling traps. We could not determine the genotypic identity of the sample in one of the three cases due to PCR amplification failure, but the multi-locus genotype of the shoots in the remaining two cases matched a genet within the block the trap was located, even though there were no rhizomal connections between shoots across the trap boundary. In both cases, the distance between the seedling trap to which the fragment dispersed and the nearest shoot of the parental genotype was less than a meter. We never observed the recruitment of a seedling into our traps (n=9 traps and 24 months of surveying).

DISCUSSION: We found that disturbance caused by simulating grazing across two seasons reduced genotypic richness. By deconstructing net changes in richness over time into the number of colonization and extinction events, we inferred that clipping did not drive genet mortality through the evolutionary mechanisms of selection or stochasticity (or, alternatively, that clipping-induced effects on these processes were too weak to result in differential extinction). This is surprising as we expected that clipping would select for more tolerant genotypes (Kollars et al. 2021) or that clipping-induced reductions in shoot density would increase stochastic loss. Rather than reducing richness through the mortality of genotypes already established in the population, we found that disturbance reduced the number of new genotypes that colonized clipped plots. This is also surprising given that disturbance both in *Zostera* meadows (Robertson

and Mann 1984, Reusch 2006, Johnson et al. 2020), and within macrophyte populations more generally (e.g., Kimmerer and Allen 1982), often facilitates sexual recruitment by reducing the competition-induced mortality of seedlings/sporelings. Interestingly, genotypic diversity recovered in the 4X treatment (a season of intense simulated grazing proceeded by a season of no simulated grazing) despite prolonged reductions in flowering effort, suggesting alternative mechanisms of colonization beyond localized seedling recruitment. We discuss several potential mechanisms underlying these unexpected results and interpret their implications for a feedback between disturbance, genotypic diversity, and resilience to that same disturbance agent.

We were particularly surprised that clipping did not increase genet extinction in our experiment. Indeed, our results contrast previous experiments demonstrating disturbance-driven loss of genets due to population bottlenecks (e.g., Whitney et al. 2019) or selection (e.g., Agrawal et al. 2012). Instead, most extinctions we observed across all treatments were of genets whose initial sampling abundance was less than 5% (*data not shown*). The more abundant genotypes typically persisted, even in the 4X treatment where we observed severe reductions in shoot density after the first year. This high temporal stability of dominant genets is consistent with previous studies in *Zostera* showing greater turnover of rarer genets and that initial genotype richness strongly predicts final richness (Reusch 2006, Becheler et al. 2014. Reynolds et al. 2017). The extinction of rare genotypes, regardless of treatment, is not surprising given that genets with few ramets likely have less reserves that can be shared intra-clonally via belowground connections (reviewed in Song et al. 2013). What is unexpected is that clipping did not act as a selection agent, especially given previous research showing genotypic variation for tolerance to clipping among genotypes collected from this site (Kollars et al. 2021). One possible explanation for the lack of discernable selection, despite genotypic variation for tolerance, is

fluctuating selection generated by the temporally concentrated nature of the selective agent (e.g., Tiffin and Rausher 1999). Grazing is seasonal and other selective agents may dominate during different times of the year. For example, heat tolerance may be important during the late summer and our previous work finds that warming and clipping favor distinct trait combinations (Kollars et al. 2021).

The observation of enhanced colonization in the absence of disturbance was also surprising, especially given that disturbance *facilitating* colonization is the cornerstone of multiple hypotheses predicting the relationship between disturbance and diversity (Connell 1978, Sousa 1979, Goodsell and Connell 2005). This result also contrasts with previous studies suggesting that physical disturbance increases eelgrass genotypic diversity by facilitating seedling recruitment (Reusch 2006, Zipperle et al. 2010, Foster et al. 2021). The reductions in flowering effort we observed in clipped treatments (see also Shaughnessy et al. 2021) might partially explain reductions in colonization if the colonizing genets are new seedlings, especially given that contributions to the seed bank come from both local and distant sources (Ruckelshaus 1998, Harwell and Orth 2002, Zipperle et al. 2010, Furman et al. 2015). However, the magnitude of local seed production is likely not the only process influencing the number of genets gained in our experiment. Colonization in the 4X and the 0X treatments were equivalent by the end of the experiment despite large differences in local flowering effort. One possible explanation for this is that the 4X treatment was the only treatment severe enough to sufficiently reduce competition by reducing shoot densities and thus facilitate the survival of new seedlings. Indeed, previous studies showing the importance of disturbance-mediated seedling recruitment to diversity involved severe reductions in shoot densities due to whole-shoot mortality (Reusch 2006, Rivers and Short 2007). Another possibility is that clipped treatments were more prone to invasion by

the asexual spread of genets from outside of the plots. We can confirm that at least 7 colonization events were from vegetative spread or rafting of detached shoots, but this number is likely higher because we did not sample all the genets that bordered a plot at the beginning of the experiment.

While we cannot conclusively explain why we observed higher colonization in the 0X plots we propose multiple possibilities. Increased flowering effort in the 0X treatment may have increased the density of seedlings due to localized seed dispersal (reviewed in Kendrick et al. 2012). However, the observation of flowering in plots in which we did not record colonization, and the high level of colonization despite low flowering effort in the 4X treatment, means this cannot be the sole explanation. Another possibility is that clipping an emerging seedling could directly increase mortality, thus reducing the number of successful sexual recruits (but not affecting the arrival of colonists, per se). This could explain why colonization does not differ between the 0X and 4X treatments given that we did not clip the 4X plots during the second year of the experiment. A final alternative is that clipping decreases facilitative interactions among shoots that buffer environmental stress aboveground (e.g., desiccation) or belowground (e.g., sulfide toxicity; Dooley et al. 2013) Lack of facilitation could, for example, explain the absence of seedlings in our experimental traps despite evidence of colonization in adjacent plots.

Though our results deviated from our initial expectations, the conditions that led to these outcomes occur in a wide range of macrophyte and sessile animal populations and communities. For example, disturbance will often have minimal effects on extinction if individuals can regenerate biomass lost to disturbance (Hulme 1996), directional selection for tolerance to biomass loss is weak or fluctuating (e.g., Tiffin and Rausher 1999, Kollars et al. 2021), and/or intra-clonal resource sharing buffers against genet extinction (reviewed in Song et al. 2013). We also predict that disturbance would decrease colonization in systems in which there is temporal

overlap of the establishment of colonists and the disturbance event, or if colonists are more vulnerable to disturbance than established individuals. Reduced colonization with disturbance will also be common when adult plants facilitate localized seed trapping and increase seedling survival via environmental buffering (reviewed by Filazzola and Lortie 2014). In contrast, if colonization and disturbance are temporally separated, disturbance may be more likely to increase diversity (e.g., Sousa 1979). Importantly, however, we only detected effects of disturbance on diversity by explicitly quantifying net change in richness and deconstructing those changes into colonization and extinction effects. As suggested by Hughes et al. (2007), we would not have discovered a relationship between disturbance and diversity if we examined the effects of clipping on final richness alone. Though it is possible to alleviate this problem by experimentally controlling for initial richness, such a design comes at the cost of ecological relevance, especially in systems composed of mosaic patches of differing levels of diversity.

Our experiment investigated the effects of disturbance over a two-year period. Developing projections for the long-term consequences of disturbance on the maintenance of genotypic diversity requires understanding how genotypic diversity reciprocally influences the realized impact of disturbance (Hughes et al. 2007). In synthesizing our work with Hughes and Stachowicz (2004) specifically, we suggest the potential for a feedback between grazing disturbance by Brant geese and genotypic diversity such that standing diversity increases resilience to grazing but grazing modestly reduces diversity by decreasing or delaying seed production. This feedback could result in lower resilience to future disturbance in the absence of other ecological or evolutionary processes that counteract the modest reductions in genotypic diversity due to grazing. The consequences of this feedback for naturally grazed *Zostera* populations requires further investigation, including documenting the spatial extent of grazing

impact on *Zostera* life history. We also encourage future studies to broaden the framework for the study of eco-evolutionary dynamics by recognizing that feedbacks between ecology and evolution extend beyond the effects of ecological processes on selection alone. Disentangling the complexities of multiple eco-evo processes operating in natural populations is essential to understanding the maintenance of genotypic diversity.

ACKNOWLEDGEMENTS: We thank all our colleagues who assisted with this project, especially B. Cameron and K. Pollard who provided extensive genotyping support. We also thank L. Dixit, K. DuBois, R. Famula, C. Gross, M. Johnson, A. Lorant, H. Nelson, I. Neylan, T. Perkins, S. Sumrall, J. Strom, M. Whalen, and D. Yim for their assistance in the lab and field. C. Dunford shared valuable information about Brant natural history in Bodega Bay, including providing us with decades of bird count data. E. Sanford, S. Strauss, K. Crawford and two anonymous reviewers provided valuable feedback that improved the manuscript.

FUNDING: Funding was provided through the following agencies: to NMK: California Botanical Society, California Native Plant Society, Center for Population Biology at the University of California - Davis, Davis Botanical Society, Hardman Foundation, Lerner-Grey Memorial Fund, Russell J. and Dorothy S. Bilinski Foundation, and the University of California Natural Reserve System; and to JJS: NSF OCE 1234345 and OCE 1829976.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

AUTHORSHIP STATEMENT: NMK and JJS conceived the study. NMK conducted the experiment, performed statistical analysis, and wrote the manuscript with significant input from JJS.

REFERENCES

Abbott, J. M., K. DuBois, R. K. Grosberg, S. L. Williams, and J. J. Stachowicz. 2018. Genetic distance predicts trait differentiation at the subpopulation but not the individual level in eelgrass, *Zostera marina*. Ecology and Evolution 8:7476–7489.

Agrawal, A. A., A. P. Hastings, M. T. J. Johnson, J. L. Maron, and J.-P. Salminen. 2012. Insect herbivores drive real-time ecological and evolutionary change in plant populations. Science 338:113–116.

Anderson, M. J. 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Canadian Journal of Fisheries and Aquatic Sciences 58:626–639.

Becheler, R., E. Benkara, Y. Moalic, C. Hily, and S. Arnaud-Haond. 2014. Scaling of processes shaping the clonal dynamics and genetic mosaic of seagrasses through temporal genetic monitoring. Heredity 112:114–121.

Bjedov, I., O. Tenaillon, B. Gerard, V. Souza, E. Denamur, M. Radman, F. Taddei, and I. Matic. 2003. Stress-induced mutagenesis in bacteria. Science 300:1399–1404.

Bonnell, M. L., and R. K. Selander. 1974. Elephant seals: Genetic variation and near extinction. Science 184:908–909.

Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into ecological theory. Trends in Ecology & Evolution 18:119–125.

Chesson, P., and N. Huntly. 1997. The roles of harsh and fluctuating conditions in the dynamics of ecological communities. The American Naturalist 150:519–553.

Connell, J. H. 1978. Diversity in tropical rain forests and coral reefs: High diversity of trees and corals is maintained only in a nonequilibrium state. Science 199:1302–1310.

Diaz-Almela, E., S. Arnaud-Haond, M. S. Vliet, E. Álvarez, N. Marbà, C. M. Duarte, and E. A. Serrão. 2007. Feed-backs between genetic structure and perturbation-driven decline in seagrass (*Posidonia oceanica*) meadows. Conservation Genetics 8:1377–1391.

Dooley, F. D., S. Wyllie-Echeverria, M. B. Roth, and P. D. Ward. 2013. Tolerance and response of *Zostera marina* seedlings to hydrogen sulfide. Aquatic Botany 105:7–10.

Dorken, M. E., and C. G. Eckert. 2001. Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). Journal of Ecology 89:339–350.

DuBois, K., J. M. Abbott, S. L. Williams, and J. J. Stachowicz. 2019. Relative performance of eelgrass genotypes shifts during an extreme warming event: disentangling the roles of multiple traits. Marine Ecology Progress Series 615:67–77.

Filazzola, A., and C. J. Lortie. 2014. A systematic review and conceptual framework for the mechanistic pathways of nurse plants. Global Ecology and Biogeography 23:1335–1345.

Foster, E., J. Watson, M. A. Lemay, M. T. Tinker, J. A. Estes, R. Piercey, L. Henson, C. Ritland, A. Miscampbell, L. Nichol, M. Hessing-Lewis, A. K. Salomon, and C. T. Darimont. 2021. Physical disturbance by recovering sea otter populations increases eelgrass genetic diversity. Science 374:333–336.

Fox, J., and S. Weisberg. 2018. An R companion to applied regression. Sage publications.

Furman, B. T., L. J. Jackson, E. Bricker, and B. J. Peterson. 2015. Sexual recruitment in *Zostera marina*: A patch to landscape-scale investigation. Limnology and Oceanography 60:584–599.

Ganter, B. 2000. Seagrass (*Zostera spp.*) as food for Brent geese (*Branta bernicla*): An overview. Helgoland Marine Research 54:63–70.

Goodsell, P. J., and S. D. Connell. 2005. Disturbance initiates diversity in recruitment of canopy-forming algae: interactive effects of canopy-thinning and substratum availability. Phycologia 44:632–639.

Hangelbroek, H. H., N. J. Ouborg, L. Santamaria, and K. Schwenk. 2002. Clonal diversity and structure within a population of the pondweed *Potamogeton pectinatus* foraged by Bewick's swans. Molecular Ecology 11:2137–2150.

Harwell, M. C., and R. J. Orth. 2002. Long-distance dispersal potential in a marine macrophyte. Ecology 83:3319–3330.

Hendry, A. P. 2017. Eco-evolutionary Dynamics. Princeton University Press, Princeton, New Jersery.

Herrera, C. M., and P. Bazaga. 2011. Untangling individual variation in natural populations: ecological, genetic and epigenetic correlates of long-term inequality in herbivory. Molecular Ecology 20:1675–1688.

Hidding, B., P. G. Meirmans, M. Klaassen, T. de Boer, N. J. J. Ouborg, C. A. M. N. Wagemaker, and B. A. Nolet. 2014. The effect of herbivores on genotypic diversity in a clonal aquatic plant. Oikos 123:1112–1120.

Hughes, A. R., J. E. Byrnes, D. L. Kimbro, and J. J. Stachowicz. 2007. Reciprocal relationships and potential feedbacks between biodiversity and disturbance. Ecology Letters 10:849–864.

Hughes, A. R., and J. J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. Proceedings of the National Academy of Sciences 101:8998–9002.

Hughes, A. R., and J. J. Stachowicz. 2009. Ecological impacts of genotypic diversity in the clonal seagrass *Zostera marina*. Ecology 90:1412–1419.

Hughes, A. R., J. J. Stachowicz, and S. L. Williams. 2009. Morphological and physiological variation among seagrass (*Zostera marina*) genotypes. Oecologia 159:725–733.

Hulme, P. E. 1996. Herbivory, plant regeneration, and species coexistence. The Journal of Ecology 84:609–615.

Hunter, C. L. 1993. Genotypic variation and clonal structure in coral populations with different disturbance histories. Evolution 47:1213–1228.

Hutchinson, G. E. 1965. The Ecological Theater and the Evolutionary Play. Yale University Press, New Haven, CT.

Johnson, A. J., R. J. Orth, and K. A. Moore. 2020. The role of sexual reproduction in the maintenance of established *Zostera marina* meadows. Journal of Ecology 108:945–957.

Kamel, S. J., A. R. Hughes, R. K. Grosberg, and J. J. Stachowicz. 2012. Fine-scale genetic structure and relatedness in the eelgrass *Zostera marina*. Marine Ecology Progress Series 447:127–137.

Kendrick, G. A., M. Waycott, T. J. B. Carruthers, M. L. Cambridge, R. Hovey, S. L. Krauss, P. S. Lavery, D. H. Les, R. J. Lowe, O. M. i Vidal, J. L. S. Ooi, R. J. Orth, D. O. Rivers, L.

Ruiz-Montoya, E. A. Sinclair, J. Statton, J. K. van Dijk, and J. J. Verduin. 2012. The central role of dispersal in the maintenance and persistence of seagrass populations. BioScience 62:56–65.

Kimmerer, R. W., and T. F. H. Allen. 1982. The role of disturbance in the pattern of a riparian bryophyte community. American Midland Naturalist 107:370–383.

Kollars, N. M. 2022. nmkollars/Field-goose: Data and Code for Ecology publication (Version v2). Zenodo. https://doi.org/10.5281/zenodo.6011003

Kollars, N. M., K. DuBois, and J. J. Stachowicz. 2021. Sequential disturbances alter the outcome of inter-genotypic interactions in a clonal plant. Functional Ecology 35:127–138.

Kollars, N. M., A. K. Henry, M. A. Whalen, K. E. Boyer, M. Cusson, J. S. Eklöf, C. M. Hereu, P. Jorgensen, S. L. Kiriakopolos, P. L. Reynolds, F. Tomas, M. S. Turner, and J. L. Ruesink. 2017. Meta-analysis of reciprocal linkages between temperate seagrasses and waterfowl with implications for conservation. Frontiers in Plant Science 8:2119.

Larkin, P. D., K. L. Heideman, D. D. Burfeind, and G. W. Stunz. 2010. The effect of boat propeller scarring intensity on genetic variation in a subtropical seagrass species. Botanica Marina 53:99–102.

Mackey, R. L., and D. J. Currie. 2001. The diversity-disturbance relationship: Is it generally strong and peaked? Ecology 82:3479–3492.

McMahon, K. M., R. D. Evans, K. van Dijk, U. Hernawan, G. A. Kendrick, P. S. Lavery, R. Lowe, M. Puotinen, and M. Waycott. 2017. Disturbance is an important driver of clonal richness in tropical seagrasses. Frontiers in Plant Science 8:2026.

Moore, J. E., and J. M. Black. 2006. Historical changes in black Brant *Branta bernicla nigricans* use on Humboldt Bay, California. Wildlife Biology 12:151–162.

Olsen, J. L., W. T. Stam, J. A. Coyer, T. B. H. Reusch, M. Billingham, C. Boström, E. Calvert, H. Christie, S. Granger, R. L. Lumière, N. Milchakova, M.-P. Oudot-Le Secq, G. Procaccini, B. Sanjabi, E. Serrão, J. Veldsink, S. Widdicombe, and S. Wyllie-Echeverria. 2004. North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina L.* Molecular Ecology 13:1923–1941.

Olyarnik, S. V., and J. J. Stachowicz. 2012. Multi-year study of the effects of *Ulva sp.* blooms on eelgrass *Zostera marina*. Marine Ecology Progress Series 468:107–117.

Pagán, J. A., A. Veríssimo, P. C. Sikkel, and R. Xavier. 2020. Hurricane-induced disturbance increases genetic diversity and population admixture of the direct-brooding isopod, *Gnathia marleyi*. Scientific Reports 10:8649.

Peng, J., C. Liang, Y. Niu, W. Jiang, W. Wang, and L. Wang. 2015. Moderate grazing promotes genetic diversity of *Stipa* species in the Inner Mongolian steppe. Landscape Ecology 30:1783–1794.

Reusch, T. B. H. 2006. Does disturbance enhance genotypic diversity in clonal organisms? A field test in the marine angiosperm *Zostera marina*. Molecular Ecology 15:277–286.

Reusch, T. B. H., W. T. Stam, and J. L. Olsen. 1999a. Microsatellite loci in eelgrass *Zostera marina* reveal marked polymorphism within and among populations. Molecular Ecology 8:317–321.

Reusch, T. B. H., C. Boström, W. T. Stam, and J. I. Olsen. 1999b. An ancient eelgrass clone in the Baltic. Marine Ecology Progress Series 183:301–304.

Reynolds, L. K., J. J. Stachowicz, A. R. Hughes, S. J. Kamel, B. S. Ort, and R. K. Grosberg. 2017. Temporal stability in patterns of genetic diversity and structure of a marine foundation species (*Zostera marina*). Heredity 118:404–412.

Rivers, D. O., and F. T. Short. 2007. Effect of grazing by Canada geese *Branta canadensis* on an intertidal eelgrass *Zostera marina* meadow. Marine Ecology Progress Series 333:271–279.

Robertson, A. I., and K. H. Mann. 1984. Disturbance by ice and life-history adaptations of the seagrass *Zostera marina*. Marine Biology 80:131–141.

Ruckelshaus, M. H. 1998. Spatial scale of genetic structure and an indirect estimate of gene flow in eelgrass, *Zostera marina*. Evolution 52:330–343.

Rusterholz, H.-P., M. Kissling, and B. Baur. 2009. Disturbances by human trampling alter the performance, sexual reproduction and genetic diversity in a clonal woodland herb. Perspectives in Plant Ecology, Evolution and Systematics 11:17–29.

Schoener, T. W. 2011. The newest synthesis: Understanding the interplay of evolutionary and ecological dynamics. Science 331:426–429.

Shaughnessy, F. J., S. L. Ferson, A. J. Frimodig, D. C. Barton, M. Hurst, and J. M. Black. 2021. Growth and flowering responses of eelgrass to simulated grazing and fecal addition by Brant Geese. Ecosphere 12:e03690.

Song, Y.-B., F.-H. Yu, L. H. Keser, W. Dawson, M. Fischer, M. Dong, and M. van Kleunen. 2013. United we stand, divided we fall: a meta-analysis of experiments on clonal integration and its relationship to invasiveness. Oecologia 171:317–327.

Sousa, W. P. 1979. Disturbance in marine intertidal boulder fields: The nonequilibrium maintenance of species diversity. Ecology 60:1225–1239.

von Staats, D. A., T. C. Hanley, C. G. Hays, S. R. Madden, E. E. Sotka, and A. R. Hughes. 2021. Intra-meadow variation in seagrass flowering phenology across depths. Estuaries and Coasts 44:325–338.

Tiffin, P., and M. D. Rausher. 1999. Genetic constraints and selection acting on tolerance to herbivory in the common morning glory *Ipomoea purpurea*. The American Naturalist 154:700–716.

Tilman, D., and A. El Haddi. 1992. Drought and biodiversity in grasslands. Oecologia 89:257–264.

Whitney, K. D., J. Mudge, D. O. Natvig, A. Sundararajan, W. T. Pockman, J. Bell, S. L. Collins, and J. A. Rudgers. 2019. Experimental drought reduces genetic diversity in the grassland foundation species *Bouteloua eriopoda*. Oecologia 189:1107–1120.

Yu, L., C. Boström, S. Franzenburg, T. Bayer, T. Dagan, and T. B. H. Reusch. 2020. Somatic genetic drift and multilevel selection in a clonal seagrass. Nature Ecology & Evolution 4:952–962.

Zipperle, A. M., J. A. Coyer, K. Reise, W. T. Stam, and J. L. Olsen. 2010. Waterfowl grazing in autumn enhances spring seedling recruitment of intertidal *Zostera noltii*. Aquatic Botany 93:202–205.

Zuk, M., R. Thornhill, J. D. Ligon, and K. Johnson. 1990. Parasites and mate choice in red jungle fowl. American Zoologist 30:235–244.

TABLE 1: Statistical effects of initial richness and clipping treatment on response variables characterizing changes in genotypic richness in *Zostera marina*. Model selection is presented as Analysis of Deviance (test statistic: *F*-test and Chi-squared test for Gaussian and Poisson distributions, respectively). *P*-values less than or equal to 0.05 are in bold. Abbreviations: "initial": initial richness; "df": degrees of freedom; "Res dev": residual deviance

			Clipping both seasons (Comparison: 0X, 1X, 2X)				Recovery of 4X (Comparison: 0X, 4X)			
Response	Distribution (link)	Factor tested	Δ df	Δ Res dev	*F* or X^2 (df)	*p*-value	Δ df	Δ Res dev	*F* or X^2 (df)	*p*-value
Net change in no. genotypes	Gaussian (identity)	Initial	1	0.86	2.06 (1, 21)	0.17	1	12.51	10.50 (1,14)	**<0.01**
		Treatment	2	8.74	10.41 (2, 21)	**<0.01**	1	0.60	0.50 (1,14)	0.49
		Interaction	2	1.44	1.71 (2, 21)	0.21	1	4.36	3.66 (1,14)	0.08
No. genotypes gained	Poisson (log)	Initial	1	14.93	14.93 (1)	**<0.01**	1	2.34	2.34 (1)	0.13
		Treatment	2	11.73	11.73 (2)	**<0.01**	1	0.13	0.13 (1)	0.72
		Interaction	2	3.03	3.03 (2)	0.22	1	2.09	2.09 (1)	0.15
No. genotypes lost	Poisson (log)	Initial	1	14.70	14.70 (1)	**<0.01**	1	19.17	19.17 (1)	**<0.01**
		Treatment	2	0.83	0.83 (2)	0.66	1	-0.01	-0.01 (1)	0.94
		Interaction	2	1.53	1.53 (2)	0.47	1	0.49	0.49 (1)	0.49

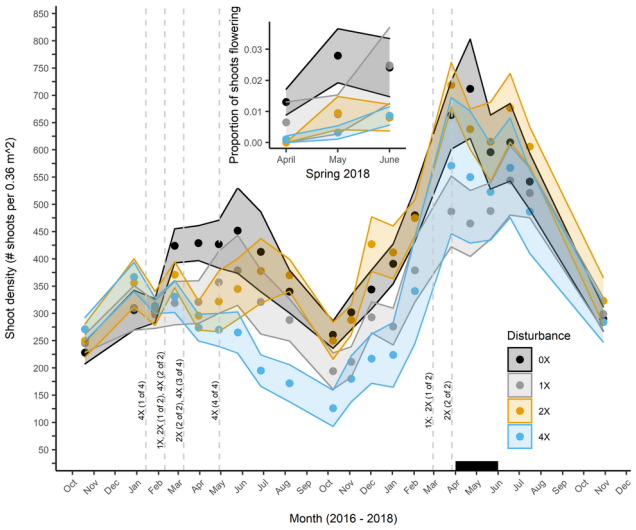
FIGURE 1: Photographs of an experiment testing the effects of simulated grazing disturbance (clipping) on changes in the genotypic richness of an intertidal *Zostera marina* meadow. *Left:* Photograph at the block level, highlighting the placement of a seed trap and the design of waterfowl exclosures. *Right*: Close-up of each plot taken in March of 2017. Starting in the top left corner and moving clockwise: 0X (not clipped), 1X (clipped once in Feb), 2X (clipped once in Feb and once in March), and 4X (clipped once in Jan, Feb, and March; note this photograph was taken before the 4th application of clipping in April).

FIGURE 2: Mean total shoot densities (vegetative + flowering) of *Zostera marina* through time in response to clipping treatments in natural assemblages of genotypes in the field. Dashed gray lines indicate timing of clipping application. *Inset:* The mean proportion of flowering shoots during the second year of the experiment (April – June 2018, indicated on the x-axis by the black box). Shaded ribbons are +/- 1 standard error of the mean.

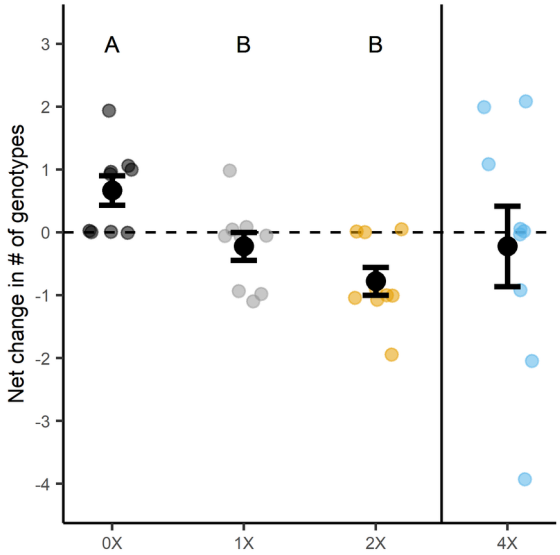
FIGURE 3: Net change in the number of genotypes in response to clipping treatments in natural assemblages of *Zostera marina*. Colored points: individual replicates; black points: treatment mean. Y-bars are standard error of the mean. The vertical black line reminds readers that we analyzed the results of the 4X treatment separately. Post-hoc Tukey's comparison tests with a p-value equal to or less than 0.05 are indicated by capital letters.

FIGURE 4: Effects of clipping treatments on the number of genotypes gained (A) or lost (B) over time in natural assemblages of *Zostera marina.* Colored points: individual replicates; black points: treatment mean. Y-bars are standard error of the mean. The vertical black line reminds readers that we analyzed the results of the 4X treatment separately. Post-hoc Tukey's comparison tests with a *p*-value equal to or less than 0.05 are indicated by capital letters.

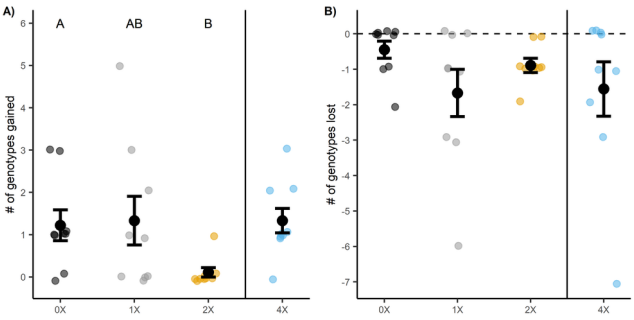
ECY_3710_Kollars_Stach_Ecol_Figure1_Revised_2.5inX6in.jpg



ECY_3710_Kollars_Stach_Ecol_Figure2_Revised_5inX6in.tiff



ECY_3710_Kollars_Stach_Ecol_Figure3_Revised_3inX3in.tiff



ECY_3710_Kollars_Stach_Ecol_Figure4_Revised_3inX6in.tiff