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A Successful Reintroduction of Columbia Spotted Frog (*Rana luteiventris*) through Repatriation of Recently Hatched Larvae

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The Columbia Spotted Frog (*Rana luteiventris*; CSF) is widely distributed across northwestern North America; however, declines in the southernmost populations, including those in Utah, have resulted in the consideration of this species for protection under the Endangered Species Act. In 1998, a conservation agreement and strategy for Utah's populations of CSF was developed and identified needed conservation actions, including range expansion. We repatriated CSF larvae during 2008–2010 from an extant population in the Provo River into Beaver Creek, a beaver-dominated stream, in the Weber River, a watershed where contemporary surveys indicate CSF have likely been extirpated. In 2011, CSF breeding was first observed in the repatriated population when 11 egg masses were documented in four depositional areas. After ten years of monitoring egg mass numbers, we consider the repatriation a success as this population continues to grow and expand with a high of 54 egg masses (2019) within ten depositional areas (2020). High quality habitat and the large number of CSF larvae transplanted likely attributed to the success of this repatriation. We used 1–2 day old CSF larvae (Gosner life stage 20–21) as the repatriation life stage that we believed would be the most likely to prevent the spread of amphibian chytrid fungus (*Batrachochytrium dendrobatidis, Bd*) from the known chytrid fungus positive donor site in the Provo River. Genetic testing of a robust sample ($n = 59$) of CSF tadpoles across the repatriation site in 2020 did not detect the presence of *Bd*, potentially indicating that we did not move *Bd* through the repatriation. Additional replicates would be required beyond this single experiment, however, to better determine if our transplant techniques are effective at preventing the spread of *Bd*.

THE Columbia Spotted Frog (*Rana luteiventris*; CSF) is widely distributed across northwestern North America. The United States Fish and Wildlife Service (USFWS) considers CSF secure in the northern portion of its range (USFWS, 2011). However, the species was petitioned for listing under the United States Endangered Species Act (ESA) in 1993 because of threats to small, isolated populations in the southern portion of its range across Utah and portions of the Great Basin (USFWS, 1993). These threats include habitat degradation and the introduction of nonnative species (Reaser, 2000; Wente et al., 2005) and more recently, disease, particularly the amphibian fungus *Batrachochytrium dendrobatidis* (*Bd*), which has been attributed to declines in anuran populations in parts of the Rocky Mountains (Muths et al., 2003; Scherer et al., 2005) with potential undocumented impacts to CSF populations (Pilliod and Scherer, 2015). *Bd* was first detected in Utah during 2001 in CSF populations in the Provo River (unpubl.). However, population declines in Utah cannot be specifically tied to *Bd* and are more likely a result from a combination of the threats identified above.

A formal conservation agreement and strategy for Utah populations of CSF was developed in 1998 (revised in 2006) to bring together partners to collectively implement conservation actions to benefit CSF populations (Perkins and Lentsch, 1998; Bailey et al., 2006). Conservation actions in the strategy included: 1) surveying historical habitat to determine CSF contemporary distribution, 2) long-term population monitoring, 3) identifying threats to CSF populations, including prevalence of *Bd*, and 4) repatriating CSF into historical habitat to increase their distribution (Bailey et al., 2006). The development of the Utah conservation agreement and strategy provided the USFWS assurances that conservation actions would continue and Utah's Wasatch Front CSF populations were removed from consideration as a candidate species under ESA in 2002 (USFWS, 2002; Bailey et al., 2006).

Distributional surveys for CSF in the early 2000s rediscovered extant populations in the Provo River upstream from Woodland, Utah where CSF had not been observed since 1960 (Thompson et al., 2003). While populations were known and being rediscovered in the Provo River watershed, their distribution in the Weber River, the watershed directly north of the Provo River, was largely unknown. A few survey records verify that CSF historically occupied the upper reaches of the Weber River watershed (Fig. 1). However, the species had not been observed in this watershed for roughly 50 years prior to this repatriation project. The objectives for this study were to: 1) repatriate CSF into the Weber River watershed to establish a self-sustaining population, and 2) assess our efforts to mitigate the spread of *Bd* from the source CSF population.

MATERIALS AND METHODS

Study area.—The Taylors Fork CSF repatriation site occurs at an elevation of 2,257 m in Beaver Creek, a low gradient (2–3% slope) tributary to the Weber River in north-central Utah (Fig. 1). Beaver Creek originates in the Uinta-Wasatch-Cache United States National Forest (USFS) and flows downstream approximately 9 km before leaving USFS land. North American Beaver (*Castor canadensis*; beaver) are common in this reach and in the Taylors Fork repatriation site. This portion of the stream does not experience high spring flow events due to extensive beaver ponds across the valley bottom. Beaver Creek parallels the road Utah SR150, which is a popular recreational highway in the Uinta Mountains. We

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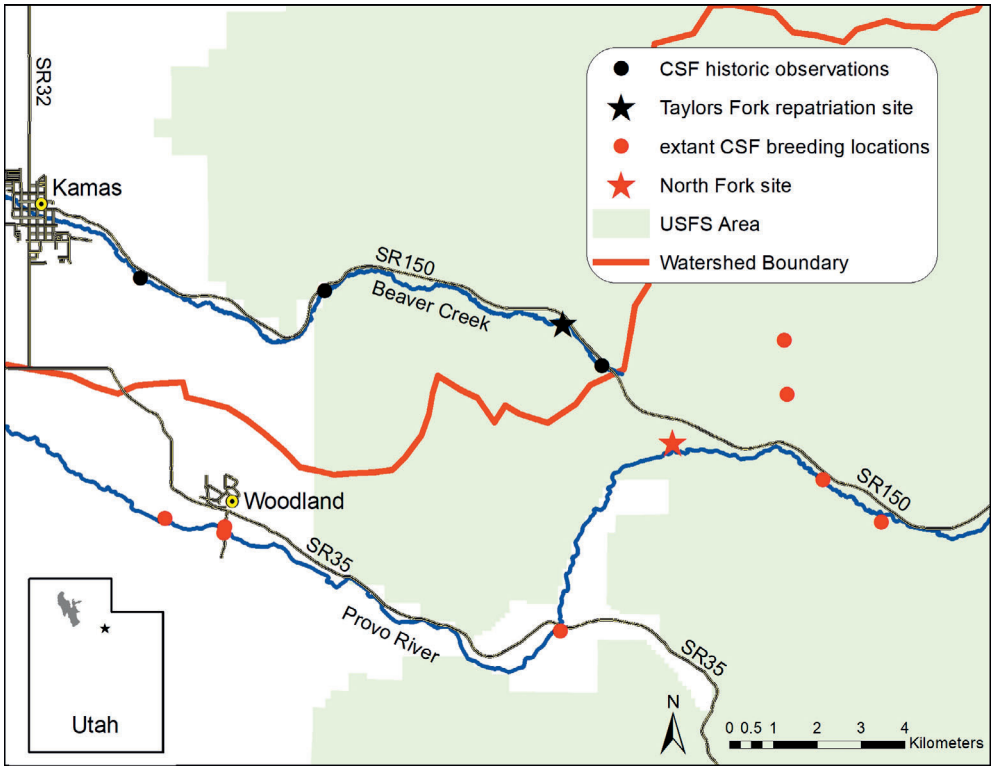

Fig. 1. Location of Taylors Fork CSF repatriation site and North Fork (CSF donor site) in the United States Uinta-Wasatch-Cache National Forest, Utah, including the location of historic CSF locations in the Weber River watershed.

repatriated CSF from the North Fork breeding site in the Provo River watershed (Fig. 1) to Taylors Fork. The North Fork site also hosts many beaver ponds within the floodplain of the Provo River at a high elevation of 2,273 m. While these two locations occur in different watersheds, they are located less than 4 km from each other (Fig. 1).

Bd *testing*.—Upon discovery of *Bd* in Utah, the Utah Division of Wildlife Resources (UDWR) contracted Brigham Young University to collect samples for *Bd* testing across Utah's amphibian populations. Between 2004–2005, samples were collected from 17 adult/juvenile CSF and Boreal Chorus Frog (*Pseudacris maculata*) at the North Fork CSF breeding site (Araos et al., 2017). These samples were sent to and analyzed at Pisces Molecular in Boulder, Colorado (1600 Range St., Boulder, CO 80301). To prevent the unintentional spread of any aquatic invasive species, including *Bd*, during repatriation efforts, a Hazard Analysis Critical Control Points plan (USFDA, 2007) was developed and identified recently hatched larvae as the best repatriation life stage to prevent further spread of chytrid fungus. The rinsing of CSF eggs to remove any aquatic invasive species as they were removed from the North Fork population and the rinsing of recently hatched larvae to remove any remaining gelatinous material were the primary control points in the plan (see repatriation methods below).

In May 2020, Taylors Fork was sampled for the presence of *Bd*. We followed guidance from Pisces Molecular (Pisces Molecular, pers. comm.). Specifically, we aimed to achieve a sample size between 35–72 individual tadpoles to target a 95% confidence in the results of the *Bd* tests. On 30 May, we collected 59 tadpoles; each individual tadpole was placed in a vial and frozen the same day of collection. The tadpoles were sent to Pisces Molecular on 1 June via overnight shipment. We are aware that non-lethal swabbing methods have shown some success for detecting *Bd* in tadpoles (Retallick et al., 2006; Hyatt et al., 2007). However, lethal sampling of tadpoles is still known to achieve higher detection rates compared to swabbing, and we determined it to be critical to maximize *Bd* detection to address the objectives of our study.

Pre-repatriation CSF surveys.—Columbia Spotted Frog distributional surveys in the upper Weber River watershed during the 2000s included the USFS reach of Beaver Creek in 2003 and 2004 (UDWR, unpubl.). The surveys were completed from the USFS boundary to the headwaters of Beaver Creek from April to early May. These two months are the peak CSF breeding period in similar elevation CSF breeding sites in the Provo River, a few kilometers away. Visual encounter surveys (Crump and Scott, 1994) were completed with a three-person crew and surveys proceeded upstream "sweeping" the entire stream corridor in a grid-like fashion. Any standing or slow-moving water within the stream corridor was surveyed for amphibian presence/absence with the expectation that CSF egg masses would be observed if they were present. Universal Transverse Mercator (UTM) coordinates were recorded for the start and finish of each survey day, and subsequent surveys were initiated at the previous ending UTM. Surveys proceeded upstream until the headwaters of Beaver Creek were reached and potential CSF habitat was no longer present.

CSF repatriation.—Prior to the repatriation, the Utah Columbia Spotted Frog Conservation Team discussed and agreed upon methods and locations for the repatriation. Utah regulatory steps for sensitive wildlife species transplants were followed (see Utah Code 23-4-21). Egg masses from the North Fork CSF breeding site in the Provo River were collected during late April 2008–2010. During each year, 15 distinct CSF egg masses were divided approximately in half at the North Fork site, rinsed with dechlorinated tap water, and

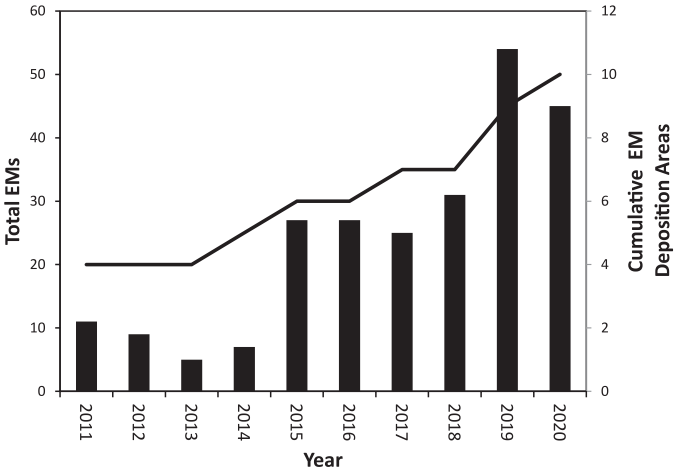

Fig. 2. Total CSF egg mass counts and cumulative depositional areas at Taylors Fork.

placed evenly into three, 5-gallon buckets that contained dechlorinated tap water. In the attempt to maximize genetic diversity, egg masses were collected across the entire North Fork breeding site from all known depositional areas that had been identified in the six years of monitoring this location. The CSF eggs were transported back to a UDWR heated warehouse, where the air stones connected to aquarium air pumps were placed into each bucket. The egg masses were visually inspected three times per day, and water temperatures in the buckets varied between 16–21°C during the two weeks the eggs developed. Within 1–2 days of hatching, all remaining gelatinous material surrounding the larvae was gently rinsed off with a curved nozzle squeeze bottle, and the tadpoles were placed in a single 5-gallon bucket filled with dechlorinated tap water. The larvae were transported to the Taylors Fork repatriation site where the water temperature in the bucket was tempered to the water temperature in the beaver pond stocking location. To acclimate the larvae, approximately 1/5 of the water from the bucket was exchanged with water from the beaver pond for five minute periods until the water temperature in the bucket was within 2°C of the stocking location. The larvae were stocked in early to mid-May in the same location each year, the north facing shoreline of an off-channel beaver pond where there was no discernible stream current.

Egg mass counts were used to evaluate CSF establishment and expansion at the Taylors Fork repatriation site. Visual encounter surveys (Crump and Scott, 1994) were completed during April and May 2011–2020 to locate and count individual CSF egg masses. Three to four weekly surveys were completed per year with each survey expanding upstream and downstream from the stocking location. Surveys swept the entire stream corridor in a grid-like fashion to examine any standing or slow-moving water within the stream corridor. Surveys continued for an additional 400 m of linear stream habitat beyond where the last CSF eggs had been observed. During each survey, the number of CSF egg masses deposited and the UTM coordinates of the deposition site was recorded. Depositional areas were used to document CSF expansion within Taylors Fork and consisted of discrete breeding habitats (e.g., individual beaver ponds) that contained one or more egg mass depositional sites.

RESULTS

Chytrid fungus.—Six of the 17 genetic samples collected from the North Fork breeding site during 2004–2005 tested positive for *Bd*. All 59 of the 2020 Taylors Fork tadpoles were negative for *Bd*.

Pre-repatriation CSF surveys.—No CSF or any amphibians were documented in the USFS reach of Beaver Creek during the 2003 and 2004 surveys. Upon completion of the surveys, this reach was considered unoccupied by CSF and the likelihood of CSF populations further downstream was considered remote since the reaches below the USFS are a cabin community where beaver are controlled to prevent flooding.

CSF repatriation.—CSF egg survival and hatch rate from incubation in five-gallon buckets was considered high and estimated to be >90% resulting in approximately 3,000 CSF larvae being stocked into Taylors Fork in each of the three years, 2008–2010. Taylors Fork was monitored starting in 2011 when 11 CSF egg masses were documented in four depositional areas (stocking site and three additional depositional areas). Yearly monitoring through 2020 demonstrated a general increase in total CSF egg masses deposited per year with a high of 54 egg masses in 2019 (Fig. 2). The repatriated population expanded each year with ten different depositional areas used by 2020 (Figs. 2, 3). The depositional areas ranged from 483 m downstream and 119 m upstream, straight line distance, from the stocking location (Fig. 3).

DISCUSSION

The CSF Taylors Fork repatriation successfully re-established a population in Beaver Creek, and currently is the only known population in Utah's Weber River watershed. Columbia Spotted Frog began breeding in Taylors Fork three years following the first larvae introduction, and total egg mass counts demonstrated an increasing trend for the ten years that this population has been monitored (Fig. 2). The number of egg masses tend to be representative of the number of breeding females in a pond breeding anuran population (Crouch and Paton, 2000; Richter et al., 2003; Stevens and Paszkowski, 2004); however, we recognize that the correlation between abundance of female CSF and egg masses is not completely understood (Hossack et al., 2013). Since females of many anuran species are reclusive and difficult to capture during the breeding season (Muths et al., 2010), egg mass counts were the most feasible method to track population establishment and expansion for our study and these methods have been used by others to monitor CSF (Hossack et al., 2013).

Moving species to supplement small populations or to repatriate extirpated populations—otherwise known as conservation translocation—is a commonly applied management action (Swaisgood and Ruiz-Miranda, 2018). Amphibians are considered a highly endangered taxonomic group (Stuart et al., 2004; Beebee and Griffiths, 2005), and translocations can play an important role in conservation and population restoration (Zeisset and Beebee, 2013). We considered our translocation effort to also be a repatriation, due to a 1964 historical record for Beaver Creek (UMNH, 2019; note that UMNH is the standard abbreviation for the Natural History Museum of Utah) and an unpublished survey

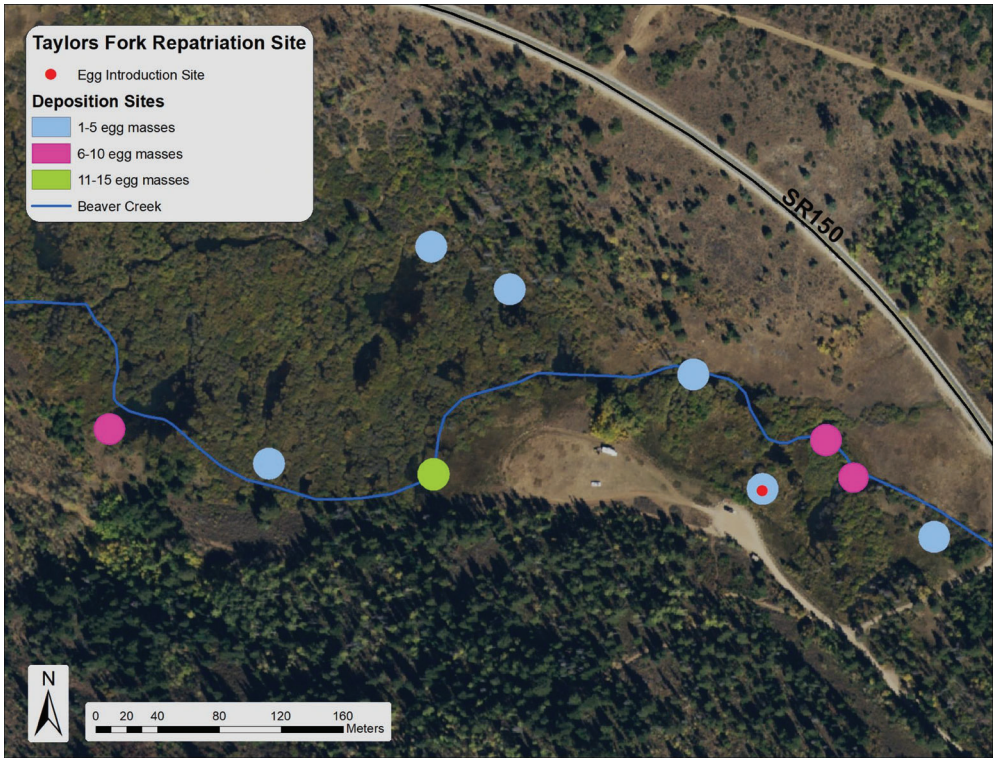

Fig. 3. A satellite image and the approximate locations of CSF egg mass deposition locations at the Taylors Fork repatriation site.

report from the Utah Natural Heritage Program. Amphibian translocation efforts have had variable success, but many have proven unsuccessful (Dodd and Seigel, 1991). We suggest that the stocking of large numbers of CSF larvae across multiple years from a nearby location with similar habitat likely were factors that contributed to the success of this repatriation. Germano and Bishop (2009) reviewed published amphibian translocations between 1991 and 2006 and found that in 25 amphibian translocation studies, success was independent of the life stage released; however, successful translocations were significantly related to the number of animals released with >1,000 animals being the most successful. We stocked an estimated 3,000 CSF larvae into Taylors Fork in each of three consecutive years thereby not relying on a single CSF year class to start the population.

We do not definitively know why CSF disappeared from Beaver Creek, but past beaver suppression efforts in the stream and herbicide drift from vegetation control efforts along the road Utah SR150 which parallels Beaver Creek may have contributed to the localized extirpation. The USFS and the Utah Department of Transportation have changed their management philosophies over the last three decades, and beavers are once again abundant in Beaver Creek and Best Management Practices (BMPs) are in place for vegetation management along Utah SR150. Some of the BMPs are the prevention of herbicide drift and using herbicides approved for riparian areas (USFS, unpubl.). Hossack et al. (2013) found that CSF populations in stable habitats (e.g., buffered by drought) tended to grow faster and were larger than populations occupying smaller habitats likely because smaller habitats are more susceptible to environmental stochasticity (Lande et al., 2003). Persistent drought can reduce the amount of habitat and cause decline or extirpation (Corn and Fogleman, 1984) potentially leading to lost or inconsistent breeding opportunities and reduced survival of larvae, especially for species with slower development (Leips et al., 2000; Daszak et al., 2005; Church et al., 2007). The Taylors Fork repatriation site consists of extensive beaver habitat in a perennial stream, which provided stable breeding habitat. The number of CSF depositional areas also has been increasing since the repatriation (Figs. 2, 3), indicating that this population continues to expand into suitable habitat. Qualitatively, the spatially larger depositional habitats (beaver ponds) appear to have a higher level of egg mass deposition; however, we did not quantify the amount of available CSF breeding habitat in each depositional area. We expect this population to continue expanding since <10% of the available habitat in Beaver Creek is currently used.

Bd has become widespread and has contributed to global amphibian declines (Rollins-Smith et al., 2002; Skerratt et al., 2007). *Bd* infects the keratinized epidermis of metamorphosed and adult amphibians as well as the tooth rows and jaw sheaths of larvae (Berger et al., 1998; Longcore et al., 1999; Vredenburg and Summers, 2001). While *Bd* is present across the CSF range, widespread population declines have not been observed (McCaffery and Maxell, 2010; Hossack et al., 2013) potentially because CSF are known to have skin peptides that are highly resistant to chytrid fungus (Rollins-Smith et al., 2002). Even if CSF populations demonstrate some resistance to chytrid fungus, other amphibians are susceptible. We attempted to prevent its spread from the North Fork donor site, which was known to be chytrid fungus positive (Araos et al., 2017).

Our attempts to mitigate the spread of *Bd* focused on precautions to not move any water or biological material from the North Fork site to Taylors Fork. Additionally, we stocked newly hatched larvae that had not developed mouth parts, primarily stage 20–21 tadpoles as defined by Gosner (1960). When CSF eggs were collected, they were rinsed with and placed in dechlorinated tap water where they were

allowed to hatch. Prior to the stocking of larvae, they again were rinsed with dechlorinated tap water to remove any remaining gelatinous egg membrane. The Taylors Fork repatriation site was not tested for chytrid fungus at the time of stocking the CSF larvae because there were no known amphibian populations to sample. Instead, a lethal tadpole sample ($n = 59$) was collected in 2020 from all known depositional areas; chytrid fungus was not detected in any sample (Pisces Molecular, pers. comm.). Because we followed the recommendations of Retallick et al. (2006) and collected entire tadpoles in place of swabbed mouthparts and we collected a robust sample (see Brem et al., 2007), we have high confidence that we did not detect chytrid fungus from our 2020 Taylors Fork population. We do note that there is conclusive evidence that the detectability of chytrid fungus in amphibian populations can be temporally and spatially variable (Kinney et al., 2011; Savage et al., 2011). We admit that it would be beneficial to have more chytrid test results from other seasons, and multiple age classes of CSF in Taylors Fork. However, outside of the breeding season it is prohibitively difficult to find a sample size of CSF that would offer test results that could be interpreted with any meaningful confidence. Additionally, if we failed to detect chytrid that is present in Taylors Fork, we would suggest that ten years of positive population growth indicates that chytrid is at a very low occurrence and has a negligible effect on the Taylors Fork population. Araos et al. (2017) also provided evidence that CSF populations are able to persist in several Utah locations despite being positive for chytrid infections. Admittedly, we cannot confirm that our repatriation methods were successful in preventing the movement of chytrid fungus with a single repatriation; therefore, we recommend further evaluation with a more robust sampling design to confirm or refute whether the stocking of newly hatched larvae is truly effective at preventing the spread of chytrid fungus.

Climate change will continue to increase temperatures and variability in precipitation, both of which are contributing to more extreme drought conditions in the western United States (Diffenbaugh et al., 2015; Martin et al., 2020). Extended drought can be detrimental to CSF populations occurring at lower elevations and their habitats (Hossack et al., 2013; Pilliod et al., 2021), and as these populations are lost, the need to repatriate CSF to different or more stable habitats will increase. Our repatriation effort demonstrates that CSF can quickly establish a self-sustaining population when introduced into suitable and similar habitat to that of the donor population, and the stocking of larger numbers of individuals across multiple years likely contributed to the success of the repatriation. We were successful in preventing the spread of chytrid fungus during the repatriation; however, we were not able to definitively identify the preventive mechanism(s).

DATA ACCESSIBILITY

The data from this study are available upon request. Unless an alternative copyright or statement noting that a figure is reprinted from a previous source is noted in a figure caption, the published images and illustrations in this article are licensed by the American Society of Ichthyologists and Herpetologists for use if the use includes a citation to the original source (American Society of Ichthyologists and Herpetologists, the DOI of the *Ichthyology & Herpetology* article, and any individual image credits listed in the figure caption) in accordance with the Creative Commons Attribution CC BY License.

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