U. S. Fish and Wildlife Service

Range-Wide Conservation Strategy for the Kentucky Arrow Darter *(Etheostoma spilotum* Gilbert*)*

Photo Credit: Dr. Matthew R. Thomas, KY Dept Fish & Wildlife Resources

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For:
Southeast Region
U.S. Fish and Wildlife Service
Atlanta, Georgia
May 2014

DISCLAIMER

This document recommends actions to maximize the conservation of the Kentucky arrow darter and the habitats upon which it depends. It does not obligate any party to undertake specific actions and may not represent the views, official positions, or approval of any individuals or agencies involved in aquatic species conservation, other than the USFWS. This is a working document subject to modification, as dictated by new findings, changes in species status, evolving priorities, and completion of conservation actions.

Recommended literature citation:
U.S. Fish and Wildlife Service. 2014. Range-wide conservation strategy for the Kentucky arrow darter (*Etheostoma spilotum* Gilbert). Kentucky Ecological Services Field Office, Frankfort, Kentucky. 40 pp.

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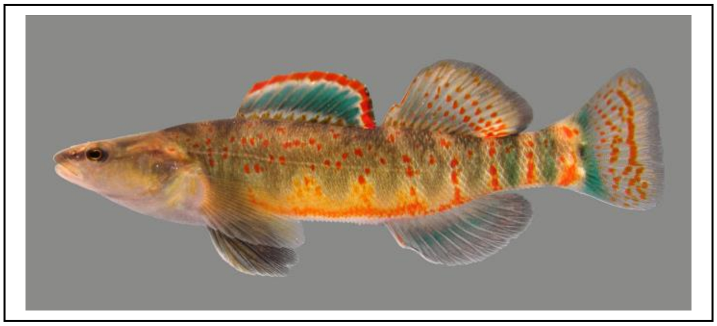

Photo credit: Dr. Matthew R. Thomas, KDFWR

Common Name Kentucky arrow darter
Scientific Name *Etheostoma spilotum* Gilbert 1887

Listing Status and Date Candidate; October 22, 2010

Lead Agency/Region U.S. Fish and Wildlife Service, Region 4

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Purpose of the Conservation Strategy: This document lays out a preliminary course of action for the conservation of the Kentucky arrow darter (KAD). It is meant to serve as guidance to direct conservation efforts and may provide a basis for eliminating the need to list this species. Conservation strategies are intended primarily for internal use by the U.S. Fish and Wildlife Service, but these strategies should incorporate input from appropriate State agencies; public participation is also encouraged. We will consider any new information or comments that the public or conservation partners offer in response to this strategy. This document was prepared by the U.S. Fish and Wildlife Service (Service), in cooperation with and input from the Kentucky Department of Fish and Wildlife Resources, Kentucky State Nature Preserves Commission, U.S. Geological Survey, the Kentucky Division of Water, the U.S. Forest Service (Daniel Boone National Forest), and a Science Advisory Team associated with the potential development of a

Candidate Conservation Agreement with Assurances (CCAA) for the species. For more information on conservation efforts for the KAD, or to provide additional comments, interested parties may contact the lead biologist for this species at the above address, telephone number, or e-mail.

Scope of Strategy and Available Information: The scope of this conservation strategy is a single species – the KAD. A considerable amount of information has been published with regard to the species' biology, life history, distribution, and threats (Bailey 1948, Lotrich 1973, Kuehne and Barbour 1983, Thomas 2008, USFWS 2010, Hitt 2014), but significant data gaps remain. More information is needed in three general areas: (1) genetics (e.g., diversity, viability, effective population size); (2) movement behavior; and (3) stressors (particularly, specific tolerances to a variety of environmental stressors). The lack of information in these specific areas may hinder the development of an effective comprehensive conservation strategy for the species.

OVERVIEW

Species Description and Life History

The KAD is a relatively large darter that reaches a maximum length of about 120 millimeters (mm) (4.7 inches [in]) (Kuehne and Barbour 1983, Etnier and Starnes 1993). It has a slender body, elongated snout, large mouth, and virtually scaleless head (Figure 1). Its background color is straw yellow to pale greenish, but the body is also covered by a variety of stripes and blotches. The back is crossed by 5 to 7 weak dorsal saddles, some of which may fuse with the 8 to 11 vertical lateral blotches (Kuehne and Barbour 1983, Etnier and Starnes 1993). The blotches are generally oval with pale centers at the front of the body but extend downward and may resemble the letters N, W, U, or V toward the back of the body. During spawning, the sides of the males are covered with scattered scarlet spots and scarlet to orange vertical bars, and the dorsal and caudal fins exhibit blue and scarlet bands and spots (Kuehne and Barbour 1983). Females remain pale straw yellow with grayish markings (Etnier and Starnes 1993).

Figure 1. Kentucky arrow darter male (left) and female (right); Photos provided by Dr. Matthew R. Thomas, Kentucky Department of Fish and Wildlife Resources.

Kentucky arrow darters typically inhabit pools or transitional areas between riffles and pools (glides and runs) in moderate- to high-gradient, first to third order streams with rocky substrates (Thomas 2008) (Figure 2). Typically, the species is observed near some type of cover – boulders, rock ledges, large cobble, or woody debris piles. Kentucky arrow darters typically occupy streams with watersheds draining an area of about 25.9 square kilometers (km^2) (10 square miles [mi^2]) or less, and many of these habitats, especially those in first order reaches, can be intermittent in nature. Lotrich (1973) observed riffle habitats in Clemons Fork (Breathitt County) that were completely dry by late summer, but isolated pools in these habitats continued to support KADs.

Figure 2. Typical KAD streams: Clemons Fork (Robinson Forest), Breathitt County (left) and Long Fork (Daniel Boone National Forest [DBNF]), Clay County (right).

Male KADs establish territories over riffles from March to May, where they are quite conspicuous in water 5 to 15 cm (2 to 6 in) deep (Kuehne and Barbour 1983). Males fan out a depression in the substrate and defend these sites vigorously. Initial courtship behavior involves rapid dashes, fin-flaring, nudging, and quivering motions by the male followed by similar quivering responses of the female, who then precedes the male to the nest. The female partially buries herself in the substrate, is mounted by the male, and spawning occurs (Etnier and Starnes 1993, p. 523). It is assumed that the male continues to defend the nest until the eggs have hatched. The spawning period extends from April to June, but peak activity occurs when water temperatures reach 13^{o}C (55^{o}F), typically in mid-April (Bailey 1948, Lowe 1979).

Young KADs can reach 50 mm (2 in) in length by the end of the first year (Lotrich 1973, Lowe 1979), and one-year olds are generally sexually mature and participate in spawning with older age classes (Etnier and Starnes 1993). Lotrich (1973) indicated mean length at age 2 of about 65 mm (2.6 in) and was unable to differentiate between older age classes (age 3+). Lowe (1979) reported four age classes, but growth was variable after age 1.

Kentucky arrow darters feed primarily on mayflies (Lotrich 1973), specifically the families Heptageniidae (genera *Maccafertium* and *Stenonema*) and Baetidae. Mayflies comprised 77 percent of identifiable food items (420 of 542 items) in 57 KAD stomachs from Clemons Fork (Lotrich 1973). Large KADs (individuals over 70 mm [2.8 in] TL) often feed on small crayfish, as 7 of 8 stomachs examined by Lotrich (1973) contained crayfish ranging in size from 11 to 24 mm (0.4 to 0.9 in). Lotrich (1973, p. 381) considered this to be noteworthy since stomachs of small KADs (<70 mm [2.8 in]) and stomachs of other darter species did not contain crayfish. He suggested that larger KADs were utilizing a different energy source, thus removing themselves from direct competition for food with other fishes in first and second order streams. This would allow these larger individuals to exploit an abundant food source and survive in extreme headwater habitats. Other KAD food items reported by Lotrich (1973) and Etnier and Starnes (1993) included larval blackflies (family Simuliidae) and midges (Chironomidae), with lesser amounts of caddisfly larvae, stonefly nymphs, and beetle larvae. Etnier and Starnes (1993) reported that juvenile KADs feed on microcrustaceans and dipteran larvae.

Common associates of the KAD include creek chub (*Semotilus atromaculatus*), central stoneroller (*Campostoma anomalum*), white sucker (*Catastomus commersonii*), emerald darter (*Etheostoma baileyi*), rainbow darter (*E. caeruleum*), fantail darter (*E. flabellare*), and Johnny darter (*E. nigrum*) (Kuehne 1962, Lotrich 1973, Thomas 2008). Within first-order or headwater reaches of these stream systems, the species is most commonly associated with creek chub, fantail darter, rainbow darter, and Johnny darter. Morphological differences between KADs and other darters make misidentifications unlikely. The species can be easily differentiated by its elongated snout, its oval or diamond-shaped blotches on its sides, and its large size (for individuals greater than 100 mm in length).

Land Ownership

Extant KAD populations occur in watersheds with both private and public ownership. Areas under public ownership include numerous watersheds on the Daniel Boone National Forest (DBNF) and two watersheds on Robinson Forest, a 59.8-km^2 (14,786-acre) research forest owned and managed by the University of Kentucky (UK). The DBNF's ownership is typically fragmented but is most concentrated within the South Fork Kentucky River drainage in Clay, Leslie, and Owsley Counties. Other KAD watersheds with DBNF ownership include two streams in the Sturgeon Creek drainage in Jackson and Lee Counties and one stream (Rock Bridge Fork) in the Swift Camp Creek system (Red River drainage) in Wolfe County. Robinson Forest is located within the Buckhorn Creek system (North Fork Kentucky River drainage) in Breathitt and Knott Counties. Almost half (22) of the extant KAD streams have all or significant portions of their watersheds in public ownership.

Taxonomy

The KAD was described from the Kentucky River basin (Sturgeon Creek, Owsley County) as *Etheostoma nianguae spilotum* (Gilbert 1887, pp. 53-54). Bailey (1948, pp. 80-84) redescribed the species, placing it and its closest relatives, *Poecilichthys nianguae* (Gilbert and Meek) and *P. sagitta* (Jordan and Swain), in a new subgenus, *Litocara*. Subsequent to this, Bailey et al. (1954, pp. 109-164) and Bailey and Gosline (1955, pp. 1-44) synonymized (combined taxonomically) *Poecilichthys* with the genus *Etheostoma* and *Litocara* with the subgenus *Oligocephalus*, in which *E. spilotum* and its relatives were regarded as a species group (group of closely related species within a genus that are grouped because they are morphologically similar and share a common ancestry). Kuehne and Bailey (1961, pp. 1-5) evaluated new material for all three members of *Oligocephalus* and determined that the group consisted of two species, *E. nianguae* (Gilbert and Meek) and *E. sagitta* (Jordan and Swain), and three forms. *Etheostoma nianguae* was distinctive morphologically and confined to the lower Osage River system in Missouri. *Etheostoma sagitta* was recognized as a polytypic species (represented by more than one subspecies), consisting of *E. s. sagitta*, an endemic form to the upper Cumberland River system, and *E. sagitta spilotum* (KAD), an endemic form to the upper Kentucky River system. The subgenus *Litocara* was later resurrected (reinstated taxonomically) by Page and Whitt (1973, pp. 611-623) to include *E. nianguae* Gilbert and Meek and *E. sagitta* (Jordan and Swain). Subsequently, the KAD's subspecific status was supported by Kuehne and Barbour (1983, p. 71), Page (1983, p. 59), and Etnier and Starnes (1993, p. 523).

Thomas (2008, p. 6) questioned the polytypic status of *E. sagitta* by arguing that (1) the two subspecies were distinguishable based on scale size and development of the lateral line (see note below), (2) the two subspecies existed in allopatry (separate ranges with no overlap), (3) the two subspecies lacked intergrades (intermediate forms), and (4) unpublished genetic data (mitochondrial DNA) suggested evolutionary independence of Kentucky and Cumberland basin populations (with no recent genetic exchange). Based on these analyses and additional morphological and genetic evidence presented by Thomas and Johansen (2008, p. 46), the two arrow darter subspecies have been elevated to species rank (Page and Burr 2011, p. 569; Eschmeyer 2013). The Cumberland arrow darter, *E. sagitta* (Jordan and Swain) is restricted to the upper Cumberland River basin, and the KAD, *E. spilotum* Gilbert, is restricted to the upper Kentucky River basin.

Cumberland arrow darters and KADs are indistinguishable based on general appearance, including pigment pattern and breeding color; however, the two species are separable based on various scale counts. Thomas (2008, p. 6) examined specimens of both species and determined that the KAD had lateral scale counts of 62 or fewer in 88% of individuals examined (vs. 63 or more in 94% of Cumberland arrow darters), pored lateral scale counts of 50 or fewer in 79% of individuals examined (vs. 51 or more in 91% of Cumberland arrow darters), and caudal peduncle scale counts of 22 or fewer in 72% of individuals examined (vs. 23 or more in 83% of Cumberland arrow darters). These differences reflect a trend toward larger scale size and a more weakly developed lateral line (a faint line of sense organs extending from the gill cover to the tail) in the KAD.

Historical and Current Distribution

The KAD's historical distribution was limited to the upper Kentucky River basin in eastern Kentucky (Kuehne and Bailey 1961, Kuehne 1962, Lotrich 1973, Branson and Batch 1983, Burr and Warren 1986, Ray and Ceas 2003). Its distribution spanned portions of five sub-basins or drainages: Red River (Rockbridge Fork of Swift Camp Creek), Sturgeon Creek, South Fork Kentucky River, Middle Fork Kentucky River, and North Fork Kentucky River (Thomas 2008).

The KAD continues to occupy portions of the upper Kentucky River basin in eastern Kentucky, including the five drainages listed above; however, recent surveys by Thomas (2009) and the U.S. Fish and Wildlife Service (USFWS 2009, 2010) revealed that the KAD has disappeared from portions of its range (Figure 3). The species was observed at only 34 of 68 historical streams (50 percent) and 45 of 100 historical sites (46 percent) during surveys completed from 2007 to 2010 (Thomas 2009, USFWS 2009, USFWS 2010). From 2010 to 2012, additional surveys were initiated by the Service within the KAD's historical range but in streams lacking previous records for the subspecies. A total of 50 new stream reaches were surveyed across the basin, but no KADs were observed during those surveys. From June to September 2013, the Kentucky State Nature Preserves Commission (KSNPC) and Service completed a probabilistic sampling study that included 80 randomly chosen sites within the species' historical range. Kentucky arrow darters were observed at only 7 of 80 sites, including 2 new localities (Granny Dismal Creek in Owsley County and Spring Fork Quicksand Creek in Breathitt County) and one historical stream (Hunting Creek, Breathitt County) where the species was not observed by Thomas (2009). With the addition of these records, we consider the species to be extant in 40 streams (or 40 unique watersheds) within the upper Kentucky River basin.

A synopsis of the KAD's current range is provided below and is arranged by sub-basin. The current number of streams and sites known to support KADs in each sub-basin is listed parenthetically after the drainage name.

Red River (1/1). The species is restricted to one stream in this drainage – Rockbridge Fork, a tributary of Swift Camp Creek in Wolfe County (Greenberg and Steigerwald 1981). Surveys in 2007 (Thomas 2008) and 2011 (Service) confirmed the species' continued presence in this stream. About 60 percent of the Rockbridge Fork watershed is located within the DBNF.

Sturgeon Creek (3/3). The species is extant in three streams – Granny Dismal Creek and Wild Dog Creek in Owsley County and Travis Creek in Jackson County. Portions of the Granny Dismal and Wild Dog Creek watersheds are located within the DBNF, with public ownership ranging from 30 (Granny Dismal Creek) to 50 (Wild Dog Creek) percent. The species was not observed by Thomas (2008) at seven historical sites surveyed in 2007-2008.

South Fork Kentucky River (17/19). Some of the best remaining populations of KADs are found within this drainage. Over 87 percent of occupied streams within this drainage have portions of their watershed in public ownership (DBNF, Redbird Ranger District) in Clay, Leslie, and Owsley Counties. These streams include the Buffalo Creek watershed in Owsley County, Bullskin Creek in Clay County, and several small, first- and second-order tributaries of the Red Bird River in Clay and Leslie Counties (Thomas 2008). These streams are characterized by relatively intact riparian zones with little or no residential development, high gradients with abundant riffles, cool temperatures, low conductivity (near baseline conditions of <100 µS/cm), and stable channels with clean cobble/ boulder substrates. In the remainder of the basin, riparian zones tend to be narrower with less canopy cover, channel substrates are composed of smaller particles because siltation and bank erosion is more prevalent, and stream conductivity is higher (>160 µS/cm). Elsewhere in the drainage, the species is known from only one watershed, Lower Buffalo Creek in Owsley County.

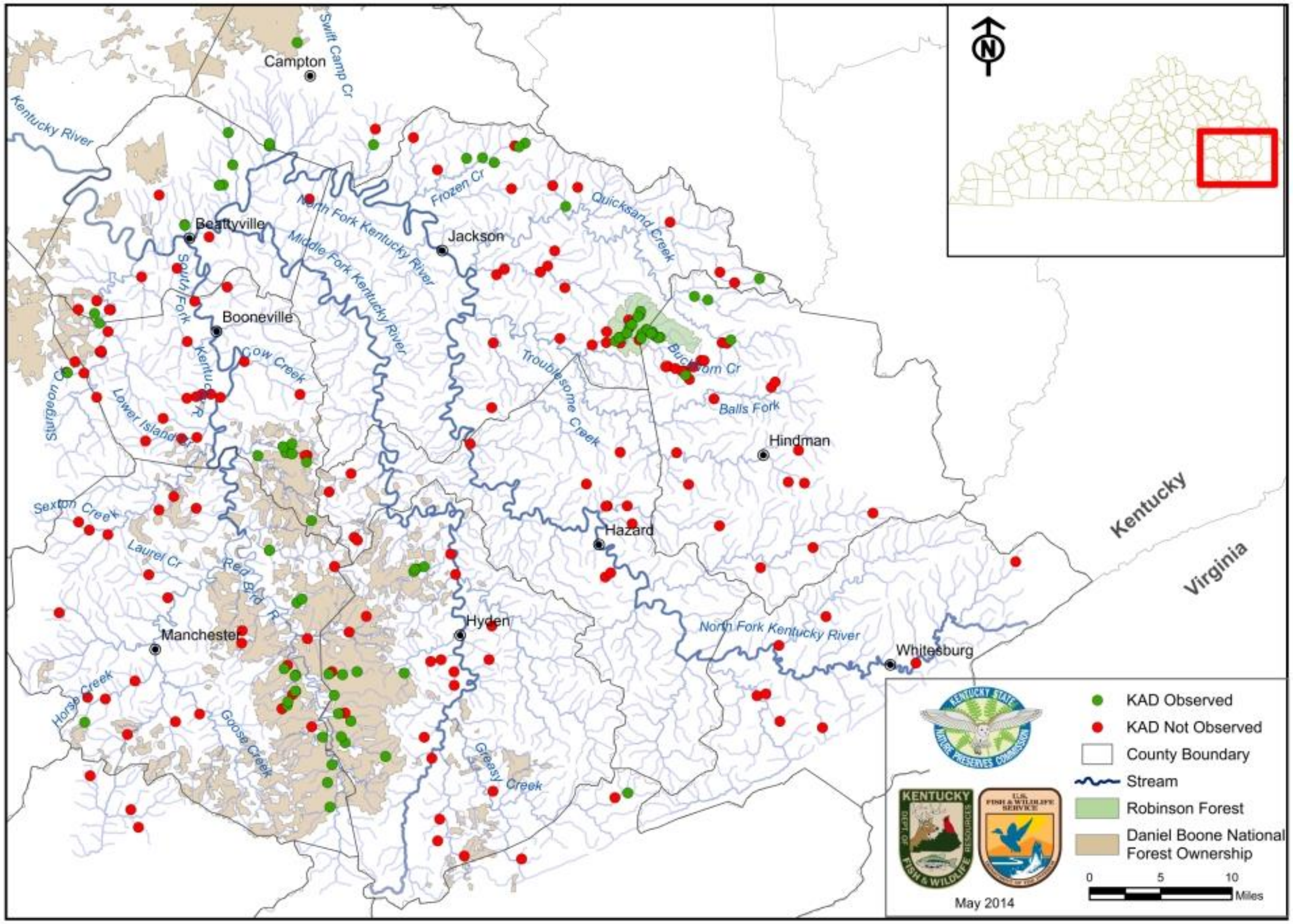

Figure 3. Current distribution of the Kentucky arrow darter based on presence-absence surveys completed from 2007-2013 at > 150 collection sites in the Upper Kentucky River drainage. Surveys were completed through cooperative efforts of the Kentucky Department of Fish and Wildlife Resources, Kentucky State Nature Preserves Commission, and the Service.

Middle Fork Kentucky River (3/3). This drainage has fewer historical KAD records than the North and South Forks of the Kentucky River. A large percentage of the basin has been disturbed by surface and underground coal mining, rural residential development is common within its narrow valleys, and an approximate 31-mile (50 km) segment of the Middle Fork has been influenced by the construction and inundation of Buckhorn Lake. During surveys from 2007 to 2009 (Thomas 2008, USFWS 2009), KADs were observed at only two streams, Hell for Certain Creek (Leslie County) and Big Laurel Creek (Harlan County). In March 2013, biologists with KDFWR and DBNF discovered an unknown population of *E. spilotum* in Laurel Creek, a second order tributary of Rockhouse Creek. Similar to Hell for Certain Creek, Laurel Creek is situated at the western edge of the Middle Fork drainage and about 90 percent of its watershed is located within the DBNF (Redbird Ranger District).

North Fork Kentucky River (16/24). The best remaining habitats in this drainage include two tributaries of Buckhorn Creek (Clemons Fork and Coles Fork), two major tributaries of Quicksand Creek (Laurel Fork and Middle Fork), and several direct tributaries of the North Fork Kentucky River (e.g., Frozen Creek). Two streams on UK's Robinson Forest, Clemons Fork and Coles Fork, support stable populations of KADs. These basins are intact and densely forested, with only minor interruption by logging roads. Both streams are high-gradient, cool, and dominated by cobble, boulder, and bedrock substrates. What appear to be stable populations also occur in two major tributaries of Quicksand Creek – Laurel Fork and Middle Fork. These basins are sparsely populated and have not been mined extensively. Outside of these areas, KADs occupy portions of 10 other streams, most of which are direct tributaries of the North Fork Kentucky River in the downstream half of the drainage.

Population Estimate/Status

Recent survey data by Thomas (2008) and USFWS (2009) revealed that sites with KADs had an average of only 3 individuals per 100-m (328-ft) sampling reach and a median of 2 individuals per sampling reach (range of 1 to 10 individuals). Surveys by the USFS from Laurel Fork and Cortland Branch of Left Fork Buffalo Creek (South Fork Kentucky River basin) produced slightly higher capture rates (an average of 5 darters per 100-m (328-ft) sampling reach) (Brandt pers. comm. 2011). The low abundance values (compared to other darters) are not surprising since Cumberland arrow darters and KADs generally are not observed in large numbers, even in those streams where disturbance has been minimal (Thomas pers. comm. 2010).

The first attempt at a population estimate was initiated in July 2013 by Eastern Kentucky University (EKU), the Service, and KSNPC (see page 22). The study was designed to estimate the KAD's current population size and average density within Clemons Fork (Robinson Forest, Breathitt County) and to compare current densities with historical densities reported by Lotrich (1973). Field surveys were completed in August 2013, but data analyses are incomplete. Preliminary results include a mean density estimate of 9.69 KADs per 150-m sampling reach and a population estimate of 986 to 2,113 KADs (95% confidence intervals). In 2014, EKU will complete additional KAD population estimates for two Red Bird Range District (DBNF) streams, Gilberts Big Creek and Elisha Creek (Clay and Leslie Counties). These estimates will be made as part of an ongoing KAD movement study and microhabitat characterization (see page 22).

Based on observed catch rates and habitat conditions throughout the upper Kentucky River basin, the most stable and largest populations of KADs appear to be located in the following streams/basins:

- Several tributaries of Red Bird River, Redbird District of DBNF (Clay and Leslie Counties);
- Hell Creek, Walker Creek, and Frozen Creek - direct tributaries of North Fork Kentucky River – (Breathitt and Lee Counties);
- Clemons Fork and Coles Fork of Buckhorn Creek, North Fork Kentucky River basin (Breathitt County).

ESA LISTING FACTORS/PRIMARY THREATS TO THE SPECIES

The main purposes of the Endangered Species Act (ESA) are to conserve endangered or threatened species, to prevent their extinction, and “to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved.” Under the ESA, when a species is able to survive on its own in the wild and the factors that previously threatened that species have been ameliorated, the species is considered “recovered,” and protection of the ESA may no longer be warranted.

The Service considers similar information in deciding whether to list a species under the ESA, delist a species (remove it from endangered or threatened status), reclassify it from endangered to threatened (or vice versa), or remove it from candidate status (species no longer requires listing). In every case, the Service assesses threats to the species using the five-factor analysis as outlined in Section 4 of the ESA.

The following discussion is an outline of the existing threats to the KAD, summarized primarily from the *Species Assessment and Listing Priority Assignment Form* for the species (USFWS 2013). The information is outlined according to each of the 5 ESA listing factors. As identified in the species assessment form, three of the Service’s five listing factors pose threats to the KAD: (a) the present or threatened destruction, modification or curtailment of its habitat or range (Factor A); (b) the inadequacy of existing regulatory mechanisms (Factor D); and (c) other natural or manmade factors affecting its continued existence (Factor E). The species’ habitat and range have been severely degraded and limited by water pollution from surface coal mining, oil/gas exploration activities, and residential areas; removal of riparian vegetation; stream channelization; increased siltation associated with poorly-implemented mining, logging, agricultural practices, and residential development; and deforestation of watersheds. Current regulatory mechanisms have been inadequate to conserve the species and its habitats. The species is also threatened due to the small, remnant nature of its populations. Their isolated nature may prohibit the natural interchange of genetic material among populations, and the small population size may reduce the reservoir of genetic diversity within populations. This can lead to inbreeding depression and reduced fitness of individuals. It is possible that some KAD populations are below the thresholds required to maintain long-term genetic and population viability.

The present or threatened destruction, modification, or curtailment of its habitat or range (Factor A)

The KAD's habitat and range have been destroyed, modified, and curtailed by a number of threats, including inputs of dissolved solids and elevation of conductivity, sedimentation/siltation, removal of riparian vegetation, bank erosion and channel instability, inputs of untreated sewage, and channel relocation or straightening. The sources of these threats include a variety of anthropogenic activities in the upper Kentucky River basin. Activities such as resource extraction (surface coal mining, logging, gas/oil well exploration), land development, rural residential land use, road construction and maintenance, inadequate sewage treatment, and agricultural practices have all contributed to the degradation of streams within the range of the species (Branson and Batch 1972, Branson and Batch 1974, KDOW 2011, Thomas 2008).

<u>Water Quality Degradation</u>

A significant threat to the KAD is water quality degradation caused by a variety of non-point source pollutants. Surface coal mining is a major source of these pollutants because it has the potential to contribute high concentrations of dissolved metals and other solids that elevate stream conductivity, increase sulfate levels, and cause wide fluctuations in stream pH (Curtis 1973, Pond 2004, Hartman et al. 2005, Mattingly et al. 2005, KDOW 2008, Palmer et al. 2010). Numerous studies have documented the fact that streams receiving discharges from mined areas exhibit water quality characteristics not observed in unmined watersheds (Curtis 1973, Dyer and Curtis 1977, Hren et al. 1984, US EPA 2003, Pond et al. 2008). As rock strata and overburden (excess material) are exposed to the atmosphere, precipitation leaches metals and other solids (e.g., calcium, magnesium, sulfates, iron, and manganese) from these materials and carries them in solution to receiving streams (Pond 2004). If valley fills are used as part of the mining activity, precipitation and groundwater seep through the fill and dissolve minerals until they discharge at the toe of the fill as surface water (Pond et al. 2008). Both of these scenarios result in elevated conductivity, sulfates, and hardness in the receiving stream.

The upper Kentucky River basin of eastern Kentucky has been mined extensively for coal, and these activities continue to be common, especially within the eastern half of the basin. As of January 2014, 157 surface mine permits were active in 7 of 9 counties located within the upper Kentucky River basin (Wahrer pers. comm. 2014). For the entire basin, 70 of the 131 current permits involved active coal removal, while the remaining 61 permits were classified as "temporary cessation" or "coal removal complete/reclamation only." No active coal removal was occurring in Jackson and Lee Counties, while Perry County had the most activity (29 permits with active coal removal). Six (6) permits were located in KAD watersheds: Buckhorn Creek (Breathitt and Knott Counties), Bullskin Creek (Clay County), and Right Fork Buffalo Creek (Owsley County).

Studies in the upper Kentucky River basin by Branson and Batch (1974), Dyer and Curtis (1977), Kuehne (1962), Thomas (2008), Pond (2010), and USFWS (2010) have clearly demonstrated that surface coal mining activities have contributed to water quality degradation (e.g., elevated conductivity) and the likely extirpation of KAD populations from numerous tributaries in the Quicksand Creek and Buckhorn Creek basins of Breathitt and Knott Counties. In general, these studies documented degraded water quality conditions in mined watersheds (e.g., elevated conductivity and other effects) and near baseline water quality conditions in unmined watersheds. Kentucky arrow darters were generally absent once conductivity levels exceeded 300 µS (USFWS 2013). Recent research by Hitt (2014) demonstrated that conductivity is a strong predictor of KAD abundance, and sharp declines in KAD abundance were observed at only 258 µS/cm (95% confidence intervals of 155-590 µS/cm). Hitt (2014) reported that conductivity was the most important variable for KAD abundance and was more than twice as important as the two next-most important variables (upstream % forest and % agricultural land uses).

Oil and gas exploration and drilling activities represent another significant source of harmful pollutants. According to Kentucky Geological survey data (KGS 2014), over 3,500 oil and gas wells exist within the upper Kentucky River basin. Demand for natural gas production in Kentucky is expected to increase in future years (KGS 2012), so threats from these activities will likely increase over this period. A variety of chemicals (e.g., hydrochloric acid, surfactants, potassium chloride) are used during the drilling process and can be harmful to aquatic organisms if they leave the drill site and enter nearby waterways. This type of release has been documented recently (2007) within the upper Cumberland River basin (Papoulias and Velasco 2013), so similar activities could produce the same result in the upper Kentucky River basin. The upper Cumberland River basin spill affected an approximate 2-mile reach of Acorn Fork, a known habitat for the federally threatened blackside dace, *Chrosomus cumberlandensis*. Initial conductivity readings downstream of the spill exceeded 30,000 µS/cm, and the fish community was decimated (Papoulias and Velasco 2013). Because oil and gas exploration activities are increasing within eastern Kentucky, events similar to the Acorn Fork spill could occur within the upper Kentucky River basin. It is also likely that these types of incidents would go unreported given the lack of federal oversight and the number and distribution of oil and gas wells that are being developed within the range of KAD.

Other nonpoint-source pollutants that affect the KAD include domestic sewage (through septic tank leakage or straight pipe discharges) and agricultural pollutants such as animal waste, fertilizers, pesticides, and herbicides. Nonpoint-source pollutants can cause increased levels of nitrogen and phosphorus, excessive algal growths, oxygen deficiencies, and other changes in water chemistry that can seriously impact aquatic species (KDOW 2006, KDOW 2011). Nonpoint-source pollution from land surface runoff can originate from virtually any land use activity and may be correlated with impervious surfaces and storm water runoff (Allan 2004). Pollutants may include sediments, fertilizers, herbicides, pesticides, animal wastes, septic tank and gray water leakage, pharmaceuticals, and petroleum products. These pollutants tend to increase concentrations of nutrients and toxins in the water and alter the chemistry of affected streams such that the habitat and food sources for species like the KAD are negatively impacted.

Physical Habitat Disturbance

Sediment (siltation) has been listed repeatedly by the Kentucky Department for Environmental Protection (Division of Water) as the most common stressor of aquatic communities in the upper Kentucky River basin (KDOW 2008, 2011). Sedimentation comes from a variety of sources, but KDOW identified the primary sources of sediment as loss of riparian habitat, surface coal mining, legacy coal extraction, logging, and land development (KDOW 2008, 2011). All of these activities can result in canopy removal, channel disturbance, and increased siltation, thereby degrading habitats used by KADs for both feeding and reproduction. The reduction or loss of riparian vegetation results in the elevation of stream temperatures, destabilization of stream banks and siltation, and removal of submerged root systems that provide habitat for fish (e.g. KADs) and macroinvertebrates (i.e. the food source for KADs) (Mattingly et al. 2005). Numerous streams within the KAD's current range have been identified as impaired (primarily due to siltation from mining, logging, agricultural activities, and land development) and have been included on Kentucky's 303(d) list of impaired waters (KDOW 2008, 2011).

Resource extraction activities (e.g., surface coal mining, legacy coal extraction, logging, oil and gas exploration and drilling) are major sources of sedimentation in streams of Kentucky and adjacent states (Paybins et al. 2000, Wiley et al. 2001, KDOW 2011). Activities associated with surface coal mining (e.g., land clearing, road construction, excavation) produce large areas of bare soil that, if not protected or controlled through various erosion control practices, can contribute large amounts of sediment during storm events. Mining companies are required to implement erosion control measures during mining activities, but sedimentation continues to be a significant stressor in some mined watersheds (KDOW 2011).

Similarly, logging activities can adversely affect KADs through removal of riparian vegetation, direct channel disturbance, and sedimentation of instream habitats. During logging activities, sedimentation occurs as soils are disturbed, the overlying leaf or litter layer is removed, and sediment is carried overland from logging roads, stream crossings, skid trails, and riparian zones during storm events. Excess sediment can bury in-stream habitats used by the species for foraging, reproduction, and sheltering, and it can disrupt the dynamic equilibrium of channel width, depth, flow velocity, discharge, channel slope, roughness, sediment load, and sediment size that maintains stable channel morphology. This can lead to channel instability and further degradation of in-stream habitats. Reductions in riparian vegetation can adversely affect the species through increased solar radiation, elevated stream temperatures, loss of allochthonous (organic material originating from outside the channel) food material, and bank instability / erosion. Direct channel disturbance occurs primarily at stream crossings during culvert, log, or rock placement. Severe impacts can occur when loggers use stream channels illegally as skid trails (M. Floyd pers. obs. 2009).

Land use practices that affect sediment and water discharges into a stream can lead to increased baseflow downstream of valley fills and to increased erosion or sedimentation patterns, which can lead to the destruction or modification of in-stream habitat and riparian vegetation, stream bank collapse, and increased water turbidity and temperature (Wiley et al. 2001, Messinger 2003). Stormwater runoff from unpaved roads, ATV trails, and driveways represents a significant but difficult to quantify source of sediment that impacts streams in the Upper Kentucky River basin. Observations made by Service personnel during field collections suggest that this is a common and widespread problem during storm events. Sediment has been shown to damage and suffocate fish gills and eggs, larval fishes, bottom dwelling algae, and other organisms; reduce aquatic insect diversity and abundance; and, ultimately, negatively impact fish growth, survival, and reproduction (Waters 1995, Wood and Armitage 1997, Meyer and Sutherland 2005). KAD habitats are also affected when riparian corridors are disturbed or significantly altered during mine preparation, logging activities, or road construction.

Overutilization for commercial, recreational, scientific, or educational purposes (Factor B)

The KAD is not believed to be utilized for commercial, recreational, scientific, or educational purposes. Individuals may be collected occasionally in minnow traps and used as live bait, but this activity does not pose a significant threat. The available information does not indicate that overutilization is likely to become a threat to the KAD in the foreseeable future.

Disease or predation (Factor C)

Disease is not considered to be a factor in the decline of the KAD. Although the KAD is undoubtedly consumed by native predators, this activity is naturally occurring and a normal aspect of the species' population dynamics. Non-native rainbow trout (*Oncorhynchus mykiss*) represent a potential predation threat (Etnier and Starnes 1993) as they are introduced annually by KDFWR into portions of three KAD watersheds: Big Double Creek (Clay County), Sturgeon Creek (Lee County), and Swift Camp Creek (Wolfe County). Annual totals of 800 and 1000 rainbow trout are introduced into Sturgeon Creek and Swift Camp Creek, respectively, but in these watersheds KAD populations occupy portions of small tributaries located outside of actual stocking locations. Therefore, it is unlikely that rainbow trout and KADs interact in these watersheds.

Up to 1000 rainbow trout are stocked annually by KDFWR within Big Double Creek, with releases occurring in March, April, May, and October in habitats occupied by KADs. KDFWR has no specific information on the feeding habits of rainbow trout in Big Double Creek, but KDFWR did support a research project (Brandt 2006) investigating the impact of stocked rainbow trout on native fishes in Rock Creek, McCreary County, Kentucky. Brandt (2006) examined the guts of 11 introduced rainbow trout obtained from 32 sampling sites within the Rock Creek watershed. The majority of stomachs were empty or contained remains of macroinvertebrates; however, gut contents from two individuals included remains of two native fishes, telescope shiner (*Notropis telescopus*) (n=2) and emerald darter (*Etheostoma obeyense*) (n=1). Brandt (2006) demonstrated that stocked rainbow trout can be piscivorous in Kentucky streams, but the magnitude of this threat was unclear.

Within Big Double Creek, stockings of rainbow trout have occurred for over 30 years (Williams pers. comm. 2014), but the KAD population continues to persist and appears to be healthy based on KDFWR surveys (KDFWR 2014). KDFWR also has no evidence suggesting that stocked rainbow trout can survive typical summer temperatures (>19°C or 66°F) within Big Double Creek (Williams pers. comm. 2014); stocked individuals are caught by anglers or perish once stream temperatures rise in warmer months. To assess the potential predation of rainbow trout on KADs or other fishes, the KFO and DBNF surveyed a 2.1-km (1.3-mile) reach of Big Double Creek on April 21, 2014, 17 days after KDFWR's April stocking event (250 trout). A total of seven rainbow trout were captured, and the gut contents of these individuals were examined. Food items were dominated by Ephemeroptera (mayflies), with lesser amounts of

Plecoptera (stoneflies), Trichoptera (caddisflies), Diptera (flies), Decapoda (crayfish), and terrestrial Coleoptera (beetles). No fish remains were observed. Based on all these factors and the absence of rainbow trout from the majority (98%) of KAD streams, we do not believe that predation by non-native rainbow trout poses a significant threat to the species. Our current information also does not indicate that disease or predation is likely to become a threat to the KAD in the foreseeable future.

The inadequacy of existing regulatory mechanisms (Factor D)

The KAD and its habitats are afforded some protection from water quality and habitat degradation under the Clean Water Act of 1977 (33 U.S.C. 1251 *et seq*.), Kentucky's Forest Conservation Act of 1998 (KRS 149.330-355), Kentucky's Agriculture Water Quality Act of 1994 (KRS 224.71-140), and additional Kentucky laws and regulations regarding natural resources and environmental protection (KRS 146.200-360; KRS 224; 401 KAR Chapters 5, 9, and 10). However, as demonstrated under Factor A, population declines and degradation of habitat for this species are ongoing despite the protection afforded by these laws and corresponding regulations. While these laws have resulted in some improvements in water quality and stream habitat for aquatic life, including the KAD, they alone have not been adequate to fully protect this species; non-point source pollutants and sedimentation continue to be a significant problem in spite of the numerous laws and regulations intended to control these stressors.

States maintain water-use classifications through issuance of National Pollutant Discharge Elimination System (NPDES) permits to industries, municipalities, and others that set maximum limits on certain pollutants or pollutant parameters. For water bodies on the 303(d) list (an official list of impaired streams compiled by the state per Section 303d of the Clean Water Act), States are required under the Clean Water Act to establish a total maximum daily load (TMDL) for the pollutants of concern that will bring water quality into the applicable standard. Eighteen (18) KAD streams have been identified as impaired by the Kentucky Division of Water and placed on the State's 303(d) list (KDOW 2011). Causes of impairment were listed as increased sediment/ siltation and total dissolved solids from coal mining, logging, loss of riparian habitat, and organic enrichment/eutrophication from agriculture. TMDLs have not yet been developed for these pollutants.

The Commonwealth of Kentucky prohibits the collection of the KAD and other fish species for scientific purposes without a valid state-issued collecting permit. However, this requirement does not provide any protection to the species' habitat. Within Kentucky, persons who hold a valid fishing license (obtained from Kentucky Department of Fish and Wildlife Resources (KDFWR)) are allowed to collect up to 500 minnows per day (a minnow is defined as any non-game fish less than 6 inches in length, with the exception of federally listed species). This regulation allows for the capture, holding, and potential use of the KAD as a bait species. While we do not currently believe this is a significant threat (see Factor B), it is a potential threat.

The KAD has been designated as "Threatened" in Kentucky by the KSNPC (KSNPC 2005), but this designation conveys no legal protection under Kentucky state law.

Other natural or manmade factors affecting its continued existence (Factor E)

The KAD has a limited geographic range and small population sizes. The existing populations are extremely localized, and geographically isolated from one another, leaving them vulnerable to localized extinctions from intentional or accidental toxic chemical spills, habitat modification, progressive degradation from runoff (non-point source pollutants), natural catastrophic changes to their habitat (e.g., flood scour, drought), other stochastic disturbances, and to decreased fitness from reduced genetic diversity. Potential sources of unintentional spills include accidents involving vehicles transporting chemicals over road crossings of streams inhabited by the species, or the accidental or intentional release of chemicals used in agricultural or residential applications into streams.

Species that are restricted in range and population size are more likely to suffer loss of genetic diversity due to genetic drift, potentially increasing their susceptibility to inbreeding depression, decreasing their ability to adapt to environmental changes, and reducing the fitness of individuals (Soule 1980, Hunter 2002, Allendorf and Luikart 2007). It is likely that some of the KAD populations are below the effective population size required to maintain long-term genetic and population viability (Soule 1980, Hunter 2002). The long-term viability of a species is founded on the conservation of numerous local populations throughout its geographic range (Harris 1984). These separate populations are essential for the species to recover and adapt to environmental change (Noss and Cooperrider 1994, Harris 1984). The level of isolation observed in portions of the KAD range makes natural recolonization following localized extirpations difficult or unlikely without human intervention.

Climate change has the potential to increase the vulnerability of the KAD to random detrimental events and to chronic stressors such as increased stream temperature (e.g., McLaughlin et al. 2002, Thomas et al. 2004). Climate change is expected to result in increased frequency and duration of droughts and the strength of storms (e.g., Cook et al. 2004). Severe droughts similar to those that affected eastern Kentucky in 2007 and 2008 could be intensified by rising mean air temperatures and reduced precipitation amounts as predicted by Maurer *et al.* (2007) and The Nature Conservancy (2013) over the next 40 years in eastern Kentucky.

The hemlock woolly adelgid (HWA) (*Adeleges tsugae*), an aphid-like insect native to Asia, represents a new threat to the KAD because it has the potential to severely damage stands of eastern hemlocks (*Tsuga canadensis*) that occur throughout the KADs' range. In some areas where KADs occur, hemlocks are the dominant riparian tree and provide the majority of shade to stream corridors. Loss of hemlocks along these streams has the potential to adversely affect the species through increased solar exposure and subsequent elevated stream temperatures, bank erosion, and excessive inputs of woody debris that will clog streams and cause channel instability and erosion (Townsend and Rieske-Kinney 2009).

Summary Threats Assessment

Three of the five listing factors considered by the Service pose threats to the KAD:

- The present or threatened destruction, modification or curtailment of its habitat or range;
- The inadequacy of existing regulatory mechanisms; and
- Other natural or manmade factors affecting its continued existence.

The KAD's habitat and range have been modified and limited by both water quality degradation and physical habitat disturbance. Water quality impacts (e.g., elevated conductivity, untreated sewage) associated with surface coal mining, oil and gas development, and other land use practices vary in magnitude across the species' range, but they are most severe in the eastern half of the range, where surface coal mining has been the most prevalent, oil and gas exploration is increasing, and residential development has been the most concentrated within the region's narrow valleys. Activities associated with surface coal mining are a major source of pollutants because they create large, physically disturbed landscapes, exposing previously buried rock strata that can contribute high concentrations of dissolved metals and other solids that elevate stream conductivity, increase sulfate and hardness levels, and cause wide fluctuations in stream pH. Past research has demonstrated that the KAD is intolerant of these conditions, as demonstrated by its disappearance from over 20 streams where these water quality changes have occurred (Branson and Batch 1974, Kuehne 1962, Thomas 2008, and USFWS 2010). Recent research by Hitt (2014) demonstrated that conductivity is a strong predictor of KAD abundance, and sharp declines in KAD abundance were detected at 258 µS/cm (95% confidence intervals of 155-590 µS/cm).

Residential areas within this portion of the range also have the potential to contribute untreated sewage from septic tank leakage or straight pipe discharges. Inputs of untreated sewage can cause increased levels of nitrogen and phosphorus, excessive algal growths, oxygen deficiencies, and other changes in water chemistry that can seriously impact aquatic species. The Kentucky Division of Water continues to identify untreated sewage as a major pollutant within the upper Kentucky River basin.

Water quality threats are less severe in the western half of the KAD's range; however, streams in this portion of the range continue to be impacted by a variety of nonpoint-source pollutants such as domestic sewage, animal waste, fertilizers, pesticides, herbicides, pharmaceuticals, and petroleum products. These pollutants tend to increase concentrations of nutrients and toxins in the water and alter the chemistry of affected streams such that the habitat and food sources for species like the KAD are negatively impacted. Based on all these factors, we considered the variation in threat magnitude from west to east and arrived at an overall threat magnitude of "moderate/high" for water quality degradation.

Physical habitat degradation associated with sedimentation and other physical habitat disturbance (e.g., loss of riparian vegetation, channelization) is widespread across the KAD's range (the geographic scope is widespread and not localized). Sedimentation/siltation is the most significant threat to physical habitat quality across the KAD's range, and sedimentation continues to be ranked by the KDOW as the most common stressor of aquatic communities in the upper Kentucky River system. We consider physical habitat threats to be of high magnitude due to their widespread occurrence and the fact that several KAD populations have disappeared from systems impacted solely by these threats.

Current regulatory mechanisms, such as the Clean Water Act, have been inadequate to prevent water quality degradation and habitat disturbance. The small, remnant nature of many KAD populations may prohibit the natural interchange of genetic material between these populations, and the small population size may reduce the reservoir of genetic diversity within populations. This can lead to inbreeding depression and reduced fitness of individuals. It is possible that some KAD populations are below the effective population size required to maintain long-term genetic and population viability. We have no information indicating that the magnitude or imminence of these threats is likely to be appreciably reduced in the foreseeable future.

Listing Priority Number

The KAD has been assigned a listing priority number of 2 on a scale of 1 (highest) to 12 (lowest), based on its high magnitude, imminent threats, and its status as a full species (USFWS 1983a, b).

Magnitude: Water quality degradation varies in intensity (severity) across the KAD's range, but it is most severe in the eastern half of the range where surface coal mining has been most prevalent and continues to occur, oil and gas exploration activities are increasing, and residential development is most concentrated in the region's narrow valleys. It has been demonstrated that these changes have contributed to KAD extirpations from over 20 streams in the eastern half of its range. The threat magnitude is lower in the western half of the range, but streams in this region continue to be threatened by a variety of nonpoint-source pollutants. For this particular threat (water quality degradation), we consider the variation in threat magnitude from west (moderate) to east (high) and arrive at an overall threat magnitude of "moderate/high."

Physical habitat disturbance continues to be a major problem across the range, and the magnitude of the threat appears to be consistent across the range. We consider physical habitat threats to be of high magnitude due to their widespread occurrence and the fact that several KAD populations have disappeared from systems impacted primarily by physical threats.

Collectively, threats of water quality degradation and physical habitat disturbance are serious and significant impediments to the survival of the KAD, and we consider them to be of "high" magnitude.

Imminence: Threats to the KAD are imminent because the effects are ongoing and will continue for some time. Annual coal production in eastern Kentucky (including counties in the upper Kentucky River basin) has declined over the past two decades, but annual production in eastern Kentucky continues to be relatively high (over 39 million tons produced in 2013) (KEEC 2014), recoverable reserves for the eastern Kentucky portion of the Appalachian Basin are estimated at 5.8 billion tons (Milici and Dennen 2009, pp. 8-11), and the species' distribution continues to be limited as a result of previous mining activities within the basin. Consequently, the potential remains for KADs to be adversely affected by water quality degradation associated with surface coal mining activities. Demand for natural gas production in Kentucky is expected to increase in future years (Kentucky Geological Survey 2012), so threats from these activities will likely increase over this period. Physical habitat disturbance (e.g., sedimentation, loss of canopy cover, channelization) associated with surface coal mining, logging, agricultural production, residential development, and state and county road construction/maintenance, is expected to continue as well and is not expected to diminish in the foreseeable future. Based on these factors, water quality degradation and physical habitat disturbance will continue to threaten the KAD's continued existence. Therefore, we consider these factors to be "imminent."

CURRENT CONSERVATION EFFORTS

A number of federal and state agencies, universities, and other groups are involved in conservation efforts for the KAD. We have organized these efforts into four general categories and provide descriptions of each below.

Candidate Conservation

DBNF Candidate Conservation Agreement (CCA). The KFO and the DBNF are working cooperatively to develop a candidate conservation agreement for the KAD on the DBNF. Over half of the species' extant streams occur on lands owned and managed by the DBNF, so conservation of these populations is essential to the species' recovery, and a DBNF-specific conservation plan is needed to guide those efforts. The DBNF and KFO met in January 2013 to discuss potential conservation actions, including (1) an evaluation of relevant Forest Plan standards to identify any impediments to KAD conservation and ways to address those impediments, (2) a review of available data that would help identify any immediate opportunities for conservation success with the KAD, (3) the development of a watershed-based threat matrix for USFS lands and watersheds, (4) identifying and ranking potential stream restoration opportunities, (5) evaluating the implications of rainbow trout stocking within USFS lands and watersheds, and (6) the development of a Candidate Conservation Agreement for the DBNF. Both agencies are currently working on action items, and completion of a draft CCA is expected in 2014.

Range-wide Candidate Conservation Agreement with Assurances (CCAA). In 2013, the KFO and KDFWR facilitated the initial steps toward development of a range-wide CCAA for the species. The process included a Science Advisory Committee (SAC) meeting (January 2013), a Stakeholder meeting (February 2013), and several other meetings and site visits by KFO, KDFWR, and coal industry representatives. The SAC meeting was moderated by KDFWR and included a total of 18 biologists, representing a variety of agencies/groups: Appalachian Wildlife Foundation (AWF), Appalachian Technical Services, Austin Peay State University, Conservation Fisheries Inc., DBNF, KDFWR, Kentucky Division of Water, KSNPC, Morehead State University, The Nature Conservancy, U. S. Environmental Protection Agency, USFWS (KFO), and the U. S. Geological Survey. The SAC reviewed and discussed the species' biology and current status/threats and identified three general research needs or priorities for the species: (1) genetics, (2) stressor identification, and (3) movement behavior.

The Stakeholder meeting was led by staff from KDFWR and was attended by a variety of stakeholders from the coal mining, forestry, transportation, and environmental consulting industries, DBNF, AWF, Eastern Kentucky University, and KSNPC. Dr. Michael Floyd (KFO) presented information on KAD life history and threats and gave a summary of CCAAs and CCAA development. The meeting also included a discussion session where KDFWR and KFO staff answered a variety of questions by potential stakeholders. Following these efforts, the KFO participated in several meetings and site visits with KDFWR and AWF to discuss future CCAA efforts and potential cooperators. In October 2013, KDFWR, Service, and other stakeholders determined that the CCAA had a low probability of success and the selection of a permit holder was unlikely. Rather than spend more time on CCAA development, they believed that conservation efforts directed at extant occurrences and habitat restoration on the DBNF and private lands held more promise to benefit the KAD.

Habitat Restoration

Bullskin Creek Restoration (Leslie County). A 0.55-km (1823-ft) stream restoration/enhancement project was completed in 2005 in the upper reaches of Bullskin Creek, Leslie County. Bullskin Creek represents suitable habitat for the KAD, and the subspecies was reported from the Bullskin Creek watershed as recently as 2007, when 2 individuals were collected approximately 12.1 stream km [7.5 mi] downstream of the restoration site (Thomas 2008, p. 4). The Bullskin project was funded through Kentucky's Wetland and Stream Mitigation Fund (managed by KDFWR's Stream and Wetland Restoration Program) and was intended to repair eroding banks and poor habitat conditions within Bullskin Creek. The project included a permanent, 9-m (30-foot) easement held by KDFWR. Habitat improvements in this reach of Bullskin Creek will benefit KADs living in upstream and downstream reaches.

Propagation/Reintroduction Efforts

KDFWR - Sugar Creek and Long Fork (Clay and Leslie Counties, DBNF). The KDFWR identified the KAD as a Species of Greatest Conservation Need (SGCN) in its State Wildlife Action Plan (KDFWR 2013). The plan identifies conservation issues (threats), conservation actions, and monitoring strategies for 301 animal species belonging to one of 20 terrestrial and aquatic habitat guilds (i.e., a collection of species that occur in the same habitat). To fully understand these conservation issues, the KDFWR developed a priority list of research and survey needs for Kentucky's SGCN. The KDFWR attempted to address two of these needs in 2008 by initiating a propagation and reintroduction study for the KAD through the Service's State Wildlife Grant program (Ruble *et al*. 2010). The study was designed to document details on the species' reproductive biology and to begin conservation actions (*e.g*., propagation followed by reintroduction or augmentation) that would preclude the need to list the KAD as threatened or endangered under the ESA. The KDFWR partnered with Conservation Fisheries, Inc. (CFI) to develop successful spawning protocols and produce the offspring needed to augment populations within the species' current range.

From 2009 to 2011, a total of 145 captive-spawned, juvenile KADs (from Big Double Creek) were produced by CFI, tagged (Northwest Marine Technologies elastomer tag), and introduced into Sugar Creek, Leslie County, a tributary of the Red Bird River in the DBNF, Redbird District (Thomas and Brandt 2012). Attempts to relocate tagged darters in August 2009, October 2009, March 2010, January 2012, and February 2012 were unsuccessful, so KDFWR and CFI made the decision to abandon efforts at Sugar Creek and begin another reintroduction effort at Long Fork, another DBNF stream and tributary of Hector Branch in Clay County.

Since August 2012, a total of 1,147 captive-spawned KADs (about 50-55 mm total length) have been tagged and reintroduced within a 1.5-km reach of Long Fork. Monitoring has been conducted on six occasions since the initial release using visual searches and seining methods. Tagged darters have been observed during each monitoring event, with numbers increasing from 18 (October 2012) to 86 (August 2013). Tagged darters have been observed throughout the Long Fork mainstem, both upstream and downstream of the release points, and one tagged individual was observed in the receiving stream - Hector Branch, downstream of its confluence with Long Fork. The majority of individuals have been found in pools (depth of 8-24 inches) with rock substrates, exposed bedrock, and some marginal cover (e.g., tree roots). Surveys in July, August, and October 2013 produced a total of 20, untagged young-of-year, indicating successful reproduction in Long Fork. Additional monitoring and releases are planned for the spring of 2014.

Research

Upper Kentucky River Basin Distributional Study and Habitat Characterization. The KFO is working cooperatively with the KSNPC to investigate the distribution, status, population size, and habitat use of the KAD within the upper Kentucky River system. One important aspect in assessing these components is to account for *imperfect detection* when surveying for the species. Studies that do not account for imperfect detection can often lead to an underestimation of the true proportion of sites occupied by a species and can bias assessments and sampling efforts (MacKenzie et al. 2002; MacKenzie et al. 2004). From June to September 2013, the KSNPC and KFO visited 80 randomly-chosen sites across the upper Kentucky River basin in order to address these concerns and meet the following objectives:

- Estimate detection probability and occupancy of KAD;
- Estimate the proportion of sites occupied by the focal species, after accounting for the detection probability;
- Draw inference into the environmental resources important to the persistence of the focal species;
- Assess the status (viability) of the focal species;

- Estimate the population size of the species within the study area; and
- Make recommendations for future efforts in the conservation of the focal species.

As expected, KADs were rare during the study and were observed at only 7 of the 80 sites. Presently, KSNPC and the KFO are in the data analysis stage of this project.

KAD Movement Study, Microhabitat characterization, and Population Estimate (EKU, KFO, and KDFWR). The KFO and KDFWR are working with EKU (Dr. Sherry Harrel and graduate student, Mr. Jonathan Baxter) to develop and implement a movement study, habitat characterization, and population estimate of two KAD streams, Gilberts Big Creek and Elisha Creek (DBNF), in Clay and Leslie Counties (Harrel and Baxter 2013). Mr. Baxter is using PIT-tags and placed antenna systems to monitor intra- and inter-tributary movement patterns in both streams, and he will be collecting seasonal (spring, summer, and fall) biotic and abiotic data from 17 100-m reaches to determine habitat use and population density/size for both streams. Preliminary findings include the following:

- 126 individuals pit-tagged
- Population estimates for Elisha Creek: 592-1429 inds (Summer) and 661-1359 (Fall) (range here and below reflects 95% confidence)
- Population estimate for Gilberts Big Creek: 175-358 (Summer)
- Maximum observed movement: 4078 m (2.5 mi) – female, downstream in Gilberts Big Creek
- Other observed movements (7 inds): 134 m (upstream), 328 m (downstream), 900 m (upstream/downstream), 950 m (downstream), 1708 (downstream), 351 (upstream), and 1282 m (downstream)

Population Estimate and Microhabitat Characterization of Clemons Fork, Robinson Forest, Breathitt County (EKU, KFO, and KSNPC). In July 2013, EKU, KFO, and KSNPC initiated a population estimate and microhabitat characterization study on Clemons Fork, Breathitt County. The study was designed to estimate the KAD's current population size and average density within Clemons Fork and to compare current densities with historical densities reported by Lotrich (1973). Additionally, population densities and habitat parameters will be compared to data from Gilberts Big Creek and Elisha Creek (both DBNF) to aid in delineation of essential habitat characteristics and development and implementation of conservation efforts. Field surveys were completed in August 2013. Data analyses are incomplete, but initial results include a mean density of 9.69 KADs per sampling reach and a population estimate of 986 to 2,113 KADs in Clemons Fork (95% confidence intervals). Preliminary findings of this study were presented at the 2013 Southeastern Fishes Council Meeting, Lake Guntersville, Alabama (November 14-15, 2013).

Rangewide Genetics Study (Austin Peay State University and KDFWR). In August 2013, Austin Peay State University (Drs. Rebecca Johansen and Mollie Cashner) began work on the first comprehensive assessment of genetic variation and gene flow patterns across the range of the KAD. Approximately 25 individuals per population from up to 12 populations across the range of the species will be genotyped using microsatellite markers. Resulting data will be used to generate robust estimates of effective population sizes and overall population and species variability. This information is essential to the development of effective conservation and recovery measures to ensure the long-term persistence of the species. Funding for this project is being provided through the Service's Section 6 program.

Landscape Model of KAD Occurrence in Response to Water Quality (USGS). Through USFWS-USGS Quick Response funding, the USGS Leetown Science Center (Dr. Nathaniel Hitt) evaluated the relationship between KAD abundance and stream conductivity in the upper Kentucky River basin (Hitt 2014). Nonlinear regression techniques were used to evaluate significant thresholds and associated confidence intervals for KAD abundance related to conductivity levels. As a contrast to KAD, Dr. Hitt also evaluated blackside dace occurrence in this regard. Data for the study were supplied by the KFO, Tennessee Field Office (USFWS), KDFWR, and KSNPC. Nonlinear regressions indicated a distinct decline in KAD abundance at 258 µS/cm (95% confidence intervals 155-590 µS/cm), above which abundances were negligible. Nonlinear threshold declines for blackside dace were observed at 343 µS/cm, and 95% confidence intervals bounded this relationship between 123-632 µS/cm. Boosted regression results indicated that stream conductivity was the strongest predictor in separate analyses of KAD and blackside dace abundance. Hitt (2014) concluded that the similar responses of these ecologically distinct taxa suggest the general importance of this water quality attribute for stream fish ecology in central Appalachia.

Summary Conservation Assessment

As described above, numerous conservation efforts are underway for the KAD, but most of these efforts are preliminary in nature. One of the more significant, ongoing efforts has been the propagation and reintroduction study by KDFWR and CFI. It has been ongoing since 2008 and has demonstrated some early successes, including development and refinement of propagation methods, persistence of introduced darters, and successful reproduction in Long Fork.

The species has also benefited from its occurrence on the DBNF, where it is currently extant in 19 streams (Red Bird, London, and Cumberland Ranger Districts). Land management decisions on the DBNF are guided by its 2004 Land and Resource Management Plan (Forest Plan) (USFS 2004), which guides the coordination of multiple uses and promotes sustained yields of products and services on the DBNF (USFS 2004). Two goals of the Forest Plan – Goal 1 (Maintain a variety of life and recover native and desirable non-native populations that are rare and declining) and Goal 3 (Management and restoration of watersheds) offer direct and indirect benefits to the KAD. The new KAD CCA between the DBNF and KFO will help organize conservation efforts on the DBNF and determine if conservation efforts in the Forest Plan are sufficient to conserve the species.

The other conservation efforts mentioned above – the CCAA development process and proposed research in particular - will provide needed conservation planning across the species' range and additional information regarding biological needs, population status, and threats. An active conservation constituency is in place for the species due to early planning/coordination associated with the range-wide CCAA.

Summary Assessment of Conservation Status (Conservation Needs)

The KAD continues to face high magnitude, imminent threats to its habitat and range (Factor A), as evidenced by recent extirpations and degradation of water and physical habitat quality across its range. Existing regulatory mechanisms have been inadequate to prevent these impacts (Factor D). Due to these threats, the species is now limited to 40 isolated watersheds, many of which are separated from each other by considerable distances. Mixing of these subpopulations is unlikely, making them vulnerable to low genetic diversity, inbreeding depression, and reduced fitness (Factor E).

Protection and management of existing KAD populations should be a top priority of any conservation strategy. Fortunately, over half of the species' known streams (about 20) are located in watersheds with significant (> 50 percent) public ownership. Development of detailed conservation strategies for these habitats would ensure that these populations are given the proper level of protection and management. Other conservation needs include the identification of unoccupied, suitable reintroduction sites; habitat restoration efforts in historical streams or watersheds; permanent protection of occupied streams through voluntary agreements, conservation easements, or land acquisitions; and funding for additional research on genetics, dispersal behavior, and stressors (the research priorities as identified by the CCAA SAC).

CONSERVATION OBJECTIVES AND ACTION PLANS

For this section, action items described under Objective 1 address some informational needs regarding the species' biology, ecology, viability, and survey methods; they do not specifically relate to any one of the 5 threats to the species described previously. The remaining 3 objectives (Objectives 2 – 4) are organized to correlate directly to Threat Factors A, D, and E, respectively.

Objective 1: Investigate the species' status, population genetics/structure, and habitat requirements.

1) Determine the most effective surveying/monitoring protocol for the species (including capture probability considerations) and integrate its use into State, Federal, and local policy as the approved method to accurately assess KAD population levels, trends, and responses to management; determine appropriate time frames for surveying, and acceptable alternative survey protocols, if needed. The Service and KSNPC initiated a range-wide distributional study in 2013 to address this action item (see pages 22-23).

2) Determine the species' genetic variation and gene flow patterns across the range. Austin Peay State University and KDFWR initiated a range-wide genetics study in 2013 (see page 24).

3) Define the spatial dimensions of KAD population structure and use this information (e.g., size, age structure, recruitment rate, spatial distribution, etc.) to estimate the species' population viability and establish consensus on a range of populations needed for recovery. The Service, EKU, and KDFWR initiated studies on the DBNF and Robinson Forest in 2013 to address this action item (see pages 22-23).

4) Investigate the species' movement behavior and habitat requirements through field investigations of selected streams throughout its range. The Service and KSNPC initiated a range-wide distributional study in 2013 that will assist in determining the species' habitat requirements (see page 23). The Service, EKU, and KDFWR initiated studies on the DBNF and Robinson Forest in 2013 that will investigate the species' movement behavior and habitat requirements (see page 23).

5) Search for new or unknown populations within the species' known range. Surveys in 2013 produced 2 new records for the species. Searches of suitable habitat in other parts of the range could produce similar results.

Objective 2: Address the present and threatened destruction, modification, or curtailment of KAD habitat.

1) Identify, prioritize, protect, and manage viable KAD populations and best remaining habitats for the species. Utilize existing federal and state laws and regulations to protect existing habitats and populations.

2) Increase public awareness of the KAD through distribution of fact sheets and other outreach materials within the Kentucky River basin. Outreach efforts by the Service (Partners for Fish and Wildlife Program), KDFWR (Stream and Wetland Mitigation Program, Kentucky Afield), and NRCS (Farm Bill programs) should incorporate information on the species' biology, status, distribution, and threats.

3) Identify privately owned lands containing high-priority KAD populations (e.g., Quicksand Creek basin). Directly target these landowners with outreach materials and (potentially) provide incentive funding to implement BMPs and/or promote other conservation activities benefiting KADs. Utilize Service (Partners for Fish and Wildlife Program), KDFWR (Stream and Wetland Mitigation Program), and NRCS (Farm Bill) programs to improve habitats in these watersheds.

4) Evaluate and identify existing stressors and sources of threats throughout the species' range; develop Best Management Practices (BMPs) and utilize existing agency programs to minimize, mitigate, and/or remove threats; encourage participation from the coal mining industry, oil and gas industry, forest management companies, private lands foresters, and county officials in the development of these recommendations. The Service and USGS cooperated on development of a landscape model of KAD occurrence in response to elevated conductivity (see page 22, Hitt 2014).

5) Investigate the species' sensitivity to increased silt, elevated temperature, dissolved ions, metals, and other pollutants using captively-bred individuals.

   <u>Specific Actions to Address Siltation/Physical Habitat Alteration</u>

   a. Identify areas within the KAD's range where water quality characteristics are near baseline levels (e.g., conductivity <100 µS/cm) but physical habitat quality is poor due to historical land use and stream channelization activities; utilize KDFWR's Stream and Wetland Mitigation Program or other mitigation programs to implement stream restoration projects.

   b. For surface coal mine projects, avoid placement of valley (hollow) fills in KAD watersheds, avoid placement of instream sediment control ponds/dams in KAD watersheds, and maximize the use of existing developed areas and rights-of-way for infrastructure supporting the development of mining operations.

   c. For activities associated with oil and gas development, including road construction, drilling, and maintenance, minimize sedimentation of streams through adherence to sediment BMPs and ensure that these actions become standard practice.

   d. In agricultural areas, identify areas where livestock have access to KAD streams; in these areas, work with NRCS liaisons to ensure that NRCS prescribed grazing standards are being implemented - exclude livestock from streams via fencing, and install alternate water supplies/infrastructure.

e. Ensure that silvicultural activities comply with Forest Stewardship Council (FSC), Sustainable Forestry Initiative (SFI), American Tree Farm (ATF), or National Alliance of Forest Owners (NAFO) certification standards, including Streamside Management Zone (SMZ) criteria as outlined in Kentucky's *Field Guide to BMPs for Timber Harvesting in Kentucky* (University of Kentucky Cooperative Extension Service and Kentucky Division of Forestry).

f. Restrict off-road vehicle access to KAD streams/SMZs on publically owned lands and encourage avoidance of these activities on private lands where possible.

g. Identify areas with beaver activity; remove dams and implement beaver eradication/control methods to safeguard aquatic connectivity.

<u>Specific Actions to Address Water Quality Impairment</u>

a. Investigate the species' sensitivity to dissolved ions, metals, and other pollutants through acute or chronic whole effluent toxicity tests.

b. For surface coal mine projects, avoid placement of valley (hollow) fills in KAD streams/watersheds and identify and isolate TDS and/or sulfate producing materials (reactive materials).

c. For activities associated with oil and gas development, including road construction, drilling, and maintenance, prevent potential spills and groundwater contamination through adherence to contaminant-related BMPs and through increased technical assistance and review by federal and state agencies.

d. Identify areas within the KAD's range where livestock are contributing fecal matter directly into streams; work with NRCS liaisons and private landowners to exclude livestock from these streams and install alternate water supplies/infrastructure.

e. Identify areas within the KAD's range where straight-pipe discharges are causing organic enrichment of streams; work with federal agencies and state and local governments to develop a list of these sources; seek funding for installation of improved sewage treatment.

Objective 3: Investigate range-wide effective regulatory mechanisms

1) Develop minimum standards for regulatory mechanisms (existing or future mechanisms) that should be in place in order to minimize threats to the species.

2) Encourage and assist in the development and implementation of a model CCAA/HCP (preferably one that is range-wide and programmatic) that details effective, measurable conservation objectives and habitat management goals.

3) Pursue conservation agreements with county road departments to minimize and mitigate impacts from bridge/culvert construction and road maintenance activities.

4) Evaluate state regulatory processes to minimize and mitigate the loss and degradation of KAD habitat resulting from surface coal mining activities, logging, agricultural land conversion, residential land development, stream channelization, flood control, and county road maintenance.

5) Enforce or develop state laws/regulations that are protective of the species.

6) Utilize existing Clean Water Act regulatory mechanisms (Sections 401 and 404) to direct stream mitigation activities to locations that contain or could contain KADs.

7) Engage county officials or planners about voluntary conservation efforts for the species.

Objective 4: Investigate other natural or man-made factors affecting its continuing existence

1) Investigate the potential use of captive-reared or translocated KADs to augment a population or re-populate a previously occupied habitat area to increase viability of the general population. KDFWR and CFI have been working on a reintroduction program on the DBNF (Redbird Ranger District) since 2008. Future efforts will follow guidelines established by George et al. (2009) for planning, executing, and monitoring propagation, translocation, reintroduction, and augmentation of KADs.

2) Investigate using Section 6, State Wildlife Grant, or other funding sources to conduct surveys and censuses of large, suitable public and private parcels that contain a substantial amount of potential KAD habitat. Estimate the number of KADs present and evaluate those sites for potential KAD population enhancement or re-establishment. Provide information and incentives to private landowners to manage their land for KAD, possibly working with partners to offer higher cost-sharing for more aggressive habitat management.

3) Increase the size and/or carrying capacity of viable population areas (and areas with KAD populations just below the "viable" threshold) through applied land management, land acquisition, or incentives to adjacent landowners to properly manage for KADs; in order to allow for the potential expansion of those populations.

4) Locate areas where reintroductions and restoration can most effectively be accomplished by creating large, contiguous tracts or habitat corridors that may or may not be occupied by KADs, specifically those directly adjacent to current managed lands.

5) Investigate the interaction of non-native rainbow trout and KADs in Big Double Creek, Clay County (DBNF), and determine if rainbow trout represent a significant predation threat to KAD.

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REFERENCES

Allan J.D. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics 35:257-284.

Allendorf, F.W. and G. Luikart. 2007. Conservation and the genetics of populations. Malden, Massachusetts, Blackwell Publishing. 642 pp.

Bailey, R. M. 1948. Status, relationships, and characters of the percid fish, *Poecilichthys sagitta* Jordan and Swain. Copeia 1948:77-85.

Bailey, R. M. and W. A. Gosline. 1955. Variation and systematic significance of vertebral counts in the American fishes of the family Percidae. Miscellaneous Publications, Museum of Zoology, University of Michigan 93:1-44.

Bailey, R. M., H. E. Winn, and C. L. Smith. 1954. Fishes from the Escambia River, Alabama and Florida, with ecologic and taxonomic notes. Proceedings of the Academy of Natural Sciences of Philadelphia 106:109-164.

Brandt, S. L. 2006. The ichthyofauna of Rock Creek, Kentucky, with observations on the impact of stocking rainbow trout on native fishes. M.S. Thesis, Eastern Kentucky University, Richmond, Kentucky. 59 pp.

Brandt. S. L. 2011. Personal communication regarding fish surveys of Laurel Fork and Cortland Fork, Owsley County, Kentucky (email correspondence). Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky.

Branson, B. A. and D. L. Batch. 1972. Effects of strip mining on small stream fishes in east-central Kentucky. Proceedings of the Biological Society of Washington. 84:507-518.

Branson, B. A. and D. L. Batch. 1974. Additional observations on the effects of strip mining on small-stream fishes in east-central Kentucky. Trans. Kentucky Acad. Sci. 35:81-83.

Branson, B. A. and D. L. Batch. 1983. Fishes of the South Fork of the Kentucky River, with notes and records from other parts of the drainage. Southeastern Fishes Council Proceedings 4:1-15.

Burr, B. M. and M. L. Warren, Jr. 1986. A distributional atlas of Kentucky fishes. Kentucky State Nature Preserves Commission Scientific and Technical Series Vol. 4.

Cook, E.R., C.A. Woodhouse, C.M. Eakin, D.M. Meko, and D.W. Stahle. 2004. Long-term aridity changes in the western United States. Science 306:1015-1018.

Curtis, W. R. 1973. Effects of strip mining on the hydrology of small mountain watersheds in Appalachia. Pages 145-157 *In* R. J. Hutnik and G. Davis. Eds. Ecology and reclamation of devastated land. Vol. 1. Gordon and Breach, New York.

Dyer, K. L. and W. R. Curtis. 1977. Effect of strip mining on water quality in small streams in eastern Kentucky, 1967-1975. USDA Forest Service Research Paper NE-372. Northeastern Forest Experiment Station, Upper Darby, Pennsylvania. 13 pp.

Eschmeyer, W. N. (ed). 2013. Catalog of Fishes. California Academy of Sciences (http://research. calacademy.org/research/ichthyology/catalog/fishcatmain.asp). Electronic version accessed April 12, 2013.

Etnier, D.A. and W.C. Starnes. 1993. The Fishes of Tennessee. The University of Tennessee Press, Knoxville, Tennessee. 681 pp.

George, A. L., B. R. Kuhajda, J. D. Williams, M. A. Cantrell, P. L. Rakes, and J. R. Shute. 2009. Guidelines for propagation and translocation for freshwater fish conservation. Fisheries 34:529-545.

Gilbert, C. H. 1887. Descriptions of new and little known etheostomoids. Proceedings of the United States National Museum 10:47-64.

Greenberg, L. A. and S. Steigerwald. 1981. New distributional record for *Etheostoma sagitta* in Kentucky. Transactions of the Kentucky Academy of Science. 42:37.

Harrel, S. L. and J. B. Baxter. 2012. Distribution, movement, and ecology, of *Etheostoma sagitta spilotum* (Gilbert), the Kentucky arrow darter, in second and third order streams in two subbasins of the Kentucky River basin. Draft research proposal submitted to the U.S. Fish and Wildlife Service, Frankfort, Kentucky. 12 pp.

Harris, L. D. 1984. The fragmented forest. University of Chicago Press. 211 pp.

Hartman, K.J., M.D. Kaller, J.W. Howell, and J.A. Sweka. 2005. How much do valley fills influence headwater streams? Hydrobiologia 532:91-102.

Hitt, N. P. 2014. Landscape modeling of Kentucky arrow darter (*Etheostoma spilotum*) occurrence and response to water quality parameters. Final report, USGS-USFWS Quick Response Research Project. U.S. Geological Survey, Kearneysville, West Virginia. 34 pp.

Hitt, N. P. and M. A. Floyd. 2012. Landscape modeling of Kentucky arrow darter (*Etheostoma sagitta spilotum*) occurrence and response to water quality parameters. USFWS and USGS

Quick Response Program Proposal. USGS Leetown Science Center, Kearneysville, West Virginia and Frankfort, Kentucky. 7 pp.

Hren, J., K. S. Wilson, and D. R. Helsel. 1984. A statistical approach to evaluate the relation of coal mining, land reclamation, and surface water quality in Ohio. U.S. Geological Survey Water Resources Investigations Report 84-4117. 36 pp.

Hunter, M. L., Jr. 2002. Fundamentals of conservation biology, second edition. Blackwell Science, Inc. Malden, Massachussetts. 547 pp.

Jordan, D.S. and J. Swain. 1883. List of fishes collected in the Clear Fork of the Cumberland, Whitley County, Kentucky, with descriptions of three new species. Proceedings of the . United States National Museum 6:248-251.

Kentucky Department of Fish and Wildlife Resources (KDFWR). 2014. Kentucky arrow darter (*Etheostoma spilotum*) reintroduction in Long Fork, Red Bird River drainage, Kentucky. Pages 44-53 *In* KDFWR's Annual Performance Report for Grant #KY-F-F11AF00728 (T-60-R). Frankfort, Kentucky. 188 pp.

Kentucky Department of Fish and Wildlife Resources (KDFWR). 2013. Kentucky's Comprehensive Wildlife Conservation Strategy. KDFWR, Frankfort, Kentucky. Available from: http://fw.ky.gov/kfwis/stwg/ (Date updated 2/5/2013).

Kentucky Division of Water (KDOW). 1996. Kentucky 305(b) report to Congress on water quality. Natural Resources and Environmental Protection Cabinet, Frankfort, Kentucky. 199 pp.

Kentucky Division of Water. 2006. Integrated report to congress on water quality in Kentucky. Volume I. 305(b) assessment results with emphasis on the Kentucky River basin management unit and Salt-Licking Rivers basin management unit. Natural Resources and Environmental Protection Cabinet, Frankfort, Kentucky. 222 pp.

Kentucky Division of Water. 2008. Draft 2008 integrated report to Congress on the condition of water resources in Kentucky. Vol. II. 303(d) list of surface waters. Kentucky Environmental and Public Protection Cabinet, Frankfort, Kentucky. 609 pp.

Kentucky Division of Water. 2011. 2010 Integrated report to Congress on the condition of water resources in Kentucky. Vol. II. 303(d) list of surface waters. Kentucky Environmental and Public Protection Cabinet, Division of Water, Frankfort, Kentucky. 716 pp.

Kentucky Energy and Environment Cabinet (KEEC). 2014. Kentucky quarterly coal report (Q4-2013 year end). Available (pdf format) at http://energy.ky.gov/Coal%20Facts%20 Library/

Kentucky%20Quarterly%20Coal%20Report%20(Q4-2013%20year%20end).pdf. Frankfort, Kentucky. 5 pp.

Kentucky Geological Survey. 2014. Kentucky oil and gas overview: future trends. KGS website, http://www.uky.edu/KGS/emsweb/oginfo/future.html.

Kentucky State Nature Preserves Commission. 2005. Rare and extirpated biota of Kentucky. (pdf file available at: www.naturepreserves.ky.gov). 19 pp.

Kuehne, R. A. 1962. A classification of streams, illustrated by fish distribution in an eastern Kentucky creek. Ecological Society of America 43:608-614.

Kuehne, R. A. and R. W. Barbour. 1983. The American darters. The University of Kentucky Press, Lexington, Kentucky. 177 pp.

Kuehne, R. A. and R. M. Bailey 1961. Stream capture and the distribution of the percid fish *Etheostoma sagitta*, with geologic and taxonomic considerations. Copeia 1961:1-8.

Lotrich, V. A. 1973. Growth, production, and community composition of fishes inhabiting a first- second-, and third-order stream of eastern Kentucky. Ecological Monographs 43:377-397.

Lowe, J. E. 1979. The life history, behavior, and ecology of *Etheostoma sagitta* (Jordan and Swain). Unpublished M.S. thesis, The University of Tennessee. 73 pp.

MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248–2255.

MacKenzie, D.I., J.A. Royle, J.A. Brown, and J.D. Nichols. 2004. Occupancy estimation and modeling for rare and elusive populations. Pp. 149–172, *In* W.L. Thompson (Ed.). Sampling Rare or Elusive Species: Concepts, Designs, and Techniques for Estimating Population Parameters. Island Press, Washington, DC. 429 pp.

Mattingly, H.T., J. E. Detar, B. K. Jones, and C. F. Walton. 2005. Factors affecting the distribution and recovery of the threatened blackside dace in Kentucky and Tennessee. Unpublished final project report prepared for the U.S. Fish and Wildlife Service, Tennessee Ecological Services Field Office. Cookeville, Tennessee. 126 pp.

Maurer, E. P., L. Brekke, T. Pruitt, and P. B. Duffy (2007). Fine-resolution climate projections enhance regional climate change impact studies. EOS Transactions American Geophysical Union 88(47):504.

Messinger, T. 2003. Comparison of storm response of streams in small, unmined and valley-filled watersheds, 1999-2001, Ballard Fork, West Virginia. Water-Resources Investigations Report 02-4303. US Geological Survey, Charleston, West Virginia.

McLaughlin, J.F., J.J. Hellmann, C.L. Boggs, and P.R. Ehrlich. 2002. Climate change hastens population extinctions. Proceedings of the National Academy of Sciences of the United States of America 99(9):6070-6074.

Meyer, J. L. and A. B. Sutherland. 2005. Effects of excessive sediment on stress, growth, and reproduction of two southern Appalachian minnows, *Erimonax monachus* and *Cyprinella galactura*. Unpublished final project report for grant #1434-HQ-97-RU-01551, Department of Interior. University of Georgia, Institute of Ecology. Athens, Georgia.

Milici, R. C., and K. O. Dennen. 2009. Production and depletion of Appalachian and Illinois Basin coal resources. Chapter H *In* B. S. Pierce and K. O. Dennen, Eds. The National Coal Resource Assessment Overview: U.S. Geological Survey Professional Paper 1625–F. 402 pp.

Noss, R. E. and A. Y. Cooperrider. 1994. Saving nature's legacy. Protecting and Restoring Biodiversity. Island Press. California. pp. 416.

Page, L. M. 1983. Handbook of darters. T. F. H. Publications, Inc., Neptune City, New Jersey. 271 pp.

Page, L. M. and B. M. Burr. 2011. Peterson field guide to freshwater fishes of North America north of Mexico (second edition). Houghton Mifflin Harcourt, Chicago, Illinois. 663 pp.

Page, L. M. and G. S. Whitt. 1973. Lactate dehydrogenase isozymes, malate dehydrogenase isozymes and tetrazolium oxidase mobilities of darters (Etheostomatini). Comparative Biochemistry and Physiology 44B:611-623.

Palmer, M. A., E. S. Bernhardt, W. H. Schlesinger, K. N. Eshleman, E. Foufoula-Georgiou, M. S. Hendryx, A. D. Lemly, G. E. Likens, O. L. Loucks, M. E. Power, P. S. White, and P. R. Wilcock. 2010. Mountaintop mining consequences. Science 327:148-149

Papoulias, D. M. and A. L. Velasco. 2013. Histopathological analysis of fish from Acorn Fork Creek, Kentucky, exposed to hydraulic fracturing fluid releases. Southeastern Naturalist 12:92-111.

Paybins, K. S., T. Messinger, J. H. Eychaner, D. B. Chambers, and M. D. Kozar. 2000. Water quality in the Kanawha-New river basin, West Virginia, Virginia, and North Carolina, 1996-98. Circular 1204, U.S. Geological Survey, Washington, D.C.

Pond, G. J. 2004. Effects of surface mining and residential land use on headwater stream biotic integrity in the eastern Kentucky coalfield region. Kentucky Department for Environmental Protection, Division of Water, Frankfort, Kentucky. 48 pp.

Pond, G. J. 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). Hydrobiologia 641:185–201.

Pond, G. J., M. E. Passmore, F. A. Borsuk, L. Reynolds, and C. J. Rose. 2008. Downstream effects of mountaintop coal mining: comparing biological conditions using family- and genus-level macroinvertebrate bioassessment tools. Journal of the North American Benthological Society. 27:717-737.

Ray, J. M. and P. A. Ceas. 2003. Fish survey and bioassessment of Station Camp Creek and Sturgeon Creek in the upper Kentucky River drainage. Southeastern Fishes Council Proceedings 46: 1-15.

Ruble, C., P. Rakes, M. Petty, and J. R. Shute. 2010. Captive propagation and reintroduction of the Kentucky arrow darter, *Etheostoma sagitta spilotum*, in the upper Kentucky River drainage of Kentucky. Conservation Fisheries, Inc. Interim performance report submitted to Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky. 8 pp.

Soule, M.E. 1980. Threshold for survival: maintaining fitness and evolutionary potential. Pages 151-169 *In* M.E. Soule and B.A. Wilcox, eds. Conservation biology. Sinauer Associates, Inc., Sunderland, Massachusetts.

The Nature Conservancy. 2013. ClimateWizard website. http://www. Climate wizard. org/. Accessed September 25, 2013.

Thomas, C. D., A. Cameron, R.E. Green, M. Bakkenes, L.J. Beaumont, Y.C. Collingham, B.F.N. Erasmus, M Ferreira de Siqueira, A. Grainger, L. Hannah, L. Hughes, B. Huntley, A.S. van Jaarsveld, G.F. Midgley, L. Miles, M.A. Ortega-Huerta, A.T. Peterson, O.L. Phillips, and S.E. Williams. 2004. Extinction risk from climate change. Nature 427:145-148.

Thomas, M. R. 2008. Distribution and status of the Kentucky arrow darter (*Etheostoma sagitta spilotum*). Kentucky Department of Fish and Wildlife Resources. State and Tribal Wildlife Grant T-9-RSI-1. 9 pp. + figures.

Thomas, M. R. 2010. Personal communication regarding the general abundance of Kentucky arrow darters (email correspondence). Ichthyologist, Kentucky Department of Fish and Wildlife Resources, Frankfort, Kentucky.

Thomas, M. R. and S. L. Brandt. 2012. Kentucky Arrow Darter (*Etheostoma spilotum*) reintroduction in Sugar Creek, Red Bird River Drainage, Kentucky: 2009-2012 monitoring results. Kentucky Department of Fish and Wildlife Resources. State and Tribal Wildlife Grant T-60-R. 11 pp.

Thomas, M. R. and R. B. Johansen. 2008. Systematics and conservation status of the arrow darter, *Etheostoma sagitta* (Percidae: *Litocara*). Page 46 In Southeastern Fishes Council Annual Meeting Abstracts (Contributed Oral Presentation), Chattanooga, Tennessee. 70 pp.

Townsend, L. and L. Rieske-Kinney. 2009. Meeting the threat of the hemlock wooly adelgid. ENTFACT-42. University of Kentucky, Cooperative Extension Service, College of Agriculture. Lexington, Kentucky. 3 pp.

U.S. Environmental Protection Agency (USEPA). 2003. Affected environment and consequences of mountaintop/valley fill coal mining. Chapter III of draft programmatic environmental impact statement. Region 3, US EPA, Philadelphia, Pennsylvania. 318 pp. (Available from: http://www.epa.gov/region3/mtntop/index.htm).

U.S. Fish and Wildllife Service (USFWS). 2009. Project report: Kentucky arrow darter surveys in eastern Kentucky. Kentucky Ecological Services Field Office. Frankfort, Kentucky. 4 pp.

U.S. Fish and Wildllife Service (USFWS). 2010. Project report: Kentucky arrow darter surveys in eastern Kentucky. Kentucky Ecological Services Field Office. Frankfort, Kentucky. 13 pp.

U.S. Fish and Wildllife Service (USFWS). 2013. United States Fish and Wildlife Service species assessment and listing priority assignment form. Available from: http://ecos. fws.gov/docs/candidate/assessments/2014/r4/E0BF_V01.pdf.

U.S. Forest Service (USFS). 2004. Land and Resource Management Plan, Daniel Boone National Forest. Winchester, Kentucky.

Wahrer, R. 2013. Personal communication regarding the number of surface mining permits in Kentucky (email correspondence). Kentucky Department of Natural Resources, Frankfort, Kentucky.

Waters, T. F. 1995. Sediment in streams: sources, biological effects, and control. American Fisheries Society Monograph 7, Bethesda, Maryland.

Wiley, J. B., R. D. Evaldi, J. H. Eychaner, and D. B. Chambers. 2001. Reconnaissance of stream geomorphology, low streamflow, and stream temperature in the mountaintop coal-mining region, southern West Virginia, 1999-2000. Water-Resources Investigations Report 01-4092, U.S. Geological Survey, Charleston, West Virginia.

Williams, J. 2014. Personal communication regarding trout stocking in Big Double Creek, Clay County, Kentucky (email correspondence). Southeastern District Fisheries Biologist, Kentucky Department of Fish and Wildlife Resources, Somerset, Kentucky.

Wood, P. J and P. D. Armitage. 1997. Biological effects of fine sediment in the lotic environment. Environmental Management 21:203-217.