

Ecological equivalency as a tool for endangered species management

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Abstract. The use of taxon substitutes for extinct or endangered species is a controversial conservation measure. We use the example of the endangered California tiger salamander (*Ambystoma californiense*; CTS), which is being replaced by hybrids with the invasive barred tiger salamander (*Ambystoma mavortium*), to illustrate a strategy for evaluating taxon substitutes based on their position in a multivariate community space. Approximately one-quarter of CTS's range is currently occupied by "full hybrids" with 70% nonnative genes, while another one-quarter is occupied by "superinvasives" where a specific set of 3/68 genes comprising 4% of the surveyed genome is nonnative. Based on previous surveys of natural CTS breeding ponds, we stocked experimental mesocosms with field-verified, realistic densities of tiger salamander larvae and their prey, and used these mesocosms to evaluate ecological equivalency between pure CTS, full hybrids, and superinvasives in experimental pond communities. We also included a fourth treatment with no salamanders present to evaluate the community effects of eliminating *Ambystoma* larvae altogether. We found that pure CTS and superinvasive larvae were ecologically equivalent, because their positions in the multivariate community space were statistically indistinguishable and they did not differ significantly along any univariate community axes. Full hybrids were ecologically similar, but not equivalent, to the other two genotypes, and the no-*Ambystoma* treatment was by far the most divergent. We conclude that, at least for the larval stage, superinvasives are adequate taxon substitutes for pure CTS and should probably be afforded protection under the Endangered Species Act. The proper conservation status for full hybrids remains debatable.

Key words: *Ambystoma californiense*; California tiger salamander; community composition; ecological similarity; endangered species management; mesocosm; superinvasives; taxon substitution.

INTRODUCTION

As a species approaches extinction, one potential conservation measure is to introduce a "taxon substitute" to replace it (Donlan et al. 2006, Griffiths and Harris 2010). Taxon substitution is defined as the introduction of a nonnative taxon to a focal region to restore an ecological function lost through extinction of a native taxon (Aslan et al. 2014). This definition implies that the introduction is an intentional restoration measure, and this is traditionally how the term has been used. However, nonnative

species resulting from unintentional introductions may also perform ecological functions similar to native taxa (Hallet et al. 2013) and also constitute potential taxon substitutes. Novel ecosystems resulting primarily from changing biotic conditions due to nonnative species and changing abiotic conditions due to climate change are coming to occupy an increasing percentage of the Earth's surface (Hobbs et al. 2013). Given this reality, the goal of restoring ecosystems to a desired historical state of "naturalness" is often virtually impossible; in some cases where nonnative species have become integral to interspecies relationships, it may even be undesirable (Kennedy et al. 2013). What are currently lacking from the literature are quantitative tests of when taxon substitutes should be intentionally introduced into a community, and when nonnative species suspected of functioning as taxon substitutes should be maintained or eradicated.

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One classic example of taxon substitution is the introduction of Aldabra giant tortoises (*Aldabrachelys gigantea*) to the Mascarene Islands to replace the extinct giant *Cylindraspis* tortoises that once occurred there. These introduced tortoises have been shown to graze on exotic grasses and disperse seeds of critically endangered native plants (Hansen et al. 2008, Griffiths et al. 2011). However, the only evidence that *Cylindraspis* performed similar grazing and seed dispersal functions are anecdotal accounts from 18th century explorers (Leguat 1708, Tafforet 1726) and inferred tortoise defenses in the native plants (Eskildsen et al. 2004). Inferences about ecological function become ever more tenuous the further back in time an extinction occurred. For example, arguments for Pleistocene rewilding in North America rely on analogies to the African savanna, hypothesizing that large herbivores in temperate grasslands would once have performed similar roles in controlling woody vegetation to those carried out by ungulates and proboscidiens in Africa today (Donlan et al. 2005, 2006, Zimov 2005). Our intention is not to be critical of these studies, and we agree with Stuart Pimm (Marris 2009) that when evaluating taxon substitutes in cases with limited baseline data, our natural desire for replication and controls may need to be rethought. However, in cases where ecological functionality of substitute taxa can be measured with replication and controls, it is clearly desirable. The question remains, however, how to best measure ecological equivalency in real systems.

Here, we experimentally quantify the ecological equivalency among three genotypic classes of an endangered species that is being replaced by hybrids with a nonnative invader. When the Central Distinct Population Segment of the California tiger salamander (*Ambystoma californiense*; CTS) was granted protection under the U.S. Endangered Species Act, one of the main threats identified was hybridization with an introduced congener, the barred tiger salamander (*Ambystoma mavortium*; BTS; U.S. Fish and Wildlife Service 2004). This action reflects the concern that hybrids can reasonably be thought of as a different taxon from the native species that they replace, and in that sense may be thought of as a taxon substitute. In this case, BTS larvae were introduced to the Salinas Valley in the 1950s for use as fish bait (Riley et al. 2003), and their genes have gradually spread away from the introduction sites due to hybrid advantage (Fitzpatrick and Shaffer 2007a). By the time CTS were placed on the endangered species list in 2004, it was estimated that ~24% of CTS's range was compromised by the presence of nonnative genes. Since then, new research has shown that three genetically unlinked regions of the BTS genome are spreading across the native range at a much faster rate than estimated for the majority of the genome, such that an additional ~25% of the native range is now impacted by these

“superinvasive” alleles (Fitzpatrick et al. 2009, 2010). Thus, the current situation is that one-quarter of CTS's native range is occupied by salamanders that we term “full hybrids,” with genomes that consist of ~70% nonnative genes (Fitzpatrick and Shaffer 2007a). An additional quarter of the native range is impacted by three superinvasive alleles (out of 68 surveyed genetic markers), rendering ~4% of the sampled genome nonnative (Fitzpatrick et al. 2010). We will refer to salamanders that have these nonnative alleles, but with genomes consisting of otherwise native genes as “superinvasives,” reflecting their rapid spread in space and time. The remaining ~50% of the range contains CTS with 100% native genomes, which we refer to as “pure CTS.”

Given the rapid spread of the superinvasives, it seems likely that they may eventually replace pure CTS (Fitzpatrick and Shaffer 2007a, Fitzpatrick et al. 2010), and given enough time, the entire species may become full hybrids. Thus, natural selection following an intentional, human-mediated introduction may result in the replacement of pure CTS by this hybrid taxon substitute. Presumably these alleles spread because they confer a selective advantage relative to native alleles, and halting their spread may be time-consuming, expensive, and ultimately impossible. Although we strongly favor the conservation strategy of protecting native lineages on the landscape where they evolved, we also recognize that pragmatism is important, and that understanding the ecological impact of this and other unintentional taxon substitutes should be a part of management decisions.

In this study, we use the CTS hybrid system as a test case for experimentally evaluating the critical question of how to quantitatively compare the relative ecological effects of potential taxon substitutes. We measure the effects of full hybrids, superinvasives, and pure CTS on other members of the California vernal pool community by stocking replicated mesocosms with realistic, field-determined densities of larval tiger salamanders and the other key members of their vernal pool food webs, and measuring the resulting densities of all community members at the end of the larval period. Tiger salamander larvae are top predators in the fishless pools that they inhabit, and can thus be expected to influence the densities of other community members, either directly through predation or indirectly through trophic cascades (Holomuzki and Collins 1987, Holomuzki et al. 1994). Our experimental design also includes a critical “no-*Ambystoma*” treatment, which serves as a null model and demonstrates the state of the community if no taxon substitutes were present. Under the assumption that the ideal conservation outcome would be to have pure-CTS community dynamics, we evaluate the conservation value of full-hybrid, superinvasive, and no-*Ambystoma* alternatives under two criteria. First, we ask whether either of the two alternative genotypic

communities is statistically indistinguishable from those with pure CTS, and treat any such communities as “ecologically equivalent” to those with native CTS (Grinnell 1924, Warren et al. 2008). Second, we ask whether any nonequivalent communities are closer to pure-CTS communities in multivariate community space than pure-CTS communities are to the alternative no-*Ambystoma* ecosystems. In so doing, we evaluate the alternative management outcomes of protecting nonequivalent *Ambystoma* or removing all hybrids from the landscapes that they have invaded. We label nonequivalent treatments that are closer to the pure-CTS treatment than the pure-CTS treatment is to the null model community as “ecologically similar” to the pure-CTS larval ecosystem (Warren et al. 2008), and cautiously view such communities as a preferable alternative to complete eradication of hybrids. We evaluate statistical distinguishability in both a multivariate community space and along univariate community axes, providing a community-level view of ecological impacts as well as of effects on specific taxa in the food web.

These experiments inform two central questions in conservation biology. First, should hybrids receive protection under the Endangered Species Act, ESA (Fitzpatrick and Shaffer 2007b)? We contend that if hybrids pass the ecological equivalency criterion, it provides compelling evidence that they should be candidates for protection. Conservation of endangered species ideally protects native lineages, and hybrids contain some, but not all, of the genetic and evolutionary legacy that we seek to conserve. However, when the restoration of hybrids to a genetically native state is impossible, or when natural genetic variation has been so reduced that pure populations are no longer viable (Johnson et al. 2010), then retaining the ecological functionality of native communities is one reasonable way to determine if hybrids or other taxon substitutes lead to desirable conservation outcomes. In these circumstances, if hybrids pass the ecological equivalency criterion, then we argue that they should be protected. If they fail, but pass the ecological similarity criterion, then their conservation status is more questionable. In this case, the individual axes along which they differ from the native control need to be considered in the context of ecosystem function, impacts on other endangered and sensitive species, and other conservation priorities. The second question we seek to answer is whether an explicitly experimental approach to ecological equivalency can be used to evaluate conservation outcomes. Our work on the CTS/BTS system suggests that the answer is an unqualified “yes.”

METHODS

We constructed replicated experimental mesocosms with four treatment levels: (1) pure-CTS larvae

(0% nonnative), (2) superinvasive larvae from the edge of the hybrid swarm (~4% nonnative for three specific genomic regions; see Fitzpatrick et al. 2010), (3) full-hybrid larvae from the center of the hybrid swarm (~70% nonnative), and (4) no *Ambystoma* larvae. We replicated each genotypic treatment level 10 times, for a total of 40 1136-L mesocosms.

Mesocosms were filled with untreated lake water that already contained phytoplankton. Each tank was stocked with zooplankton from four tiger salamander breeding ponds in the Ohlone and Sunol Wildernesses (Alameda County, California, USA). Macroscopic taxa were collected from 14 breeding ponds from the same site. Stocking densities of these taxa were based on surveys of 15 geographically and ecologically diverse salamander breeding ponds conducted in 2011. These densities were: 21 water boatmen (*Corisella* sp.), 18 California clam shrimp (*Cyzicus californicus*), one predaceous diving beetle larva (Dytiscidae), six snails (Gastropoda), 11 backswimmers (*Notonecta* sp.), and 102 Pacific chorus frog tadpoles (*Pseudacris regilla*). These six taxa are the only macroscopic taxa that were present in the majority of the 15 surveyed breeding ponds at a density such that at least one individual would be expected in a body of water the size of our mesocosms (that is, one per 2.48 m²). A previous experiment that used identical mesocosm stocking densities for most of these taxa (excluding beetle larvae and backswimmers) recovered CTS size distributions that closely mimicked those observed in nature, suggesting that they replicate the dynamics of natural vernal pool communities (Searcy et al. 2015). Pure CTS were collected from three ponds at the Jepson Prairie Preserve (Solano County), superinvasives from three ponds at Fort Ord National Monument (Monterey County), and full hybrids from three ponds at Garlinger Ranch (Monterey County). Half of each of the genotypic replicates received four larvae and half received eight, mimicking the range of high- and low-density conditions that we measured in nature. Larval salamanders were added to the tanks on 26 March, initiating the experiment.

Chlorophyll, periphyton, and zooplankton densities were measured at monthly intervals, and mesocosms were checked every three days for metamorphosed amphibians, which were weighed, measured, and removed. The experiment ended on 26 June, when the shallowest tank was 10 cm deep; a previous experiment demonstrated that at this point, tiger salamander larvae are susceptible to death from heat stress. After removing the last salamander larvae, we drained the tanks and counted the remaining *Corisella*, *C. californicus*, snails, and *Notonecta*. No dytiscid larvae or *P. regilla* remained in the tanks at this time. See the Appendix for details, with count data sorted by individual tank in Table S11.

Analyses

A MANOVA was used to test the association between salamander genotype and community composition. The nine response variables were: densities of chlorophyll, cladocera, copepods, and periphyton on 24/25 May, final counts of *Corisella*, *C. californicus*, Gastropoda, and *Notonecta*, and number of *P. regilla* that successfully metamorphosed. Chlorophyll, cladocera, copepod, and periphyton densities from 24/25 May were used because the effect of larval genotype on these variables was strongest at this sampling point. We suspect that the effect decreased over the remainder of the experiment as salamanders metamorphosed and communities in some tanks had over one month to recover from tiger salamander predation. We used contrasts to test the significance of all pairwise differences between the four treatments. Here, and for all other analyses where multiple nonindependent comparisons were made, we employed a sequential Bonferroni correction to interpret the resulting *P* values. Next, we performed a discriminant function analysis to determine which of the community variables best differentiated the four treatments. We also used one-way ANOVAs and Tukey comparisons to test for significant differences within each response variable.

We used ANOVAs to test for differences in metamorph traits among the three salamander genotypes. These traits were: date, mass, and snout-vent length (SVL) at metamorphosis, change in SVL, growth rate, and survivorship. The model for each test included three terms: genotype, larval density, and their interaction. If the genotype term was

significant, contrasts were used to test for significant differences between the three pairwise genotype combinations.

We used ANCOVAs to test for treatment differences in the reaction norm between size and date of metamorphosis. The ANCOVA models consisted of larval genotype as the main effect, date of metamorphosis as the covariate, and their interaction term. Models were created for both mass and SVL at metamorphosis. When the interaction term was significant (indicating significant variation in the slope of the reaction norm), contrasts were used to test for significant differences between all pairwise treatment combinations. All statistical analyses were performed using JMP 10 (SAS Institute 2012).

RESULTS

Out of 180 salamander larvae originally introduced to the mesocosms, 140 survived to the end of the experiment. Of these, 18 were not close to completing metamorphosis when the experiment was terminated, and were thus classified as desiccation-related mortalities (see Appendix); all analyses of larval traits were based on the remaining 122 individuals. One tank in which all larvae died prior to the initiation of metamorphosis was excluded from the community composition analysis.

Community composition was significantly altered by larval genotype (MANOVA: Wilks' $\lambda = 0.046$, $P < 0.001$; Fig. 1). Pairwise contrasts between the four treatments revealed that all pairs were significantly different except for pure CTS vs. superinvasive and pure CTS vs. full hybrid (for all

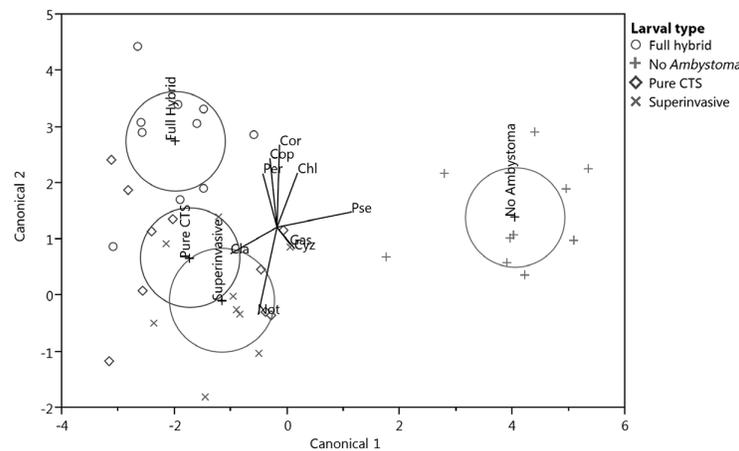


FIG. 1. Canonical centroid plot showing variation in community composition between the four tiger salamander treatments. The centroid for each treatment is labeled with a cross and the 95% CI is a large circle. Individual tanks ($N = 39$) are represented by different symbols for full-hybrid, no-*Ambystoma*, pure-CTS, and superinvasives treatments. Biplot rays show the loadings of each response variable onto the first two canonical axes (97% of the total variation). Abbreviations for response variables are: Chl, chlorophyll; Cla, cladocera; Cop, copepod; Cor, *Corisella*; Cyz, *C. californicus*; Gas, Gastropoda; Not, *Notonecta*; Per, periphyton; and Pse, *P. regilla*.

contrasts with no *Ambystoma*, $P < 0.001$; for full hybrid vs. superinvasive, $P = 0.005$; for full hybrid vs. pure CTS, $P = 0.054$; for pure CTS vs. superinvasive, $P = 0.45$). The Mahalanobis distances between the centroid of the pure-CTS treatment and the centroids of the superinvasive, full-hybrid, and no-*Ambystoma* treatments were 2.5, 5.2, and 34.2, respectively. This implies that community composition with superinvasives is 2.1 and 13.7 times closer to a community with pure CTS than are communities with either full hybrids or no *Ambystoma*, respectively.

The first canonical axis explained 83% of the variance and the second axis explained an additional 14%. The first canonical axis primarily separated no-*Ambystoma* tanks from the other three treatments. The variables that made the greatest contribution to this axis were *P. regilla* tadpole and cladocera densities, with no-*Ambystoma* tanks having high densities of *P. regilla* and low densities of cladocera. The second canonical axis primarily separated full-hybrid tanks from the other three treatments. Chlorophyll, copepod, *Corisella*, *Notonecta*, and periphyton densities contributed most to this axis, with full-hybrid mesocosms having low densities of *Notonecta* and

high densities of the other four groups. One-way ANOVAs revealed significant differences in seven of the nine response variables (all except copepod and *Corisella* densities; Fig. 2), although the *Notonecta* ANOVA became marginally nonsignificant after Bonferroni correction.

Given our sample size, we had 80% power to detect a significant pairwise difference along each univariate axis when two treatments differed by more than 0.89 standard deviations. Thus, when we say that two treatments are statistically indistinguishable, as Fig. 2 shows for the pure-CTS and superinvasive treatments, we know that they differ by less than 0.89 standard deviations on each community metric. On the axis with the least variance (*C. californicus*), 0.89 standard deviations corresponds to a 59% change in density, while on the axis with the greatest variance (cladocera), 0.89 standard deviations corresponds to a 421% change in density. These numbers represent the limit for detection of statistical significance, given our sample size, while the actual differences between the pure-CTS and superinvasive treatments on the univariate axes were substantially less: 0–66% (mean 32%) or 0.00–0.31 SD (mean 0.18 SD). In comparison, the differences between the

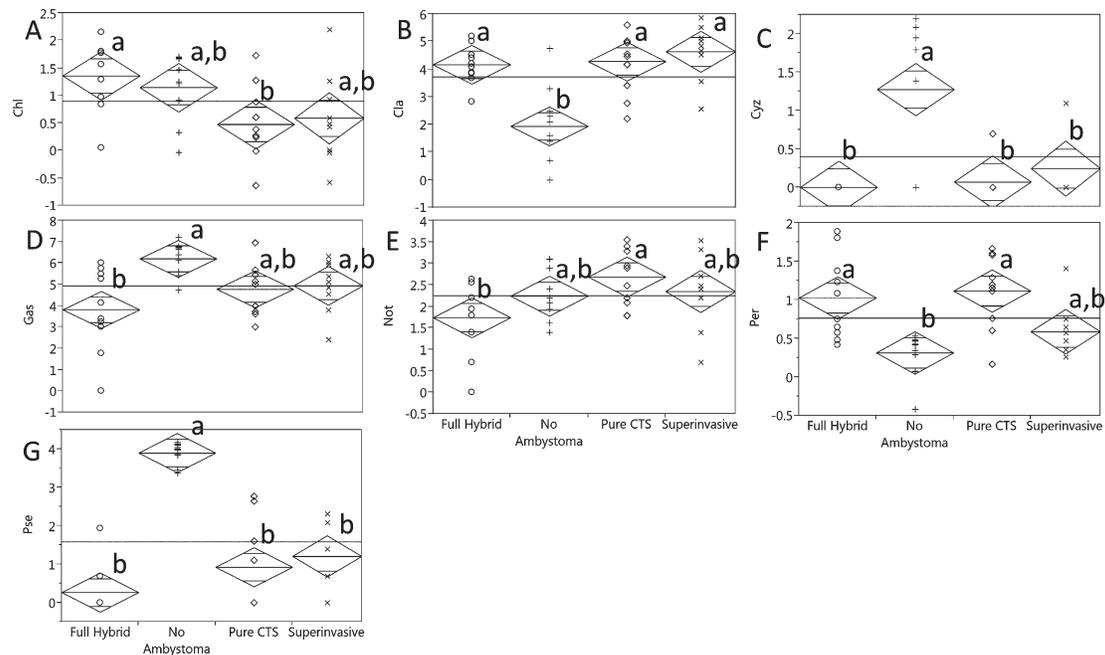


FIG. 2. One-way ANOVAs for all significant differences in community composition metrics. Abbreviations for response variables are as in Fig. 1. Lowercase letters show significance groupings based on Tukey comparisons. In each panel, the horizontal line is the grand mean and diamonds show 95% CI. Lines within each diamond are the mean \pm SE for that treatment. (A) Chl ($F = 3.8$, $df = 3, 35$, $P = 0.019$). (B) Cla ($F = 12.4$, $df = 3, 35$, $P < 0.001$). (C) Cyz ($F = 12.4$, $df = 3, 35$, $P < 0.001$). (D) Gas ($F = 5.4$, $df = 3, 35$, $P = 0.004$). (E) Not ($F = 2.9$, $df = 3, 35$, $P = 0.047$). (F) Per ($F = 7.7$, $df = 3, 35$, $P < 0.001$). (G) Pse ($F = 40.4$, $df = 3, 35$, $P < 0.001$). Measurements of Chl and Per are concentrations; scale numbers in all other panels have been log-transformed.

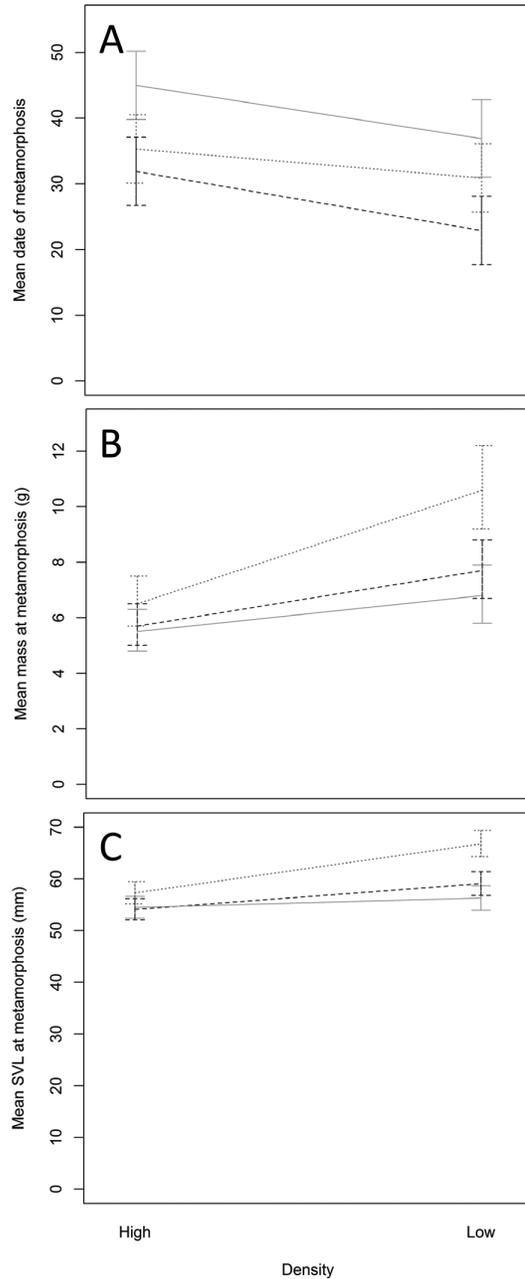


FIG. 3. Interaction plots for the response of three metamorph traits to larval genotype and density ($N = 39$). Full hybrids are represented by the dotted line, pure CTS by the dashed line, and superinvasives by the solid line. Vertical bars show 95% CI for each treatment \times identity combination. (A) Mean date of metamorphosis (days after 30 April) (for Genotype, $F = 12.3$, $df = 2$, $P < 0.001$; for Density, $F = 10.3$, $df = 1$, $P = 0.004$; for Genotype \times Density, $F = 0.4$, $df = 2$, $P = 0.67$). (B) Mean mass at metamorphosis (for Genotype, $F = 9.9$, $df = 2$, $P = 0.001$; for Density, $F = 31.5$, $df = 1$, $P < 0.001$; for Genotype \times Density, $F = 2.0$, $df = 2$, $P = 0.16$). (C) Mean SVL at metamorphosis (for Genotype, $F = 17.7$, $df = 2$, $P < 0.001$; for Density, $F = 31.2$, $df = 1$, $P < 0.001$; for Genotype \times Density, $F = 4.6$, $df = 2$, $P = 0.02$).

full-hybrid and pure-CTS treatments on the univariate axes were 7–163% (mean 86%) or 0.09–0.93 SD (mean 0.47 SD), and the differences between the no-*Ambystoma* and pure-CTS treatments were 47–1855% (mean 412%) or 0.32–2.59 SD (mean 1.08 SD).

The three larval salamander genotypes differed among themselves in all metamorph traits except for growth rate (Fig. 3; Appendix). Pure CTS metamorphosed significantly earlier than full hybrids ($P = 0.04$), which in turn metamorphosed significantly earlier than superinvasives ($P = 0.009$), although the difference between pure CTS and full hybrids was not significant after Bonferroni correction. Full hybrids metamorphosed at a significantly larger size than pure CTS and superinvasives in terms of both mass and SVL. However, because of differences in size at the start of the experiment, superinvasives grew significantly more than full hybrids ($P = 0.02$), which in turn grew significantly more than pure CTS ($P < 0.001$), resulting in no significant differences in larval growth rate among the genotypes. Both pure CTS ($P < 0.001$) and full hybrids ($P < 0.001$) had significantly higher larval survivorship than superinvasives. Density had a significant effect on all metamorph traits as well (Fig. 3; Appendix), with larvae in low-density tanks metamorphosing earlier and at a larger size, with both a higher growth rate and higher survivorship. Body length was the only trait for which there was a significant genotype \times density interaction, with full hybrids showing a greater difference in body length as a function of density than superinvasives ($P = 0.006$).

Finally, we found significant variation in the slope of the reaction norm for both mass and SVL at metamorphosis among genotypes (Fig. 4). Size and date of metamorphosis were positively correlated in full hybrids and negatively correlated in pure CTS and superinvasives, suggesting that full-hybrid genotypes are uniquely capable of capitalizing on perennial pond hydroperiods to maximize their time in the high-productivity larval environment. Contrasts between full hybrids and CTS (for mass, $P = 0.02$; for SVL, $P = 0.008$) and between full hybrids and superinvasives (for mass, $P = 0.03$; for SVL, $P = 0.04$) were all significant, although only the SVL contrast between full hybrids and CTS was significant after Bonferroni correction (the rest were marginally nonsignificant).

DISCUSSION

Identifying when taxon substitutes, be they intentional or accidental, constitute a reasonable conservation alternative is one of the most difficult issues in conservation management. Our analysis illustrates an experimental approach to this problem by evaluating taxon substitutes based on their position in a

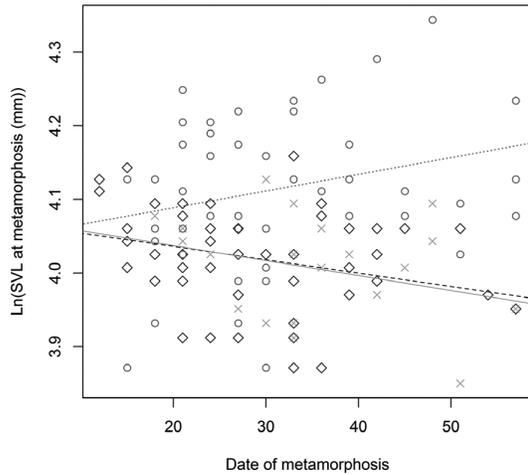


FIG. 4. Reaction norms showing the relationship between ln-transformed SVL (originally measured in mm) and date of metamorphosis (days after 30 April) for each treatment: dotted line, full hybrid; dashed line, pure CTS, and solid line, superinvasive. The slope of the reaction norm is significantly greater for full hybrids than for pure CTS or superinvasives (for Genotype, $F = 18.9$, $df = 2$, $P < 0.001$; for Date, $F = 0.5$, $df = 1$, $P = 0.50$; for Genotype \times Date, $F = 4.4$, $df = 2$, $P = 0.01$). The relationship is qualitatively identical for mass at metamorphosis. Data points for each individual tiger salamander ($N = 122$) are shown by different symbols: open circles, full hybrid; diamonds, pure CTS; crosses, superinvasive.

multivariate community space. This approach is particularly important when objective criteria are needed to determine whether certain taxa (e.g., hybrids) should be extended protection under the ESA, or should be used to rescue genetically depauperate lineages. In our case study, superinvasive larvae were ecologically equivalent to pure-CTS larvae and thus constitute a reasonable taxon substitute, at least in the larval phase of their complex life cycle. The community effects of superinvasives and pure CTS overlapped in multivariate space (Fig. 1) and were statistically indistinguishable on all univariate axes (Fig. 2). Full-hybrid larvae, on the other hand, are not ecologically equivalent to pure CTS. The 95% CI on the community effects of pure CTS and full hybrids were at the edge of overlapping in multivariate space ($P = 0.054$) and differed significantly on the chlorophyll and *Notonecta* axes. Instead, we found full-hybrid larvae to be ecologically similar to pure-CTS larvae, in that their niche centroid falls closer to the pure-CTS centroid than the pure-CTS centroid does to the no-*Ambystoma* centroid. This indicates that full hybrids fill some of the ecological role of pure CTS in the pond community, even though there are detectable differences between their two roles. We conclude that superinvasive salamander larvae play an equivalent ecological role to pure CTS and should

probably be extended continued protection under the ESA, while it remains equivocal whether full hybrids should be considered an adequate taxon substitute for pure CTS.

Food web equivalency: what makes taxa substitutable?

Our approach of evaluating taxon substitutes based on their position in a multivariate community space essentially compares their equivalency in food web dynamics. In natural vernal pools and our mesocosms, *Ambystoma* larvae are often the top predator. To mechanistically understand our results, we focus on their effects on three groups: (1) salamander prey, (2) food of those prey, and (3) taxa that are differentially impacted by full hybrids compared to pure CTS.

Salamander prey include *C. californicus*, *P. regilla*, and snails, and they reached their highest densities in the no-*Ambystoma* treatment. In addition, each of these taxa were at their lowest densities in the full-hybrid treatment. This matches well with findings showing that full hybrids are more voracious predators than pure CTS on two native amphibian species (Ryan et al. 2009). Our results extend this earlier result, demonstrating that full-hybrid larvae have strong predatory effects on invertebrate as well as vertebrate prey, whereas the community effects of superinvasive larvae are indistinguishable from those of pure CTS.

In contrast, cladocera and periphyton were at their lowest densities in the no-*Ambystoma* treatment, indicating that they experience predator release when tiger salamander larvae of any genotype are present. This is not surprising for periphyton, because snails and *P. regilla* tadpoles, which are both tiger salamander prey (Anderson 1968), mainly consume periphyton. Much more surprising was our observation that cladocera also appear to be experiencing predator release from tiger salamander larvae. Cladocera can be the chief prey item of salamander larvae (Dodson and Dodson 1971, Holomuzki and Collins 1987), and increasing tiger salamander density in whole-pond manipulations has been shown to decrease the density of cladocera in other systems (Benoy 2005). However, these studies took place in communities that lacked another important crustacean (*C. californicus*, clam shrimp) that is simultaneously an important predator of cladocera and an important prey of *Ambystoma*. Our data suggest that the indirect link to cladocera through clam shrimp is stronger than direct consumption by tiger salamander larvae, especially as the *Ambystoma* larvae become larger and shift toward consuming fewer, larger prey (i.e., clam shrimp) rather than large numbers of small zooplankton (i.e., cladocera; Brophy 1980).

Chlorophyll and *Notonecta* fall into a third category, where the significant difference is between the

full-hybrid and pure-CTS treatments rather than between the three salamander treatments and the no-*Ambystoma* treatment. Notonectids are small, powerful predators, and are not a favored prey item of tiger salamander larvae (Dodson and Dodson 1971, Brophy 1980, Holomuzki and Collins 1987). Only large larvae consume notonectids and, as full hybrids grew significantly larger than both pure CTS and superinvasives, they may have been the only genotype large enough to eat substantial numbers of *Notonecta*. Alternatively, their ability to drive all other prey items to low densities may have forced full hybrids to include this less preferred prey in their diet. We lack a strong hypothesis for why chlorophyll density was highest in the full-hybrid treatment, but it is an important result because it appeared to lead to increased turbidity, which could have additional community-level consequences.

Morphology/phenology

Because of differences in breeding phenology, the superinvasive larvae commenced the experiment at a smaller size than the other two genotypes, making comparisons of size and date of metamorphosis difficult to interpret. The smaller size and later date of metamorphosis in superinvasives may be attributable solely to this difference in initial conditions. As a result, the most telling morphological/phenological comparison between the three genotypes is among the reaction norms for size at metamorphosis, because this reveals the life history strategy of each genotype independent of initial size. Consistent with field observations, the mean size of metamorphosing pure CTS decreased through time (Trenham et al. 2000, Searcy et al. 2014). Because size at metamorphosis is correlated with juvenile and adult fitness (Searcy et al. 2014), this implies that the most fit pure CTS emerged first, with progressively less fit animals metamorphosing later in the season. Superinvasives almost exactly matched the reaction norm found in pure CTS, whereas full hybrids showed the opposite pattern (Fig. 4). This suggests that superinvasives retain the pure CTS life history strategy of emerging early if they are fit enough to do so. Full hybrids, on the other hand, delay metamorphosis as long as conditions remain conducive to continued growth, resulting in the largest, most fit individuals emerging at the end of the season.

As a result, full hybrids seem better able than pure CTS/superinvasives to take advantage of any factor that would allow them to metamorphose at a larger size. Previous experiments have shown that full-hybrid mean fitness increases more dramatically with increased hydroperiod than that of pure CTS (Johnson et al. 2013). Here we show that within this mean effect, it is also the fittest full-hybrid individuals that are emerging latest. In addition, our results indicate that full

hybrids are more fully capable of taking advantage of low conspecific densities, because their size at metamorphosis increased more dramatically under low-density conditions than in either pure CTS or superinvasives (Fig. 3). This greater plasticity may reflect the less constrained climatic regime in native barred tiger salamander habitat in the Great Plains, where summer precipitation occurs throughout their emergence period, rather than the rain-free emergence period that characterizes the mediterranean climate of California.

Conservation

The ideal conservation outcome for any endangered species is to preserve healthy, native populations across its original range. However, this may not be possible for certain species, communities, or ecoregions. North American grasslands will presumably never be rid of invasive Eurasian grasses, Australia will have cane toads (*Rhinella marina*) as part of its new amphibian community assembly, and west Texas mutations are now part of the Florida panther (*Puma concolor coryi*) genome (DiTomaso 2000, Urban et al. 2007, Johnson 2010). In the CTS system, if natural selection favors certain regions of the BTS genome, then it may be beyond the capacity of government agencies and land managers to stop the spread of those genes. Given these realities, conservation biologists must explore scientifically justifiable middle grounds, and the concepts of ecological equivalency and similarity provide those opportunities. Our experimental analyses suggest that superinvasive larvae act as reasonable taxon substitutes for pure CTS because they fulfill the ecological equivalency criterion, and we suggest that they should continue to be protected under the ESA. It is somewhat less clear whether this protection should also be extended to full hybrids. Given that the vernal pool community with full hybrids is more similar in ecological space to a pure CTS pond than one lacking *Ambystoma*, we tend to favor the presence of full hybrids if the only realistic alternative is the removal of all tiger salamanders from the landscape. However, the low densities of prey species in full-hybrid pools is a definite concern, given the high number of other threatened species, many of which are tiger salamander prey, that currently inhabit the same pools as pure CTS (Fitzpatrick and Shaffer 2004).

Finally, we agree with the idea that an overarching conservation goal should be to manage wild populations to increase the fraction of native genes, even if pure native genotypes (and ecosystems) are currently unachievable. The most operationally feasible strategy that has so far been proposed for the CTS system is to decrease the hydroperiod of manmade ponds so that they more closely resemble natural vernal pools (Fitzpatrick and Shaffer 2004, 2007a, Johnson et al.

2013). Our results provide additional evidence that this could be a beneficial strategy. Not only do pure CTS emerge earlier on average than hybrids (Fig. 3), but also the most fit individuals emerge at the beginning of the season. Thus, if ponds dry earlier, the animals that are truncated from the late-metamorphosing end of the distribution will be the ones with lowest fitness that were making the smallest contribution to population persistence. In contrast, full hybrids that emerge latest are largest and have the highest fitness, and thus removing these animals will have a disproportionately larger effect on the full-hybrid population. Assuming that hybrids that emerge first tend to have the most native genotypic composition, the combined effect of these truncations will be to increase the fraction of natives (or native genes) in the overall population.

Caveats

An evaluation of taxon substitutes based on ecological equivalency should ideally investigate as large a portion of the life history of the potential substitutes as possible. We investigated only the aquatic phase of the tiger salamander life cycle and ignored the terrestrial phase. Given that larval tiger salamanders are top predators in the fishless ponds they inhabit, whereas adult tiger salamanders are lower-level predators in their grassland habitat (Holomuzki and Collins 1987, Holomuzki et al. 1994), this strategy makes sense, although the possibility remains that the terrestrial phase of the tiger salamander life cycle is an important component of understanding why the superinvasive alleles are spreading so rapidly. As with many other conservation decisions, we base our recommendations on the incomplete data that are available, rather than the full understanding that would come from complete ecological knowledge. For the time being, we recommend that superinvasives be extended continued protection under the Endangered Species Act due to the ecological equivalency of their larvae with those of pure CTS. However, we encourage future studies examining the ecological equivalency of the terrestrial life stages of both genotypes, and recommend that management should be both adaptive and based on the fullest data that are available at any point in time.

At a broader level, we strongly support the idea that there also needs to be some flexibility in assessing an overall ecological similarity criterion. If certain univariate axes have high conservation value (e.g., densities of other sensitive/endangered taxa), then substitutes that differentially affect these axes may be unacceptable even if they pass the ecological similarity criteria based on the position of their treatment centroid. This potential weighting of the univariate axes based on their conservation importance is why we have remained equivocal about the role of full hybrids

as acceptable substitutes. Although full hybrids pass a general ecological similarity criterion, they also severely depress prey densities, many of which are themselves endangered taxa.

CONCLUSION

Understanding the ecological role of novel taxa, be they invasives, hybrids, or intentionally introduced taxon substitutes, is a critical, but often poorly understood problem in conservation biology. Our approach of measuring their effects on the whole community in multivariate space should be useful in many other systems, and we advocate this as one general approach to evaluating the potential of taxon substitutes as a conservation strategy. The ultimate goal of conservation should be to preserve intact ecosystems, and thus the most relevant metric for evaluating taxon substitutes is their ecosystem function and effect on community composition. Our results clearly demonstrate that, in one well-studied system, larval superinvasives are ecologically equivalent to pure CTS larvae, and thus merit continued protection under the ESA.

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