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LANDSCAPE HOMOGENIZATION THREATENS THE GENETIC INTEGRITY OF THE ENDANGERED SAN DIEGO FAIRY SHRIMP *BRANCHINECTA SANDIEGONENSIS* (BRANCHIOPODA: ANOSTRACA)

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ABSTRACT

Genetic threats to the integrity and persistence of endangered species can be significant and develop rapidly. These threats include the breakdown of locally adapted gene pools, and more extreme phenomena such as the dissolution of species boundaries. Anthropogenic homogenization of the landscape is often the underlying cause, manifested as an increase in biotic connectivity, and a decrease in structural and spatial habitat heterogeneity. In southern California, the narrowly endemic San Diego fairy shrimp *Branchinecta sandiegonensis* is federally endangered due to high levels of vernal pool habitat loss. Human disturbance is associated with the increased presence of the widely distributed generalist *B. lindahli* in the native range of *B. sandiegonensis.* Regional sympatry for these closely related species has now become local co-occurrence in anthropogenically created basins and disturbed pools, with possible hybridization. To assess this threat, we developed a new morphological hybrid index that ranges from 1.0 for typical *B. lindahli* adult females to 3.0 for typical *B. sandiegonensis*. Index scores in undisturbed habitats that are typical for each "pure" species are ⩽1.3 and ⩾2.6 respectively. In disturbed areas, females have a wider range of intermediate scores. Using mitochondrial DNA markers that are diagnostic to each species, we also determined that putative hybrids at all mixed sites share their maternal lineage with the more common species at the site. We hypothesize that anthropogenic activities have increased genetic, taxonomic and functional homogenization in southern California's vernal pools, and may constitute a significant threat to the species integrity and persistence of the San Diego fairy shrimp.

KEY WORDS: Anostraca, endangered species, habitat disturbance, morphological hybrid index, vernal pool

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INTRODUCTION

The worldwide loss of natural habitats in extent and functionality has resulted in biodiversity losses and numerous species being classified as threatened or endangered. Although the initial listing of endangered species is usually due to habitat loss, the likelihood of recovery is often worsened by degradation of the remaining habitat and homogenization of the landscape (Olden et al., 2004; Olden, 2006; Devictor et al., 2008a, b). Some of the most urgent threats that arise from these factors correspond to changes in species distributions.

Anthropogenic disturbance can alter the abiotic environment, resulting in reduced fitness for sensitive endemics and specialists, while increasing suitability for generalists (Olden et al., 2004; Devictor et al., 2008a). When human activities homogenize entire landscapes, this problem is exacerbated by the loss of biotic and abiotic heterogeneity that underlies community structuring. Generalists usually benefit from these changes with expanded ranges, while range and local population sizes of rare, endangered or threatened specialists tend to contract. Formerly unique communities become more similar, and contact between previously allopatric species initiates the breakdown of habitat partitioning and novel competition scenarios (Mooney and Cleland, 2001). Invading species can also introduce diseases or parasites into naive populations of endemics, resulting in fitness losses that range from minor to catastrophic (Warner, 1968; van Riper et al., 1986; Weldon et al., 2004). Finally, increased contact between closely related taxa that do not have efficient pre-mating isolating mechanisms can lead to the loss of species integrity via intergradation or hybridization (Wolf et al., 2001; Levin, 2002; Riley et al., 2003), even if hybrids are infertile. In some cases, the extinction risks due to hybridization meet or exceed those posed by novel ecological interactions (reviewed by Rhymer and Simberloff, 1996; Perry et al., 2002; Olden et al., 2004; Mallet, 2005). However, increased contact between closely related congeners often goes unnoticed (Geller et al., 1997; Roman, 2006) despite the critical importance of identifying and preventing the spread of hybrids throughout an endangered species' range (Perry et al., 2002).

Southern California's vernal pools are a unique set of temporary wetlands, and a rich source of native biodiversity. The pools typically occur on flat mesa tops scattered within chaparral vegetation, where soils contain an impermeable layer and retain water. During the rainy season, vernal pools hold standing water long enough to support specially adapted faunal (Ripley and Simovich, 2008; Bauder et al., 2009) and floral communities (Bauder, 1987; Bauder et al., 2009) and exclude upland plants. It has been estimated that

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>95% of the vernal pools which were historically present in this region are now gone (Long et al., 1992; Bauder and McMillan, 1998; King, 1998). Much of the habitat that does remain is severely disturbed due to past and current vehicle usage and grazing. In some areas, recreational vehicles, utility vehicles or military training have created dirt roads through pools, cleared and denuded the landscape that surrounds them, and/or created basins where none existed historically by compacting soils. In the most severe cases, the surrounding landscape is reduced largely to non-native grasses and other weeds, and hydrology has been altered so that native vernal pool plants no longer persist. For these reasons, numerous vernal pool plants and animals have been federally listed as threatened or endangered (USFWS, 1980, 1991, 1992, 1993a, b, 1994).

The most prominent endangered species in southern California's vernal pools is the San Diego fairy shrimp, *Branchinecta sandiegonensis* Fugate, 1993 (USFWS, 1997). Pristine and near-pristine coastal vernal pools in southern California and northern Baja California, Mexico contain only this species of fairy shrimp (Simovich and Fugate, 1992; Fugate, 1998; Eriksen and Belk, 1999). In contrast, the versatile fairy shrimp, *B. lindahli* Packard, 1883 is widespread across the western United States and Mexico and found in a variety of ephemeral wetlands including dry lakes and depressions in dirt roads (Maeda-Martínez et al., 1997; Eriksen and Belk, 1999). Until relatively recently, the occurrence of *B. lindahli* in southern California was thought to be limited to desert playas and a few disturbed coastal pools (Fugate, 1998; Eriksen and Belk, 1999). It now also occurs in a variety of human created and disturbed pools in this region (Eriksen and Belk, 1999, personal observation).

The potential homogenization of southern California's vernal pool habitat is alarming, in light of the fact that *B. lindahli* and the federally endangered *B. sandiegonensis* can hybridize in the laboratory (Fugate, unpublished data; mentioned in Fugate (1998), and confirmed recently by C. Shanney, unpublished data). Motivated by anomalous specimens collected throughout southern California by us and others, e.g. Mark Angelos (personal communication), we sought to determine whether hybridization is likely to be occurring in the field as well. Our goals in this study were as follows:

1. Survey multiple *B. lindahli* and *B. sandiegonensis* populations across a disturbance gradient, and evaluate individual morphology in the context of published species descriptions.
2. Assay maternal species lineages using mitochondrial DNA.
3. Propose a morphological hybrid index for the identification of *B. lindahli*/*B. sandiegonensis* hybrid adult females.

MATERIALS AND METHODS

Field Collections

Sexually mature female *Branchinecta* were collected from sites that range from those typical for *B. sandiegonensis* or *B. lindahli* in southern California, USA, to disturbed sites in which apparent intermediates had been seen or were suspected (Fig. 1, Table 1). *Branchinecta sandiegonensis* was collected from vernal pools at the Del Mar Mesa Reserve (California Department of Fish and Game) and McAuliffe Park (City of San Diego School District). Del Mar Mesa is the type locality for this species (Fugate, 1993), and has had little human activity or disturbance for at least twenty years. The majority of pools in McAuliffe Park are relatively undisturbed.

Branchinecta lindahli was collected from temporary wetlands in the desert, more than 50 km from the nearest known populations of *B. sandiegonensis*. This included four sites near Twentynine Palms in the Mojave Desert of San Bernardino County (Means, Melville, Emerson and Dale) and one from DiGorgio Road in Borrego Springs (Colorado Desert, San Diego County). The Twentynine Palms sites are all desert dry lakes and experience heavy military and/or recreational vehicle usage. The Borrego Springs site is a depression in a lightly used dirt road (these are hereafter referred to as a "road pool").

Three disturbed coastal sites were sampled for putative hybrids (Table 1). Carmel Mountain Preserve (City of San Diego) is less than 4 km from Del Mar Mesa but was frequented by recreational vehicles until 1999, and the major road in the preserve is still used by utility vehicles. Most basins are turbid, highly disturbed road pools with little or no native vegetation. Three road pools were sampled from Marine Corps Base Camp Pendleton. Across the installation, a complete range of disturbance levels can be found, from near pristine vernal pools to highly disturbed road pools frequented by vehicles that include tanks. At these two sites, many of the basins may be artificial due to soil compaction by vehicles. The Salk site (San Diego School District) was scraped down to bedrock over thirty years ago, and pools have reestablished on it. This vacant lot experiences foot traffic, bicycle and pet use, but not vehicular traffic. Pools near Salk contain primarily *B. sandiegonensis*, including McAuliffe Park (0.5 km away). Although the Salk pools have atypical hydrology and soil condition, they are usually clear and do have some vegetation that is characteristic of pristine vernal pools.

For Carmel Mountain, Camp Pendleton, Dale Lake, Del Mar Mesa, and Salk, mature females were brought live to the lab, analyzed and then frozen in 100% ethanol for genetic analysis. Specimens from Borrego Springs and McAuliffe were relaxed in carbonated water prior to field preservation in ethanol. For Twentynine Palms, females reared in the lab from previous soil collections were relaxed in carbonated water and preserved in ethanol.

Morphological Analyses

Male and female morphology (Fugate, 1993; Eriksen and Belk, 1999; Rogers, 2002a), allozymes (Fugate, 1992) and species specific mtDNA haplotypes (Bohonak, 2005; Vandergast et al., 2009) all indicate that *B. lindahli* and *B. sandiegonensis* are "good species" in the sense that they constitute independent evolutionary lineages and have fully diagnostic genetic and morphological characters. Although characters on the male second antennae are most frequently used for species identification in this genus (Eriksen and Belk, 1999), male *B. sandiegonensis* and *B. lindahli* are not differentiated enough to reliably identify hybrids. We focused instead on female dorsolateral projections (emphasized in Fugate, 1993; Eriksen and Belk, 1999; Rogers, 2002a). Adult females were scored for each thoracic segment between 3 and 11 as described in Tables 2 and 3, and illustrated in Fig. 2 (see also Rogers, 2002a).

For each of nine characters, every female was assigned a score of 1 for the state typical in *B. lindahli*, 3 for the state typical in *B. sandiegonensis*, and 2 for states that are atypical for a particular segment in both species. We conducted a variety of exploratory data analyses, including principle component analysis (PCA) in Data Desk v. 6.2.1 (Velleman, 1997). Based on the results discussed below, we defined the morphological hybrid index as the simple mean of the nine characters. This index was calculated for every female in the data set, and then compared among species and sites.

Maternal Lineage Determination

To provide information on behavioral or genetic biases that may underlie hybridization, we determined which species' mtDNA lineage (inherited maternally) was present in 104 females (45 *B. sandiegonensis*, 30 *B. lindahli* and 31 putative hybrids). DNA was extracted from the head of each individual using Qiagen DNeasy kits (Valencia, CA, USA). The mtDNA species lineage was identified with a rapid diagnostic used extensively in the Bohonak lab (Vandergast et al., 2009). The protocol is based on PCR amplification of species-specific haplotypes of the mtDNA gene cytochrome oxidase subunit 1 (CO I) with a cocktail of nested primers. The two species are divergent enough in their mtDNA that the mis-identification rate is zero (or nearly zero) for non-hybrids (Vandergast et al., 2009: Single-step protocol, Tables 2 and 3). PCR products were separated by size in agarose gels, and visualized with ethidium bromide staining.

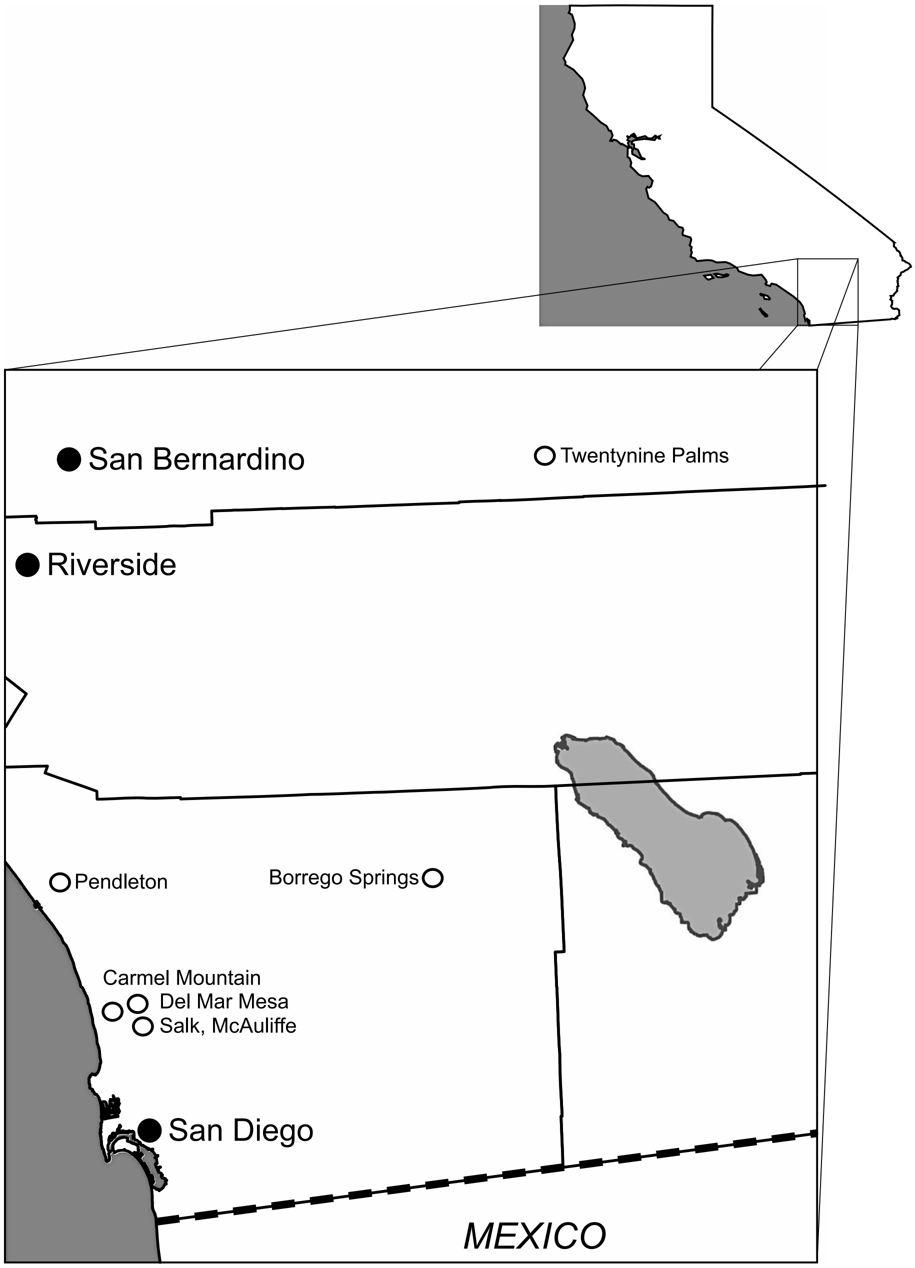

Fig. 1. Map of southern California, USA, highlighting the seven field sites.

Table 1. Site descriptions. V, vernal pools; R, road pools; P, desert playas. Clear pools are visually clear, except in the days following a major precipitation event. Turbid pools are visually opaque at all times, and are largely devoid of aquatic vascular plants (see Eriksen and Belk, 1999 and references therein). Additional location information provided in Appendix A, which can be found in the online version of this journal, which can be accessed via http://booksandjournals.brillonline.com/content/1937240x.

Site (no. of pools)	Pool type	Dates collected	Description
Coastal sites			
Del Mar (2)	V	01/2011	Clear pool, characteristic native flora
McAuliffe (2)	V	02/2008, 02/2009	Clear pool, characteristic native flora
Salk (2)	V	11/2010, 01/2011	Clear pool, some native flora
Pendleton (3)	R	11/2010	Turbid pool, no flora
Carmel Mountain (5)	R	10/2010, 11/2010, 01/2011	Turbid pool, no flora
Desert sites			
Twentynine Palms (4)	P	05/2010, 01/2011	Turbid pool, no flora
Borrego Springs (1)	R	01/2011	Turbid pool, no flora

Table 2. Character states and scoring criteria. See Fig. 2 for an illustration of character states and scoring in non-hybrids.

	Typical states	
	B. lindahli	*B. sandiegonensis*
Thoracic segments		
T3	a or e	c
T4	a	c or d
T5	a	c
T6	a	c
T7	a	c
T8	a	c
T9	a	e
T10	a	e
T11	a	e

RESULTS

The nine characters we analyzed were highly, but not perfectly, correlated. For 280 individuals analyzed in the PCA (means of 14.7 per pool and 38.3 per site), 77% of the variance could be explained by the first component (PC1) (Fig. 3). All characters loaded on PC1 with nearly the same values (−0.35 to −0.30), suggesting that a simple mean of these characters is appropriate for species classification. PC2 (explaining 11% of the variance) highlighted more subtle correlations among characters in hybrids. Scores for thoracic segments T4-T8 loaded positively on PC2 (0.16 to 0.35), while T3 and T9-11 all loaded negatively (−0.38 to −0.33). This suggests two suites of characters that are developmentally (and possibly genetically) correlated. Carmel Mountain contained females with a wide variety of character combinations. In contrast, individuals from Pendleton more uniformly resembled *B. sandiegonensis* in segments T4-T8, and the other characters resembled *B. lindahli* (or had atypical character states).

Individuals from relatively undisturbed areas did not overlap in hybrid index scores, confirming the presence of "pure" species at expected sites (Table 4, Fig. 4). In Del Mar Mesa and McAuliffe, females had hybrid index scores between 2.6 and 3.0, and *B. sandiegonensis* mtDNA lineage haplotypes. Shrimp from the five desert wetlands all possessed hybrid index scores between 1 and 1.2, and *B. lindahli* mtDNA haplotypes.

As expected, samples from Camp Pendleton and Carmel Mountain had intermediate hybrid index values encompassing a far wider range of scores than found elsewhere (Fig. 4). Fugate (unpublished data) observed similar mixtures of character states in the hybrid offspring of no-choice crosses. Despite the fact that both species can be found at Camp Pendleton and Carmel Mountain, the hybrid-containing pools that we sampled at these sites contained only *B. lindahli* mtDNA haplotypes (Table 4). At Salk (morphologically, a *B. sandiegonensis* site), two putative hybrids were found among the 59 females analyzed. These two females possessed *B. sandiegonensis* haplotypes (Table 4). Overall, we inferred diagnostic values for the hybrid index as follows: 1.0-1.3 is indicative of *B. lindahli*, 2.6-3.0 corresponds to *B. sandiegonensis*, and 1.4-2.5 are morphological intermediates that we interpret to be hybrids. Using these criteria, presumed hybrids were present at Camp Pendleton (12 of 12 females analyzed), Carmel Mountain (36 of 45), and Salk (2 of 59).

We also noted the presence of one gynandromorph at Carmel Mountain, and one at Emerson Lake. Gynandromorphs, also known as intersex individuals, contain both male and female characteristics (reviewed by Narita et al., 2010). These particular individuals possessed highly developed male second antennae, but female gonads and ovisacs. They were identified as *B. lindahli* but not used in the hybrid index analysis.

Table 3. Scoring criteria. Each segment was assigned a score of 1 for the state typical in *B. lindahli* (a or e, depending on the segment), 3 for the state typical in *B. sandiegonensis* (c, d, or e, depending on the segment), or 2 for states that are atypical in that segment in both species. See Fig. 2 for an illustration of character states and scoring in non-hybrids.

	Segment-specific scores for each character state				
	a	b	c	d	e
Thoracic segments					
T3	1	2	3	2	1
T4	1	2	3	3	2
T5	1	2	3	2	2
T6	1	2	3	2	2
T7	1	2	3	2	2
T8	1	2	3	2	2
T9	1	2	2	2	3
T10	1	2	2	2	3
T11	1	2	2	2	3

DISCUSSION

Our results suggest that the endangered San Diego fairy shrimp *B. sandiegonensis* is hybridizing with its congener *B. lindahli*, which could compromise the genetic integrity of *B. sandiegonensis*. Although females from undisturbed sites in the traditionally recognized ranges of these two species exhibit the species-specific character states published in species descriptions and taxonomic keys, those from human disturbed pools within the San Diego fairy shrimp's range contain a mosaic of morphological character states. Until hybrids of *Branchinecta* are thoroughly studied in a controlled laboratory setting, it is unclear if specific character combinations found in the field can be interpreted in terms of F1 (first-generation) hybrid crosses, or various back crosses. Similarly, quantifying the degree of introgression and the fitness of hybrids will also require the development of a genetic hybrid index based on nuclear genetic markers that possess fixed differences between unambiguously pure populations of *B. lindahli* and *B. sandiegonensis*.

As discussed by numerous authors (Levin et al., 1996; Rhymer and Simberloff, 1996; Wolf et al., 2001; Levin, 2002), hybridization has negative consequences for the integrity (and sometimes persistence) of species. These can be broadly characterized as: 1) outbreeding depression (lowered fitness in suboptimal gene complexes), which can lead to population declines if coupled with gene pool introgression; or 2) "genetic swamping" if backcrossing

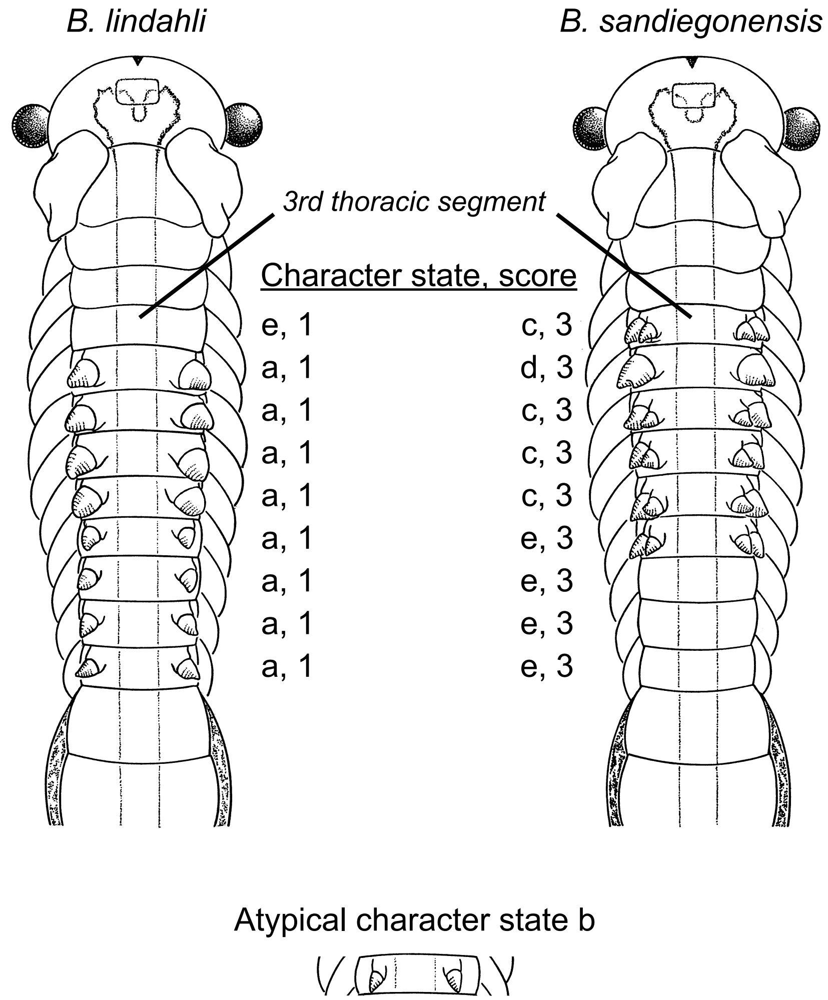

Fig. 2. Comparison of dorsolateral projection character states in female *B. lindahli*, *B. sandiegonensis* and atypical females. Illustrations modified and reproduced with permission from Eriksen and Belk (1999). Character states:
(a) One pair of simple processes located on opposing sides of the thorax, projecting laterally.
(b) One pair of simple processes located on opposing sides of the thorax, projecting dorsally.
(c) Two distinct pairs of processes located on opposing sides of the thorax. Each spine is distinct. One process per pair projects laterally, and one projects dorsally.
(d) One pair of bi-lobed processes located on opposing sides of the thorax (these are thicker than simple spines, project laterally, and appear to represent the fusion of state b).
(e) No processes.

results in assimilation of one genome (Levin, 2002; Olden et al., 2004).

If broader regional surveys verify a relationship between recent disturbance and hybridization, conservation and management plans may need to account for this challenge to recovery, which is explicitly mentioned the U.S. Fish and Wildlife Service (2008) five-year review of the San Diego fairy shrimp.

Hybridization in Anostracans

The potential for hybridization appears to be somewhat common in fairy shrimp. Anostracan hybrids have been produced in the lab from no choice mating experiments (Wiman, 1979a, b; Fugate, 1992; Dumont and Adriaens, 2009; Sugumar, 2010), although less frequently documented in wild populations (Brendonck and Riddoch, 1997; Kappas et al., 2009). In some cases, relatively porous species genomes even permit hybridization between populations that have evolved on different continents (Wiman, 1979a; Dumont and Adriaens, 2009; Sugumar, 2010). Rogers (2002b) found that males of all species of *Branchinecta* tested were willing to amplex with females of other species of *Branchinecta* (including dead ones). Numerous species pairs of *Branchinecta* have produced hybrids in laboratory trials (Fugate, 1992; Maeda-Martínez et al., 1992), including *B. lindahli* and *B. sandiegonensis* in preliminary experiments by C. Shanney (unpublished data) and Fugate (unpublished data). Thus, distinct species of *Branchinecta* seem to be susceptible to hybridization and introgression when they are brought into in contact.

One possible mechanism for reproductive isolation and speciation in *Branchinecta* is cytoplasmic incompatibility

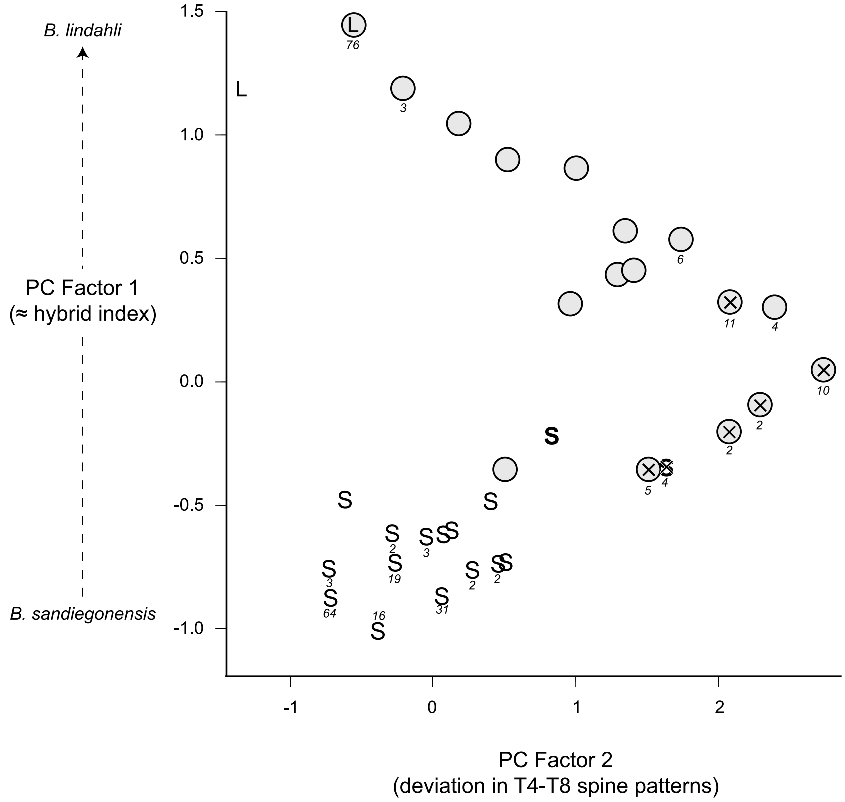

Fig. 3. Scatterplot of the first two principal components from the PCA. L, *B. lindahli* (29 Palms and Borrego Springs); S, *B. sandiegonensis* (Del Mar Mesa, McAuliffe and Salk); circles, individuals from Carmel Mountain; crosses, individuals from Camp Pendleton. The two hybrids from Salk are indicated by bold letters. Small numbers represent the number of overlapping points.

induced by *Wolbachia* or similar bacteria. *Wolbachia* are maternally inherited intracellular bacteria found in the reproductive tissues of many invertebrates. Because males and females infected with different strains of *Wolbachia* may not be reproductively compatible, these parasites can initiate lineage isolation and speciation (reviewed by Werren, 1998; Werren et al., 2008; Brucker and Bordenstein, 2012). Numerous lines of evidence suggest that *B. lindahli* harbors feminizing endoparasitic bacteria (Sassaman and Fugate, 1997; Krumm, 2006). Such bacteria cause male biased populations and, when feminization is incomplete, gynandromorphs.

Although we did not use mtDNA data to identify hybrids, those data can provide some insights into the processes that may drive hybridization. In this study, the presence of only *B. lindahli* mitochondrial haplotypes in two sites with high frequencies of morphological intermediates (Table 4) suggests that either hybrid offspring fitness or fertilization success depend on particular gender/species combinations in the parents. The second possibility could occur if interactions where one species is infected by endoparasites such as *Wolbachia* but the other is not, result in asymmetrical reproductive incompatibility. Other possibilities exist as well including unequal initial ratios of the species.

Table 4. Summary of hybrid index scores, morphological interpretation and mtDNA lineages across sites. Hybrid index scores of 1.0-1.3 represent *B. lindahli*, 1.4-2.5 represent putative hybrids, and 2.6-3.0 represent *B. sandiegonensis*. Hybrid N is the number of individuals that were morphological hybrids. Hybrid N^* is the number of morphological hybrids analyzed genetically.

Site	Hybrid index score					mtDNA maternal lineage species (total N, Hybrid N^*)
	N	Range	Mean	Hybrid N	Morphological ID	
Del Mar	40	2.7-3.0	2.88	0	*B. sandiegonensis*	*B. sandiegonensis* only (14, n/a)
McAuliffe	50	2.6-3.0	2.88	0	*B. sandiegonensis*	*B. sandiegonensis* only (14, n/a)
Salk	59	2.3-3.0	2.83	2	*B. sandiegonensis* (2 hybrids)	*B. sandiegonensis* only (19, 2)
Pendleton	12	1.9-2.4	2.22	12	Hybrids	*B. lindahli* only (10, 10)
Carmel Mountain	45	1.0-2.4	1.82	39	Hybrids (6 *B. lindahli*)	*B. lindahli* only (22, 17)
Twentynine Palms	71	1.0-1.2	1.00	0	*B. lindahli*	*B. lindahli* only (22, n/a)
Borrego Springs	3	1.0-1.0	1.00	0	*B. lindahli*	*B. lindahli* only (3, n/a)

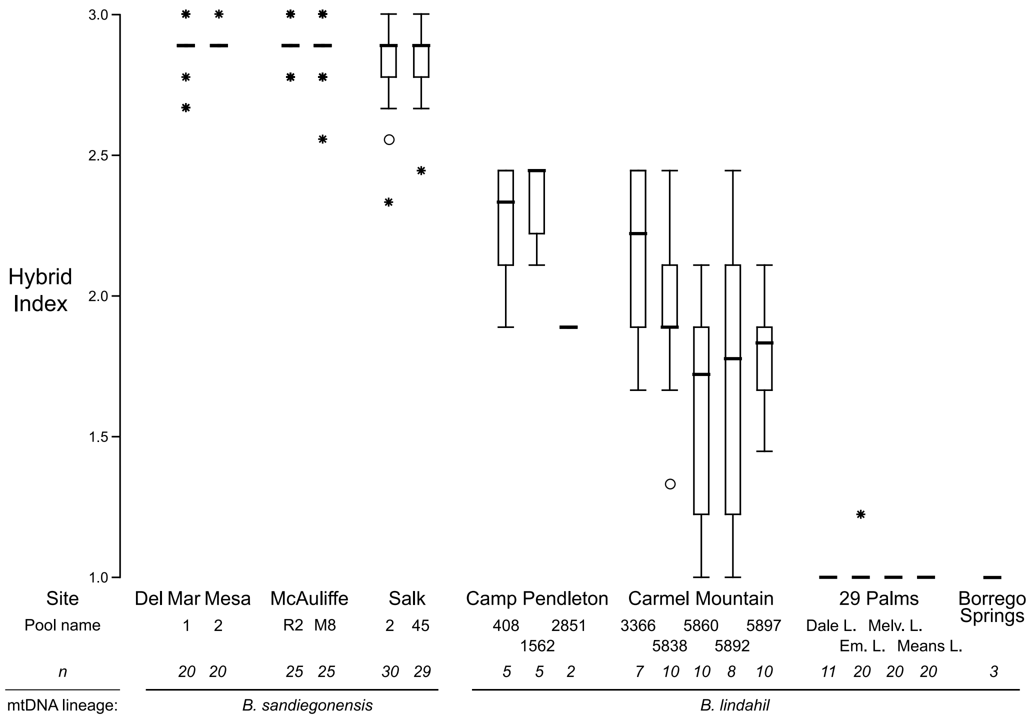

Fig. 4. Box plot comparing hybrid index scores across the 19 pools. Each pool is labeled by site name, pool number (or name) and the mtDNA lineage found in that pool. Only one mtDNA lineage is found per pool (Table 4). Female fairy shrimp from the first six pools correspond morphologically to typical *B. sandiegonensis*, and those from the last five correspond to typical *B. lindahli*. Each box depicts the median for the pool, 25th and 75th percentiles (box hinges), whiskers extending to the minimum and maximum data values occurring within 1.5× range above and below the box hinges, outliers (circles) and extreme outliers (stars) (Velleman, 1997).

Causation

Any conclusions about the causes and consequences of hybridization between these species are coupled with assumptions about the current and past distributions of hybrid and non-hybrid populations. Current distributions are well characterized. *Branchinecta sandiegonensis* is the only branchinectid found in the relatively clear, cool, shallow, and undisturbed vernal pools of coastal southern California (Simovich and Fugate, 1992; Fugate, 1998; Eriksen and Belk, 1999; City of San Diego, 2005; Bauder et al., 2009). The fact that many undisturbed *B. sandiegonensis* vernal pool complexes are fixed for unique mtDNA lineages (Bohonak, 2005) is consistent with the hypothesis that this species has long been established in those same pools. The San Diego fairy shrimp is not currently found in inland desert playas that contain *B. lindahli*, because it cannot persist in the warmer, highly turbid pools with high solute levels (Gonzalez et al., 1996; Hathaway and Simovich, 1996; Eriksen and Belk, 1999). It is reasonable to assume that the San Diego fairy shrimp never occupied those habitats. Currently, *B. lindahli* is a widespread, opportunistic species with a wide tolerance for many ephemeral wetlands, including desert playas (Maeda-Martínez et al., 1997; Eriksen and Belk, 1999). We concede that the past distribution of this species is open to interpretation. However, because the species are distinct despite ineffective intrinsic barriers to hybridization, it is reasonable to assume that *B. sandiegonensis* evolved in a habitat distinct from *B. lindahli* (in terms of abiotic pool conditions and/or geographic location).

In coastal San Diego County, both species and putative hybrids can currently be found in highly disturbed pools and artificial depressions such as road ruts. The majority of these disturbances are due to vehicular and recreational activity. Disturbed and compacted basins that do contain *B. sandiegonensis* are always in areas where natural vernal pools existed historically. However, because hybrids have not been previously recognized, most disturbed pools thought to contain *B. sandiegonensis* may actually contain hybrid populations (most consultants and researchers identify *Branchinecta* and other fairy shrimp only from male specimens, and we have not been able to determine a reliable way to delineate male hybrids).

Mechanisms for Regional Dispersal and Gene Flow

Most vernal pool plants and arthropods possess drought-resistant propagules. Wind is often mentioned as a source of propagule dispersal, but it is unlikely to be significant beyond a spatial scale of tens of meters (Brendonck and Riddoch, 1999). The encysted embryos of *Branchinecta* fairy shrimp are also amenable to transport by birds (Proctor et al., 1967; Figuerola and Green, 2002; Green et al., 2005). However, most coastal vernal pools are too small and ephemeral to attract a significant number of birds, and desert playas inhabited by *B. lindahli* do not share a significant avian flyway with coastal pools to the west. Recreational activities and vehicular disturbance are the most prominent correlates of *B. lindahli* presence in coastal pools. If the

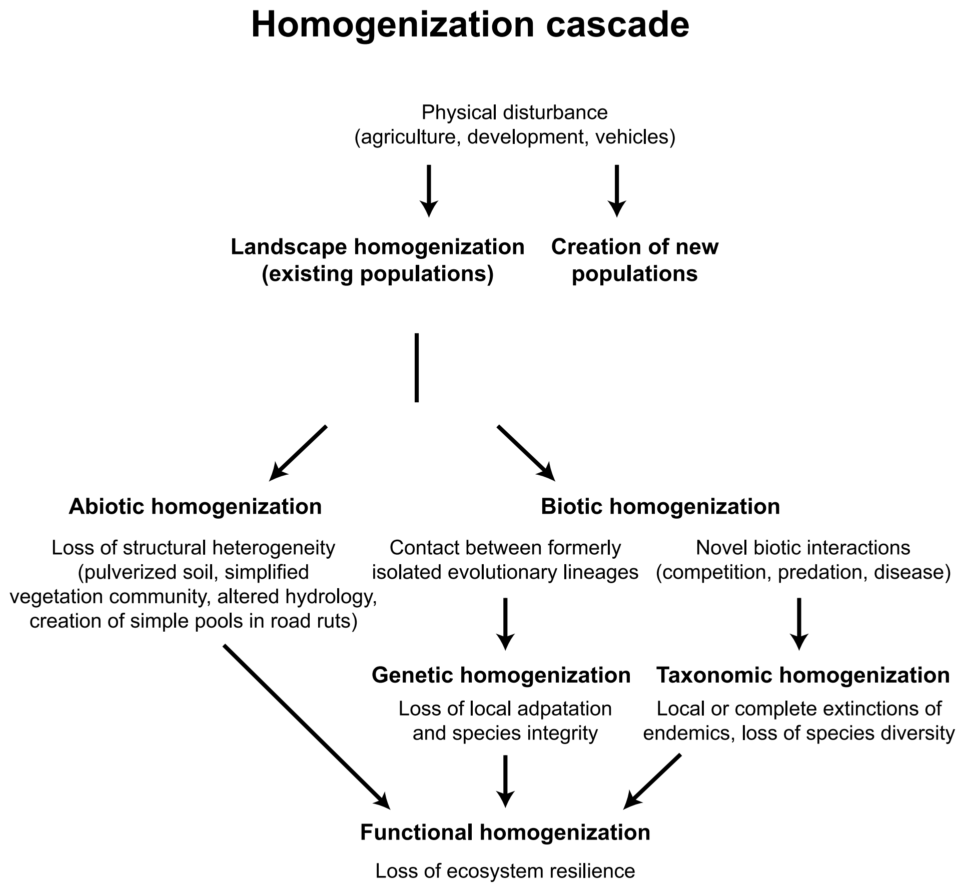

Fig. 5. Conceptual model showing the impacts of a "homogenization cascade" on habitat, community, species-level and genetic characteristics in disturbed landscapes.

source of coastal *B. lindahli* colonization is inland desert playas, it is possible that vehicles are the major vector across these large distances (Waterkeyn et al., 2010).

Across the broad spatial scale that includes both coastal and inland southern California, we hypothesize that the most significant increases in biotic connectivity have come from private, commercial and governmental off-highway vehicles transporting cysts in adhered mud. The desert playas we sampled are heavily utilized by recreational vehicles, some of which may also use dirt roads in coastal areas. Across San Diego County, utility vehicles (e.g., phone, electrical) frequently use access roads that go directly through pools. It is also possible that soil containing fairy shrimp cysts might also be transported long distances by pets, recreational activities such as foot traffic and off-road biking, and military vehicles moving between installations. Bohonak (2005) found evidence that gene flow is higher among *B. sandiegonensis* complexes that are accessible to recreation activities than those which are in fenced preserves. Belk (1977) similarly noted that range expansions of two fairy shrimp in Arizona were associated with highly disturbed conditions and man-made ponds.

Homogenization

If human activities are facilitating increased hybridization in these fairy shrimp, there are likely to be additional consequences for the entire system in which it is found. Over the past several decades, there has been a growing realization that non-native species (whether from near or far) threaten native biodiversity in a variety of ways (Wilcove et al., 1998; Enserink, 1999; Mooney and Cleland, 2001; Levin, 2002; Perry et al., 2002). The proliferation of invasive species represents only one aspect of a broader trend towards biotic and abiotic homogenization over large geographic scales (Olden et al., 2004; Olden, 2006; Devictor et al., 2008a). For vernal pools, these effects can be summarized conceptually as a "cascade of homogenization" (Fig. 5). First, physical disturbance of vernal pools and/or their surrounding uplands decreases their structural integrity and promotes spatio-temporal abiotic homogenization. Disturbance of coastal pools, particularly by heavy and/or tracked vehicles, can cause severe structural disturbance by changing soil structure and properties, altering hydrology, increasing erosion, creating artificial basins in roads, and crushing vegetation in and around the pools (Iverson et al., 1981; Thurow et al., 1993; Prosser et al., 2000; Grantham et al., 2001; Quist et al., 2003; Bhat et al., 2007; Perkins et al., 2007; Warren et al., 2007). The net effect of frequent vehicle incursions into coastal vernal pool habitats is to convert them into (or create de novo) turbid, less heterogeneous wetlands that are more conducive to generalist species.

Historically, undisturbed coastal vernal pools in southern California supported a diverse ecosystem of endemic algae, plants and animals that include *B. sandiegonensis* (Holland and Jain, 1977; Bauder, 1987; Zedler, 1987; Sawyer and Keeler-Wolf, 1995; Bauder et al., 2009). Following the nomenclature developed by Olden et al. (2004) (see also Olden, 2006), biotic homogenization impacts this community and its species in three ways:

1. Genetic homogenization occurs when formerly isolated evolutionary lineages are brought into contact. Hybridization can occur between the newly sympatric lineages if they are closely related and/or have not evolved strong mechanisms for reproductive isolation. This can constitute a major threat to the more specialized species in the pair via genetic swamping/assimilation (if hybrids are at least somewhat viable), or by local extirpation (if hybrids are inviable or infertile). Perry et al. (2002) has gone so far as to suggest that hybridization in North American freshwater systems constitutes an extinction threat as high as competition and predation from introduced species.
2. Taxonomic homogenization occurs as communities become more similar in species composition. Novel competition and predation scenarios may drive local species extinctions (Devictor et al., 2008a), decreasing richness and increasing similarity. Undisturbed vernal pools in coastal southern California that are at least 15 cm deep have highly varied crustacean communities, with at least 6 indicator species and 10 or more total species. In pools that are highly disturbed, crustacean diversity declines to only 1-2 common species and *B. sandiegonensis* is replaced by *B. lindahli* (Ripley and Simovich, 2008; Bauder et al., 2009). Although the potential for competition between "pure" *B. lindahli* and *B. sandiegonensis* has not been yet been studied experimentally, *B. lindahli* can decrease the fitness of *B. longiantennae* (a rare endemic from elsewhere in California) in laboratory experiments (Jensen, 1999).
3. Finally, functional homogenization would be expected as a consequence of homogenization in one or more biotic (and abiotic) factors. The loss of genotypes and species (Devictor et al., 2008a) often results in a net loss of ecosystem resilience, with varied and often unpredictable changes in community composition (Olden, 2006; Crowl et al., 2008).

In conclusion, the potential for hybridization between the fairy shrimp *B. sandiegonensis* and *B. lindahli* has significant repercussions for conservation of the endangered *B. sandiegonensis*. If this phenomenon is widespread, management efforts will need to move beyond habitat protection to limiting biotic and abiotic homogenization of the southern California vernal pool landscape.

The index presented here is a tool for assessing the degree of hybridization that has already taken place, monitoring further changes, and tracking the effectiveness of alternative habitat management or remediation scenarios. This hybrid index is relatively easy to score, and does not require access to molecular tools. We suggest that taxonomic training required for federal permits be expanded to include female characters. Previous suggestions that female characters be used for identifying fairy shrimp, particularly in mixed species pools (Rogers, 2002a, b), have not yet been translated into policy changes.

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Appendix A. Site locations.

Site	Pond	Longitude (N)	Latitude (W)
29 Palms	Dale Lake	34.1278	115.70648
29 Palms	Emerson Lake	34.43242	116.40333
29 Palms	Means Lake	34.40956	116.50988
29 Palms	Melville Lake	34.46414	116.58422
Borrego Springs	DiGeorgio Rd.		
Carmel Mountain	3366	32.93254	117.21986
Carmel Mountain	5838	32.9291	117.22031
Carmel Mountain	5860	32.9274	117.22023
Carmel Mountain	5892	32.93146	117.21451
Carmel Mountain	5897	32.92707	117.22051
Del Mar Mesa	1	32.94639	117.16202
Del Mar Mesa	2	32.94467	117.1664
Salk	2	32.90548	117.15759
Salk	45	32.90514	117.15595
McAuliffe	M8	32.91177	117.1573
McAuliffe	R2	32.91119	117.1567
Pendleton	408	33.28394	117.43754
Pendleton	1562	33.27849	117.43676
Pendleton	2851	33.31073	117.35693