Plan for the Reintroduction of Ash Meadows Naucorid (*Ambrysus amargosus*) into Springs within the Point-of-Rocks Spring Complex

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I. Background and Rationale

Due to extensive modification of thermal springs and spring outflows throughout Ash Meadows, many species of aquatic organisms have suffered extreme population reductions and have been extirpated from portions of their former ranges. Conspicuous among these are two endemic naucorid species, *Ambrysus amargosus* (Ash Meadows naucorid) and *A. relictus* (Warm Springs naucorid). Due to its extremely restricted range, loss of habitat, and potential impacts from non-native species, *A. amargosus* was designated threatened, and received protection under the federal Endangered Species Act (U. S. Fish and Wildlife Service 1985). Important components of the recovery plan for Ash Meadows aquatic species include redirecting spring flows into their natural channels and reestablishing native aquatic communities, including reintroducing species into habitats within their historic range (Sada 1990; sect. 23). Currently, distribution of *A. amargosus* is restricted to short, upstream reaches of two spring systems within the Point of Rocks springs complex, a total area of approximately 24 m^2. These include the Refuge spring (so named because it is the source of water for the Devils Hole pupfish refuge) and Middle spring complex, a group of three very short channels west of the Refuge spring. Historically, all of the Point of Rocks springs with sufficient flow were likely inhabited by naucorids, including Grindstone spring (so named because it flows through a limestone outcrop with numerous native American matates) and the outflow of Kings Pool, the type locality for the species (La Rivers 1953).

Although observed in Grindstone spring periodically prior to 1997, no naucorids were collected from this system during a year of extensive monthly sampling between 1997 and 1998 (Parker et al. 2000). Multiple surveys since then have also been unsuccessful in confirming the presence of naucorids in this system. Causes for this apparent local extinction are unclear, but are most likely related to chronically small population size and poor habitat quality caused by extensive vegetation encroachment, and resulting low current velocities, deposition of fine sediments and reduced prey populations.

Naucorids were extirpated from the Kings Pool system sometime in the 1960s or 70s when the entire spring discharge was diverted into a concrete channel for distribution to irrigated crops. The Kings Pool outflow channel was reconstructed in 1996 (Scoppettone et al. 2005), creating extensive areas of suitable naucorid habitat. Naucorids were re-introduced into the system in 1997-98 and the population grew dramatically, reaching densities of 128-230/m^2 within the upper 200 m of the channel during summer 1998. Periodic surveys revealed that naucorids persisted within the upper 100 m of the system until early 2002, but after summer 2002 no naucorids have been observed and they are, once again, considered extirpated from the Kings Pool system. Causes of this apparent extinction are not known, but are probably related to relatively rapid, large scale habitat shifts that, in turn, caused large fluctuations and overall decline in the naucorid population. For example, upstream encroachment by cattails, and resultant large scale changes in flow and substrate composition, allowed the population of non-native crayfish to expand into upper reaches of the channel, both reducing naucorid habitat and increasing predation by an exotic predator. In addition, succession via rapid growth of riparian and upland vegetation has resulted in extensive canopy closure and reduced sunlight along much of the upper channel which has reduced primary production within the system. There has also been an increase in diversity and abundance of aquatic invertebrate predators (primarily odonates), which coupled with lower overall productivity, likely increased competition and predation on early naucorid instars.

Although cattails are still present in the upper Kings outflow channel, their abundance is drastically reduced due to manual removal and shading by a dense Mesquite canopy. As a result, crayfish are no longer abundant within the upper 100 m of the channel. In general, habitat conditions have become more stable and although primary production is much reduced and invertebrate predator populations are larger, there remain extensive areas with relatively fast current velocities (i.e., > 30cm/s) and diverse substrate particle size distributions favored by naucorids (Parker et al. 2000, Whiteman and Sites 2007). In Grindstone Spring, habitat restoration in 2006 involving vegetation removal and addition of gravel-pebble substrate particles throughout the lower 30 m of the channel has created nearly 15 m^2 of suitable naucorid habitat. Although many of the spring channels within the Point of Rocks system are connected, habitats at and below their confluences are inhospitable to naucorids, functionally isolating populations within short headwater reaches and preventing natural recolonization. Reintroduction into these restored habitats is, therefore, the only means by which to expand the range of this species, thereby reducing its risk of extinction.

II. Purpose

The purpose of this document is to present a detailed plan and guidelines for reestablishing Ash Meadows naucorid populations within Grindstone spring and Kings Pool outflow. Specifically, this plan addresses (1) timing and frequency of naucorid introductions, (2) sources, numbers and life stages of organisms to be transferred, (3) habitat conditions in both source and recipient systems prior to, during, and following introductions, and (4) population monitoring to determine success of introductions.

Initial naucorid transfers will follow a two-step process in which Grindstone spring will be stocked in 2009 and Kings Pool outflow in 2010. Depending on growth of the Grindstone Spring population, it could provide an additional source of individuals to be stocked into the much larger Kings Pool system.

III. Procedures

<u>Phase I – Grindstone Spring, 2009</u>

1. In late February-early March, overhanging vegetation should be cleared along channels in the Refuge and Middle spring systems to promote primary production and increased prey abundance, thereby increasing naucorid reproductive output. Overhanging vegetation should also be cleared along the lower Grindstone channel (the 30 m below the limestone outcropping) in preparation for naucorid introduction.

2. Also in late February-early March diversion of water from the head of the Refuge Spring to the Devils Hole pupfish refuge should be terminated and the entire spring discharge allowed to flow down the natural channel. Prior to terminating the diversion the entire refuge tank and ouflow should be thoroughly searched for naucorids, including using a long handled dip net to sweep along the tank sides and sediment surface. Any naucorids captured should be released into lower Grindstone spring following procedures outlined in #4, below.

Three transects should be located along the main Refuge spring channel and marked with stakes so they can be resurveyed after the diverted flow is returned to the channel. At each transect, wetted channel width should be measured (to nearest 0.5 cm) and depths should be measured (to the nearest 0.1 cm) at 3-5 equally spaced points along each transect. These measurements should be repeated several hours after flow has been returned to the channel and again during the naucorid population surveys in May and July (see below). In addition, the downstream extent of suitable naucorid habitat will likely increase with additional flow and habitat maintenance (vegetation clearing and substrate addition) should be extended as far downstream as possible to maximize population growth prior to collecting animals for reintroduction.

3. In mid to late-May naucorid populations should be surveyed and population sizes estimated in Refuge and Middle Spring systems using procedures described in the draft Naucorid Management Plan (Parker and Goodchild 2009). These estimates will provide guidance for how many naucorids can be removed for reintroduction into Grindstone Spring. If the population estimate exceeds 500 late instar and adult naucorids, then up to 25 individuals (< 5% of the population) can be removed for reintroduction. If an estimate is fewer than 500 animals, no more than 5% of the estimated population should be removed. If the estimate is below 100, no animals should be removed, and measures should be taken to improve habitat conditions to promote population growth. The population estimate for the Middle Spring complex should be the sum of all three channels and the number of naucorids removed from each should be proportional to their contributions to the total population estimate.

4. The following procedures should allow for collection and transfer of naucorids while minimizing risks to the animals and disturbance to source or recipient habitats.

   a. All late instar and adult naucorids (>5.0 mm TL) collected during surveys to estimate population sizes should be retained in a plastic bucket containing approximately 2 liters of spring water. A large piece of nylon window screening should be placed into the bucket to provide substrate on which naucorids can cling. This material should be loosely wadded up to provide a large amount of substrate surface area which will reduce encounters among naucorids while they are being held. Separate buckets should be used for the different source springs.

   b. If additional naucorids need to be collected, they should be collected with an aquarium net in the same manner as during population sampling and transferred to the holding bucket. Additional sampling should begin at the lowest downstream site and proceed in an upstream direction, taking care not to resample sites disturbed during population sampling.

   c. Monitor water temperature within the holding bucket to insure that it remains within ± 2 °C of the temperature of the spring from which the naucorids are being collected. Add additional water to the bucket as needed to maintain the temperature within this range.

   d. When a sufficient number of naucorids has been collected (e.g. 25 if the population estimate >500), they should be transferred to a bucket containing approximately 2 liters of water from the spring into which they will be introduced. Water from the recipient spring

should be collected very shortly before the transfer to minimize temperature change. Naucorids can be transferred by moving the screen to which they are clinging into the new bucket. Inspect the screen carefully and remove any invertebrates other than naucorids to prevent unintended transfers among springs. An attempt should also be made to minimize the amount of water transferred between buckets (and thus springs) when transferring the naucorids.

e. Naucorids should be transported to the recipient spring as soon after collection, and should be released as far upstream in the system, as possible. In Grindstone Spring the release site should be below the trail crossing near the limestone outcrop, at the first place where the channel is relatively open and free of extensive overhanging vegetation. Naucorids can be released into the system without having to be handled by placing the window screening to which they are clinging directly into the spring flow.

f. Record the number of each life stage transferred from each source population and the date and time of each transfer. Again, only late instar nymphs and adults (> 5 mm TL) should be used in these introductions. Early instar nymphs should be released unharmed into the spring from which they were collected.

5. Also in mid to late-May, 10 artificial substrate baskets, as described in Parker et al. (2000), should be deployed in the Kings Pool outflow; 5 each approximately 25 m and 50 m downstream from where the channel exits the spring pool. These samplers should be collected, processed, and redeployed at monthly intervals through August. Results obtained from this sampling will be compared with those reported in Parker et al. (2000) and should reveal changes in benthic community structure that have occurred in response to successional changes along the reconstructed channel. Of particular interest will be changes in density of *Hyallela*, the principle prey of *A. amargosus*, and abundances and diversity of invertebrate predators.

6. Additional transfers from the Refuge and Middle spring systems into Grindstone Spring should be made in July in conjunction with the annual population censuses. Prior to these transfers population sizes should be estimated to determine the maximum number of individuals that can be transferred (see 3 above) . In addition, the Grindstone Spring population size should be estimated, with particular focus on the abundance of early intar nymphs that will indicate whether successful reproduction has taken place.

7. In late August-early September, the newly established Grindstone Spring population should be surveyed to estimate both population size and relative abundances of different instars. Subsequent annual censuses, as described in the draft Naucorid Management Plan, should be sufficient to track the long term success of this reintroduction.

Phase II – Kings Pool Outflow, 2010

1. In late February-early March, overhanging vegetation should be cleared along channels in the Refuge, Middle and Grindstone spring systems to promote primary production and increased naucorid reproduction.

2. In mid to late-May naucorid populations should be surveyed and population sizes estimated for Refuge, Middle and Grindstone springs as described in 3 above.

3. Collection and transfer of naucorids from Refuge, Middle and Grindstone springs into the Kings Pool outflow should follow procedures described in #4 above. The release site in the Kings Pool system should be along the margin of the channel within 5 m of the springpool outflow. Releasing them near the stream margin where flow velocity is reduced will slow downstream transport.

4. Additional transfers of naucorids from the other three Point of Rocks springs into the Kings system should be carried out in July.

5. Artificial substrate baskets should be deployed in May and sampled monthly through August 2010, as described in 5 above. This sampling should allow introduced naucorids and associated benthic invertebrates to be monitored through the summer, and should eliminate the need to do additional population sampling. Subsequent annual censuses should be sufficient to track the long term success of this reintroduction.

Table 1. Timeline for reintroduction of Ash Meadows naucorids into Grindstone Spring and Kings Pool outflow, 2009-10.

2009	
February – March	Channel maintenance at Refuge, Middle and Grindstone springs. Return flows from pupfish refuge tank to Refuge Spring channel. - monitor habitat changes - survey for naucorids in refuge tank and outflow. Release any naucorids found into lower Grindstone Spring.
May	Estimate naucorid population sizes in Refuge and Middle springs. Transfer naucorids from Refuge and Middle springs to lower Grindstone Spring. Deploy artificial substrate baskets to monitor benthic invertebrates in Kings Pool outflow.
June	Collect invertebrates from artificial substrate baskets in Kings Pool outflow, and redeploy.
July	Conduct annual survey of naucorid, and associated macroinvertebrate populations from all springs. Transfer additional naucorids from Refuge and Middle springs to upper Grindstone spring. Collect invertebrates from artificial substrate baskets in Kings Pool outflow, and redeploy.
August	Survey naucorid population in Grindstone Spring, determine if there has been reproduction by presence of early instars. Collect invertebrates from artificial substrate baskets in Kings Pool outflow
September-December	Analyze samples from Kings outflow substrate baskets.

2010	
February-March	Channel maintenance at Refuge, Middle and Grindstone springs.
May	Estimate naucorid population sizes in Refuge and Middle, and Grindstone springs.
	Transfer naucorids from Refuge, Middle and Grindstone springs to Kings Pool outflow.
	Deploy artificial substrate baskets to monitor benthic invertebrates in Kings Pool outflow.
June	Collect invertebrates from artificial substrate baskets in Kings Pool outflow, and redeploy.
July	Conduct annual survey of naucorid, and associated macroinvertebrate populations from all springs.
	Transfer additional naucorids from Refuge, Middle, and Grindstone springs to Kings Pool outflow.
	Collect invertebrates from artificial substrate baskets in Kings Pool outflow, and redeploy.
August	Collect invertebrates from artificial substrate baskets in Kings Pool outflow, survey naucorid poplation.
September-December	Analyze samples from Kings outflow substrate baskets.

IV. Literature Cited

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