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JOURNAL OF CRUSTACEAN BIOLOGY, 36(5), 761-766, 2016

Journal of Crustacean Biology

COLLECTING AND PROCESSING FRESHWATER CRAYFISHES

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KEY WORDS: Astacoidea, cave crayfishes, ecology, Parastacoidea, preservation

DOI: 10.1163/1937240X-00002466

INTRODUCTION

Freshwater crayfishes (sometimes called "crawfish" and by a wide variety of common and/or local names (Hart, 1994)) are a taxonomically diverse group with over 640 described species worldwide (Crandall and Buhay, 2008). Crayfishes are a monophyletic group that is a sister group to the clawed lobsters (Nephropoidea Dana, 1852) (Crandall et al., 2000). Crayfishes are classified into two superfamilies, Astacoidea Latreille, 1802 (Northern Hemisphere) consisting of two families (Cambaridae Hobbs, 1942 and Astacidae Latreille, 1802), and Parastacoidea Huxley, 1879 (Southern Hemisphere) with a single family, Parastacidae Huxley, 1879 (see Hobbs, 1974). Some of the groups are in need of a complete taxonomic reworking and there have been a number of more recent studies that can aid in appropriate assignments (Fratini et al., 2005; Larson et al., 2016).

The separation of these superfamilies represents a historical biogeographic division of Pangaea into the Laurasian and Gondwanan supercontinents (approximately 200 mya). Consequently, the freshwater crayfishes constitute a very old organismal group and show a distribution consistent with Triassic dinosaurs (Breinholt et al., 2009). Furthermore, the phylogenetic connection of the Southern Hemisphere crayfishes shows a distinct linkage of southern South America, Madagascar, New Zealand, and Australia to the exclusion of continental Africa, a biogeographic distribution of considerable historical debate (Toon et al., 2010). The antiquity of crayfishes is supported by recent fossil evidence from Colorado and Utah with fossil crayfishes and burrows associated with Permian and Early Triassic (265 mya) deposits (Hasiotis et al., 1993) and by time-tree analyses calibrated with multiple fossil records (Bracken-Grissom et al., 2014).

ECOLOGY

Freshwater crayfishes occupy four main habitat types: streams, lakes/ponds, burrows, and caves. Species within each habitat type have distinct morphological features that are common to the crayfishes that inhabit these ecosystems. For example, primary burrowers spend their entire life cycles in burrows and some have vaulted carapaces to accommodate larger gill surface areas in the oxygen-poor terrestrial habitat. Burrowers often dig down to the groundwater table and have robust pinchers for digging and burrow protection. Stream specialists typically have large abdomens (pleons) for swimming and predator escape and are highly intolerant of low oxygen content in the water. Cave species have the typical suite of cave-adapted morphologies (see below). Lake/pond species are typically similar to stream species but with less developed abdomens. Crayfishes can often have exotic coloration patterns that can provide camouflage with respect to the stream substrate. Most crayfishes have a life span of about two years, although some surface and cave species can live beyond 20 years. Freshwater crayfishes are omnivorous and typically nocturnal. They are voracious eaters and can be extremely destructive when introduced to nonnative habitats. A few species are particularly invasive (e.g., *Orconectes rusticus* (Girard, 1852)) and/or were distributed in the aquaculture trade (e.g., *Procambarus clarkii* (Girard, 1852)) and via bait-bucket introductions. To prevent such invasions and their destructive impact (Jones et al., 2009), it is therefore critical to keep aquarium crayfishes out of natural waters and not move native crayfishes from one location to another. These introduced species wreak havoc on natural ecosystems, but the vast majority of freshwater crayfishes are extremely narrowly distributed and hence are endangered due mainly to the destruction of the freshwater ecosystems in general.

COLLECTION

Crayfish collecting is a wonderful experience and one that is a fond remembrance of most people who grow up in crayfish country. Often the best collecting technique for freshwater crayfish is to find a local 9 to 12 year old, and he or she will collect all the crayfish you might want. The collecting approaches for freshwater crayfish usually depend on the species of crayfish and on the main habitat type within

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DOI:10.1163/1937240X-00002466

which the target species occurs. When targeting sampling for species delimitation studies and other taxonomic work, it is important to collect nominal taxa from their type locality for reference and to keep voucher specimens for molecular-based studies.

Streams

Streams are without a doubt the easiest and most pleasant of the crayfish habitats within which to collect. Unlike the collection of fish, stream poisons or electro-shockers do not work particularly well because the crayfish are shocked or poisoned under rocks and usually do not float up to be captured. Instead, the simplest approach is to find a shallow stream and flip rocks and catch the crayfish by hand. A good trick is to put your hand on top of the crayfish and pin it against the substrate before you worry about picking it up without getting pinched. Stream crayfish, especially, are fast. If you find them too quick for your reaction time, then grab a dip net and place it behind the crayfish and move your other hand in front of it to scare it into your net (Fig. 1). Be sure to sample in the different microhabitats of the streams too. You can often find different species in the slow, side areas with leaf litter compared to riffle areas, or to stream pools. Crayfish are generally nocturnal, thus they are out and about at night, which makes for relatively easy collecting. Using a flashlight, you will see that the eyes of crayfish reflect the light and thus they are easily identifiable in streams.

Lakes and Standing Waters

Collecting from lakes, ponds, or pools of big rivers can be more challenging than from streams because the deep water provides for greater escape opportunities for crayfish and often there are much larger substrate options to hide under. Here, again, flipping rocks and collecting by hand is effective along the lake's edge. The best way to collect is to use a mask and snorkel to allow for short surface dives to the substrate for searching. If you have a reasonable amount of patience, an alternative technique is to simply use a standard minnow trap and bait it with fish or chicken. There are also traps especially made for commercial crayfish harvesting that would work well for collecting from lakes and ponds. The principle is the same as with a baited minnow trap. The art of crayfish trapping has seen a wide variety of designs and has been an ongoing activity for a long time (O'Roke, 1922). A variety of traps designed especially for freshwater crayfishes based on a minnow trap model and other designs are available (http://www.terrybullard.com).

Road-side ditches and other standing water habitats are also popular for a variety of crayfish species. Indeed, *Cambaroides* Faxon, 1884 is found exclusively in such habitat. These shallow water, often temporary habitats can be collected most easily in the rainy season by dragging a net or seine through the water or with a quick survey using a dip net being careful to keep the net at the bottom of the water and to move from deep to shallow, especially across weedy areas. Often these habitats dry out over the summer (especially in the southern USA), and the crayfish burrow down with the receding water table. Dragging a net through the temporary water is much easier than digging up burrows.

Fig. 1. Collecting crayfish by dip net is one of the most effective methods for collecting along roadside ditches, temporary waters, rivers, streams, and lakes.

Burrows

A more challenging group of freshwater crayfishes to collect is the primary burrowers. Burrowing crayfishes have been classified as primary burrowers (those that spend their entire life cycles in burrows), secondary burrowers (those that occupy burrows for the majority of their life cycle but venture into open waters for foraging or reproductive purposes during rainy seasons and when the water table rises), and tertiary burrowers (those that live mainly in open waters but construct burrows during drought or when the female is gravid) (Hobbs, 1981). For secondary and tertiary burrowers, specimens often can be collected by hand, dip net, or seine. These techniques are especially good for collecting juveniles and/or reproductively active individuals, including females in berry (with eggs or young attached to the abdomen). The primary burrowers, however, are a different story and finding them presents a problem because they do not necessarily occur near standing water (Fig. 2). They can be found in forest depressions, open fields, and other habitats. You can often identify the occurrence of burrowing crayfish by finding the characteristic "chimneys" of the burrows, the dirt piled up, often in stacked balls, from the crayfish burrowing activity that creates a volcano-like structure atop the opening in the ground. The best way to collect such crayfishes is to simply dig for them (Fig. 3). Burrowing systems themselves have been classified into three categories as well. Type I burrows occur in permanent waters or are connected to permanent waters, type II burrows are connected to the water table, and type III burrows are independent of the water table (Horwitz et al., 1986). These latter burrow systems receive enough water through regular rainfall that they keep abundantly moist; this type of burrow is unique to Australian crayfishes. Most other primary burrowers have type II burrows. The favorite collecting technique is similar for the three types, and that is to simply excavate the burrows by hand with the help of a trowel or shovel. As one excavates, the crayfish moves to the bottom of the burrow, so depending on the species, you can dig for a long time before you encounter the crayfish. Crayfish burrows also make great habitats for a variety of animals, including snakes, many of which can be poisonous (e.g., tiger snakes in Australia, cottonmouths in the USA), so due caution should be exercised when putting one's hand down a crayfish burrow! Burrowing crayfish often can be collected on rainy or otherwise moist nights when they venture out of their burrows to forage. Again, a good flashlight or head-lamp will reflect their eyes, and a quick hand can grab them before they get back into their burrows. Another approach for the more patient is to bait the burrows with a piece of meat (chicken) on a string. Gently slide the bait into the burrow, and when the crayfish grabs the bait, gently retract the bait with the string from the burrow. The crayfish is typically very reluctant to give up its prize and will come out still holding onto the bait. This technique can also be used in lakes and ponds.

Digging up type I burrows can be more difficult, as the crayfish can escape into the permanent waters to which the burrows are connected once you start disturbing the burrows. A "yabby pump" can aid significantly in capturing

Fig. 2. Burrowing crayfish can exist independent of flowing water, as in crayfish that have dug burrows throughout a school's playground in central Arkansas, USA.

Fig. 3. Some burrowing crayfish have burrows that are quite extensive and require substantial effort to excavate and collect the inhabitants.

the inhabitants of burrows. "Yabby" is the Australian name for certain freshwater crayfish species typically of the genus *Cherax* Erichson, 1846, although the name also refers to marine yabbies or ghost shrimps (Thalassinidea). The yabby pump is placed in the mouth of the burrow and the plunger pulled to create a suction that pulls the crayfish out of the burrow and into the tube (typically PVC pipe). The crayfish (hopefully), mud, and water are then flushed out onto the ground where the crayfish can be collected by hand. For the yabby pump to work efficiently, the connecting water level must be relatively high compared to the burrow or there will not be enough water to flush the burrow effectively.

Caves

Perhaps the most exciting and certainly the most exotic habitat from which to collect crayfishes is the cave habitat. Stygobitic species are found in four genera within Cambaridae (Hobbs and Barr, 1972; Hobbs, 1977; Hobbs et al., 1977) and show convergent morphological evolution of adaptations to the subterranean environment (Fig. 4). Cave crayfishes are the most difficult to collect because of the technical difficulties of the cave environment. Caving takes special care and expertise, along with special gear including headlamps, helmets, ropes, and other light sources. One should follow established caving safety approaches as outlined by caving organizations like the National Speleological Society (www.caves.org). Cave crayfishes also pose a particular problem in that they have access to much more of the underground aquifer than you do (Buhay and Crandall, 2005), so cave collecting takes a certain amount of luck as well as skill. Cave crayfishes are typically not abundant in a given cave and in the potentially small amount of exposed water within the cave. Crayfishes are easy to spot in caves if they are present as they have no pigmentation and do not usually flee from light. They will flee as soon as you place a hand or foot into the water, so you must be quick with a dip net or hand depending on the water depth. You can also use a baited trap to effectively collect cave crayfish from exposed sections of the underground aquifer within the cave.

Note that in most areas collecting freshwater crayfishes only requires a current fishing license. In some regions, however, more extensive permitting is required to legally collect certain species. This is especially true for cave environments because caves are often on private land that require permission to enter the cave. Cave crayfishes are often endangered, requiring special collecting permits to collect from managed areas in state and national parks (see Martin, 2016).

Preservation

Freshwater crayfishes should be preserved in 70% ethanol, an argument made by H. Hobbs Jr. throughout his career. His opinion was based on the fact that crayfishes fixed in formalin become brittle and are nearly impossible to work with after a few years of preservation. DNA can be isolated from specimens collected by Hobbs 70 years ago, and such samples have been imperative in studies on the status of species (Crandall et al., 2009). Given that 70% ethanol is safer, more readily available, and preferred for long-term preservation, there is simply no reason to use formalin. Those who use formalin argue that it "preserves color," which it will for a week or so. But nothing preserves color like a color photograph. If one envisions DNA studies, it is best to sample tissue and preserve it in 95-100% ethanol and keep these tissues frozen until DNA extraction. It is best to extract DNA promptly upon returning to the laboratory and then to preserve the DNA in 100% ethanol for long-term storage. Gill tissue works best for mitochondrial DNA extraction because the gills contain a large number of mitochondria. Gills can also be taken from the specimen with minimal invasion by simply sliding the forceps under the carapace to take gill tissue. If laboratory-grade ethanol is not locally available, isopropanol ("rubbing alcohol") works fine for specimen preservation. Isopropanol or rum work fine for short-term preservation. Specimens and tissue samples will preserve better if kept cool. After collecting, we typically wrap specimens in ethanol or isopropanol-soaked paper towels and bag them in plastic bags for transport to the laboratory, where they are placed in 70% ethanol for long-term storage in a museum collection.

Fig. 4. Cave crayfish demonstrating the typical cave adaptations of loss of pigment, loss of eyes, and elongated antennae.

ACKNOWLEDGEMENTS

I thank Jennifer Buhay and David Stern for helpful comments on the manuscript. This work was supported by the US National Science Foundation (EF-0531762).

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RECEIVED: 26 June 2016.
ACCEPTED: 27 June 2016.
AVAILABLE ONLINE: 13 July 2016.