

Survey techniques for giant salamanders and other aquatic Caudata

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Abstract.—The order Caudata (salamanders and newts) comprise ~13% of the ~6,800 described amphibian species. Amphibians are the most threatened (~30% of species) of all vertebrates, and the Caudata are the most threatened (~45% of species) amphibian order. The fully aquatic Caudata family, the Cryptobranchidae (suborder Cryptobranchoidea), includes the the world’s largest amphibians, the threatened giant salamanders. Cryptobranchids present particular survey challenges because of their large demographic variation in body size (from three cm larvae to 1.5 m adults) and the wide variation in their habitats and microhabitats. Consequently, a number of survey techniques (in combination) may be required to reveal their population and demography, habitat requirements, reproduction, environmental threats, and genetic subpopulations. Survey techniques are constrained by logistical considerations including habitat accessibility, seasonal influences, available funds, personnel, and equipment. Particularly with threatened species, survey techniques must minimize environmental disturbance and possible negative effects on the health of targeted populations and individuals. We review and compare the types and application of survey techniques for Cryptobranchids and other aquatic Caudata from a conservation and animal welfare perspective.

Key words. Survey techniques, giant salamander, amphibian, Caudata, Cryptobranchid, conservation

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Introduction

Amphibians are suffering from one of the greatest rates of decline and extinction of any vertebrate class. One of the most unique, iconic, and threatened amphibian clades in the Caudata are the fully aquatic Cryptobranchids (family Cryptobranchidae; suborder Cryptobranchoidea). All three Cryptobranchids are fully aquatic and include the world’s largest amphibians: the Critically Endangered, Chinese giant salamander (*Andrias davidianus*), the Near Threatened, Japanese giant salamander (*A. japonicus*), and the North American giant salamander (*Cryptobranchus alleganiensis*), commonly known as the Hellbender (CNAH 2011).

The conservation potential of Cryptobranchids extends beyond their immediate conservation needs. As iconic species, Cryptobranchids offer an ideal opportunity to develop public awareness and government and

institutional support for water catchment management. In Japan, *A. japonicus* has become a national symbol, attracting publicity including parades with large floats, education and environmental awareness campaigns, and village conservation programs. Similarly, in the People’s Republic of China, the release of *A. davidianus* from farm stock has received widespread government support and formal public recognition, and this species is becoming a symbol for watershed conservation. There is also an increasing momentum toward establishing *C. alleganiensis* as an icon for watershed conservation in the USA (Browne et al. 2012a, b).

However, in addition to public and government support, the conservation of Cryptobranchids and other aquatic Caudata relies upon scientific knowledge of their conservation genetics, population demography and size, habitat and microhabitat variables, reproduc-

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Figure 1. *Andrias davidianus* is the largest and most threatened Cryptobranchid, and can reach 200 cm in total length and 59 kg in weight. Image Robert Browne.

tion and life stage survival, and environmental threats. The most appropriate survey techniques to achieve this knowledge will depend on survey objectives in concert with logistical constraints including the type of habitat surveyed (Dodd 2009). The choice of survey techniques must consider interacting factors, including the species' autecology, targeted life stages, and season, as well as water depth, velocity, and clarity (Dodd 2009). Survey techniques must minimize environmental disturbance and possible negative effects on the health of the targeted individuals and populations through the spread of pathogens and trauma to individuals.

The conservation needs of Cryptobranchids vary widely between the three species. *Andrias davidianus* was until recently considered almost extinct in nature. However, recent evidence shows that there are a number of relict populations distributed throughout China. The few remaining populations (in lowland areas) are fairly genetically homogenous, probably due to anthropogenic transport and the building of canals over China's ~6,000 year history of civilization. Nevertheless, there are genetically distinct populations remaining (Tao et al. 2005), and ongoing molecular studies may reveal even finer population structure (R. Murphy, pers. comm.) and further Evolutionarily Significant Units (Crandall et al. 2000).

Andrias davidianus has a considerable aquaculture potential, and more than 1000 licensed aquaculture facilities are in production in China with up to 106 individuals in stock. In concert with aquaculture, there are an increasing number of restocking programs using aquaculture brood stock. However, aquaculture brood stock is subject to genetic drift, a process that reduces genetic diversity over generations. Additionally, the source of the aquaculture brood stock is often unknown, and examples such as the unmanaged release and escape of aquaculture stock of Pacific salmon (*Oncorhynchus* spp.) have resulted in a loss of genetic variation or out breeding in wild populations (Reisenbichler and Rubin 1999). Therefore, surveys are needed at all potential release sites to reveal the presence of relictual populations to avoid compromising the long-term conservation of *A. davidianus* and

other Cryptobranchids. Their population genetics must also be assessed to enable the provision of genetically competent individuals for release (Reisenbichler and Rubin 1999)

Consequently, the major conservation needs of *A. davidianus*, besides watershed restoration, limiting wild harvest, and pathogen management, are assessing the presence of relictual populations and their conservation genetics, and then matching the genetics of released stock with those found in nature. When these requirements are satisfied, the survey focus must include selecting suitable release sites, then release of juveniles or adults, and ongoing assessment of the survival and reproduction of released individuals. Because there are few remaining *A. davidianus* in nature, it will be difficult for surveys to associate habitat variables with carrying capacity (Zhang et al. 2002). However, surveys can identify remaining populations, provide genetic samples, and assess the success of restocking programs (Wang et al. 2004).

The conservation of *A. japonicus* relies on the maintenance of the populations that generally still remain in suitable habitats (Tochimoto et al. 2008). Although *A. japonicus* was harvested in the past, strict protection is now in place to prevent this species from exploitation. However, threats include habitat modification and other anthropogenic changes, including pollutants, and the introduction of *A. davidianus* in some systems. Consequently, the conservation needs of *A. japonicus* include surveying



Figure 2. Genetic drift and selection for color traits in *A. davidianus* have resulted in orange, piebald, and albino strains. Image Robert Browne.



Figure 3. *Andrias japonicus* is the second largest Cryptobranchid and reaches 150 cm in total length and 44 kg in weight. Image Sumio Okada.

population densities and demography, habitat variables including channelization and watershed characteristics, assessing the effects of obstacle removal to migration, such as dams, and the provision of artificial habitats on survival and recruitment (Browne et al. 2012a, b).

The conservation needs of *C. alleganiensis* include identifying the most enigmatic threat to any Cryptobranchid and perhaps any amphibian species. *Cryptobranchus alleganiensis* has generally been declining over most of its range (Wheeler et al. 2003; Foster et al. 2009), to some extent due to habitat degradation and modification. However, *C. alleganiensis* still survives in near historic numbers in some locations, and some habitats modified by siltation and agricultural development still support substantial numbers of *C. alleganiensis*. However, the recruitment of *C. alleganiensis* has failed for decades over a substantial part of its range due to unknown causes, and many of these declining populations are now comprised of only a few old individuals (D. McGinnity, pers. comm.).

Cryptobranchus alleganiensis is subject to many ongoing surveys; however, these research activities have not revealed the cause of poor recruitment (Wheeler et al. 2003; Foster et al. 2009). Addressing this problem will require targeting the life history stage where the failure of recruitment occurs, from mating success through fertilization, to egg development, and larval and juvenile survivorship. Surveys will need to correlate recruitment to different life history stages with environmental variables such as pollutants. Attempts to reproduce *C. alleganiensis* in captivity for restocking are in the early stages of development, and no larvae have been produced. However, the production of large numbers of individuals from wild eggs has been successful and their release to natural habitats is underway. The cryopreservation of sperm is now being undertaken to perpetuate the genetic variation of populations with poor or no recruitment (National Geographic 2010; Michigan State University 2010). In addition, research has been initiated to provide a suite of

reproduction technologies to produce genetically competent individuals (D. McGinnity, pers. comm.).

Cryptobranchids present particular survey challenges because of their large variation in body size, from three cm larvae to 1.5 m adults. Additional challenges include the wide variation in their aquatic habitats (deep turbulent water, shallow riffles, pools, lakes) and varied microhabitats (crevices, large rocks, pebble bed in riffles) (Nickerson and Krysko 2003; Tao et al. 2004; Okada et al. 2008). The habitats of *A. japonicus* and *C. alleganiensis* are relatively accessible, but, the habitat of *A. davidianus* includes difficult to survey, rugged, remote, fast-flowing interior rivers in the mountainous areas of central China (Tao et al. 2004).

Effective survey methods depend on associating the life stages of target species with their microhabitats. Adult Cryptobranchids live in cavities, under large rocks, and in bank-side dens. Because of the low population densities of the relic populations of *A. davidianus*, recent surveys have relied on the observation of adults, electrofishing and the use of bow hooks (Wang et al. 2004). Surveys for adult and subadult *A. japonicus* in their habitats of slow flowing rivers have largely relied on direct observation with some netting (Okada et al. 2008). In contrast, surveys of adult and subadult *C. alleganiensis* have used a wide variety of techniques, including rock turning while snorkeling or, in deeper water, scuba diving or trapping (Nickerson and Krysko 2003; Foster et al. 2008). Recent innovations in survey techniques for *C. alleganiensis* include the use of artificial spawning sites to reveal reproductive success. The use of video cameras has the potential to increase observations of mating, brooding by males, and the development of oocytes and larvae. Environmental DNA (eDNA) detection (Goldberg et al. 2011) has the potential to both detect Cryptobranchids and to estimate their standing biomass and population. Radiotelemetry offers an opportunity to survey the movements and survival of an increasing size range of Cryptobranchids over an extended period (Kenward 2001).

Andrias japonicus and *C. alleganiensis* larvae and early juveniles are encountered less frequently than adults due to their particular microhabitats and to the low larval recruitment of *C. alleganiensis* in some regions (Nickerson and Krysko 2003; Okada et al. 2008). In contrast, the larvae of *A. davidianus* were commonly found in surveys of shallow mountain streams in the Qin Ling Mountains until their populations rapidly declined in the early 1980s (Zhang et al. 2002). Okada et al. (2008) found recently-hatched larvae of *A. japonicus* in pools under leaf litter or undercut banks, whereas more developed *A. japonicus* larvae were found under rocks and in gravel beds. Adults can be found in bunk burrows or among deeper rocks or branches. Although little is known about the microhabitat of the larval stages of *C. alleganiensis*, observations suggest that both larvae and small juveniles inhabit interstitial spaces under river gravel in riffles (Nickerson and

Krysko 2003; Foster et al. 2008). Juvenile and subadult *C. alleganiensis* most frequently occur in clean, rock-based streams, although they are also found in deeper pools with rocks, vegetation, and snags (Nickerson and Krysko 2003).

The efficacy of survey methods can vary through the interaction of climate and season with diel activity cycles. For example, the nocturnal activity of *C. alleganiensis* in streams of southeastern North America is positively correlated with high water levels (Humphries and Pauley 2000). Nocturnal surveys are most productive in late spring and early summer, whereas wire mesh baited traps were most efficient from early winter to late spring (J. Briggler, pers. comm.). Recent survey innovations for *C. alleganiensis* include the use of artificial breeding dens for adults, egg masses, and larvae, and the placement of natural rocks to provide habitat. Safeguarding the health and reproductive success of Cryptobranchids is critical when choosing survey techniques. Techniques necessitate minimal disturbance to the habitat, the use of sanitary procedures to prevent pathogen dissemination, and the protection of nest sites. If possible, several survey techniques should be used concurrently to improve survey accuracy and minimize sampling bias (Nickerson and Krysko 2003).

Survey design needs to incorporate the recognition of potential biases through the choice of technique, surveyed microhabitat, species, and life stage (Dodd 2009). Nowakowski and Maerz (2009) tested the efficacy of surveys of larval stream salamanders by comparing the mark-recapture success of passive leaf litter trapping and dip netting. Significant size bias occurred, with traps capturing a higher proportion of large individuals and dip netting yielding a greater proportion of smaller size classes. The survey efficiency of first and second order streams was greater at low salamander densities with time-constrained opportunistic sampling, but greater with quadrat sampling when salamanders were at high densities (Barr and Babbitt 2001). Nowakowski and Maerz (2009) concluded that the physical dynamics



Figure 4. *Cryptobranchus alleganiensis* has been the subject of the most diverse and innovative survey methods of all Cryptobranchids. Image Dale McGinnity.



Figure 5. Natural rock placed in stream to provide habitat and sampling locations for *C. alleganiensis*. Image Kenneth Roblee.

of water bodies and geographic region are primary considerations when selecting the most promising season for surveying different life stages.

An important consideration when surveying Cryptobranchids and other aquatic Caudata is the prevention and spread of infectious diseases. Chytridiomycosis (Chytrid; *Batrachochytrium dendrobatidis*) is an infectious disease of particular conservation concern for amphibians. Chytrid is an emerging pathogen that can regionally extirpate up to 90% of species and 95% of individuals in naive populations, at least among frogs (Lips et al. 2005). However, the effect of chytrid on Cryptobranchids has not been significant. One strain of chytrid has been suggested as endemic to populations of *A. japonicus* (Goka et al. 2009), and an undetermined strain of chytrid is found on mainland Asia in South Korea and may eventually impact *A. davidianus* (Yang et al. 2009).

Chytrid has been shown to be pathogenic in captive populations of *C. alleganiensis* (Briggler et al. 2007, 2008), although with apparently few, if any, pathological effects on natural populations. Nevertheless, good sanitation is a primary consideration in surveying Cryptobranchids, and other amphibians as a precaution against spreading chytrid. The same sanitary procedures will also prevent the spread of pathogens to other species of animals and plants. Another main pathogen currently threatening Cryptobranchids and other amphibians is *Ranavirus* (Geng et al. 2011). To prevent the spread of both amphibian chytrid and *Ranavirus*, equipment should be thoroughly sanitized when moving among aquatic systems, including all instruments, containers (e.g., measuring boards, weighing containers, and other instruments and equipment used), human body parts (hands), and clothing (especially, boots and waders) that come into contact with amphibians and their environment.

We review and compare the types and application of survey techniques for Cryptobranchids and other aquatic Caudata from a conservation and animal welfare perspective. Reviews or comparative studies of survey techniques for Cryptobranchids include Nickerson and

Krysko (2003; *C. alleganiensis*), Wang et al. (2004; *A. davidianus*), Okada et al. (2008, 2006; *A. japonicus*), and Dodd (2009) for general survey techniques of amphibians.

Survey techniques we review include: 1) *Wading, turning substrate, netting, and snorkeling*, 2) *Scuba/hookah diving*, 3) *Nocturnal spotlighting*, 4) *Bow-hooks/trot-lines*, 5) *Questionnaires*, 6) *Electrofishing*, 7) *Underwater camera systems*, 8) *Passive integrated transponders (PIT tags) and mark-recapture*, 9) *Radiotelemetry*, 10) *Modular artificial spawning dens and rock substrate placement*, 11) *Wire mesh baited traps*, 12) *Population genetic techniques*, and 13) *Environmental DNA (eDNA) detection*.

Review of survey techniques

1. Wading, turning substrate, netting, and snorkeling

Wading and turning substrate, coupled with snorkeling and downstream netting and seining, are widely used techniques for surveying *C. alleganiensis* and other Cryptobranchids (Taber et al. 1975; Peterson et al. 1983, 1988; Nickerson and Krysko 2003). These techniques are considered the most effective techniques in relatively clear shallow streams and pools less than one meter in depth with a substrate of rocks and other loose shelters (Nickerson and Krysko 2003). Cryptobranchids can be surveyed through blind searches by reaching beneath large rocks or within hollow logs or holes in banks. These techniques have resulted in the detection of hundreds to thousands of *C. alleganiensis* in some surveys (Taber et al., 1975; Peterson et al. 1983, 1988).

Snorkeling is another common technique for surveying *C. alleganiensis* (Nickerson and Krysko 2003) and other salamanders and is most effective in clear waters from 0.5 to < 3.0 m in depth. This method has proved more efficient than wading and turning substrate in surveys of *C. alleganiensis* in the gilled larval stage (Nickerson et al. 2002).

Foster et al. (2008) turned rocks to survey for adult and larval *C. alleganiensis* and captured 157 in 317 person hours (0.5 individuals per person hour (pph)). Bank searching through turning substrate within four meters of the stream bank yielded 14 juveniles in 55 person hours (0.25 pph). Bank searches of four of the seven inhabited sites yielded no *C. alleganiensis*, but at three sites bank searching was more efficient than rock turning (Foster et al. 2008). Capture rates of *C. alleganiensis* in four streams in the White River drainage, Missouri, varied from zero to 2.5 pph (Trauth et al. 1992). Okada et al. (2008) used diurnal wading and substrate surveys with one to three people searching under piled rocks or leaves (by hand or with dip-nets) to observe 227 *A. japonicus* at a rate of 1.4 pph.



Figure 6. Turning heavy rocks, combined with snorkeling with face masks and nets is an effective means to survey juvenile and adult *C. alleganiensis*. Image Robert Browne.

2. Scuba/hookah diving

Deep water habitats have not generally been well surveyed for Cryptobranchids, although standard scuba diving equipment and surface-based air compressor systems (hookah dive systems) are being used increasingly for surveying *C. alleganiensis* in fast-flowing, deep water two to nine meters in depth. Scuba diving allows for prolonged submergence giving the diver the capability to systematically check all available cover and often capture all individuals observed.

Standard scuba diving equipment provides unlimited mobility in terms of the area a worker can survey. In contrast, divers using a stationary anchored boat, canoe, or bank-side hookah system are limited by air line length.



Figure 7. Snorkeling and turning small substrate is a good technique for surveying small to large *C. alleganiensis* in water of moderate depth. Image Robert Browne.

Nevertheless, free-floating hookah systems are available that allow hookah divers to work in moderately fast waters with unlimited mobility as the compressor floats freely behind the divers. If conditions are not favorable for use of a free-floating hookah system, then a boat or canoe can be used to provide a semi-mobile platform for a stationary hookah compressor.

Boat-mounted hookah systems enable dives of one hour (hr) to more than 1.5 hr duration, and can be used at multiple sites during a full day of fieldwork without the need to refuel. Hookah systems require the use of a dive harness fitted with lead weight (usually 20-25 kg) sufficient to hold a diver in place in fast currents. The streamlined profile of hookah systems reduces the fatigue experienced by divers using standard scuba equipment. Divers also must be capable of working in fast moving water and have the physical strength to move large cover objects to successfully locate Cryptobranchids. For safety reasons, all diving requires a minimum of two divers, so that a “buddy system” is in place. If using a hookah dive system, a topside operator is required to monitor conditions and equipment. All divers must have appropriate certification and must surface when air cylinder pressure drops to 500 psi.

3. Nocturnal spotlighting

Nocturnal spotlighting has the advantage of producing minimal substrate disturbance, as rocks are lifted after the protruding heads of *C. alleganiensis* are observed. Spotlighting also allows observation of migratory and other behaviors. A spotlight survey of *C. alleganiensis* in West Virginia, USA, showed that increased nocturnal activity is correlated with high water levels, and suggested that spotlight surveys for mature adults are best conducted in May and June in this region (Humphries and Pauley 2000). Kawamichi and Ueda (1998) used nocturnal surveys combined with wading for *A. japonicus* in streambeds, and this technique, without substrate turning, is the most common survey technique for *A. japonicus*.



Figure 8. Artificial spawning dens for *C. alleganiensis* are used to increase the number of nesting sites and allow monitoring of egg production and larval survival. *Image Noelle Rayman.*

Nocturnal snorkeling/scuba surveys follow the same protocol as wading surveys, except that the observers are swimming and using dive lights to spot salamanders. Nocturnal snorkeling/scuba surveys have been conducted with some success in Missouri and Arkansas, USA, especially during the spawning period. Boats with halogen spotlights powered by generators have been used to survey for *C. alleganiensis* in Missouri (Wheeler 2007; Nickerson and Krysko 2003).

4. Bow-hooks/trot-lines

Bow-hooks or trot-lines can be an efficient survey technique in detecting the presence of Cryptobranchids at low population densities (Wang et al. 2004; Liu et al. 1991). Wild populations of *A. davidianus* have declined dramatically during the past 40 years, and in many regions bow-hooks may provide the most practical survey technique (Liu 1989; Wang 1996; Zhang and Wang 2000; Zhang et al. 2002).

Wang et al. (2004) surveyed *A. davidianus* using bow-hooks made of small pieces of bamboo fitted with four or five sharp hooks. In this study, only one *A. davidianus* was captured with the bow-hooks, whereas none were observed during night surveys and eight were captured by electrofishing. Bow-hooks were found to be an effective survey technique for *A. davidianus* in the remote and rugged Huping Mountain National Nature Reserve, an area of particular conservation significance (Zhang et al. 2002; Tao et al. 2004). Protection now forbids the use of hooks for surveying *A. japonicas*, although they can be captured without a hook by using bait on a stick (Tochimoto 2005). Bottom-set bank lines have been used in surveys of *C. alleganiensis* in sections of river with no rocks or logs, or that were unsuitable for wading and substrate turning (Dundee and Dundee 1965; Wortham 1970; Nickerson and Krysko 2003).

5. Questionnaires

Questionnaire surveys were conducted by Wang et al. (2004) with local fisheries managers and villagers to analyze the past and present distribution and status of *A. davidianus*. A total of 72 answered questionnaires concluded 1) *A. davidianus* were abundant prior to the 1980s, when individuals could be found easily and captured, 2) populations have since dramatically declined, and it is now difficult to capture *A. davidianus*, and 3) the main reasons for declines are excessive poaching, habitat fragmentation, and pollution. Responses to questionnaires also suggested that *A. davidianus* inhabited areas where 82 subsequent nocturnal surveys failed to detect them, so questionnaire results were neither verified nor discredited.

In another example of questionnaire survey, Tochimoto et al. (2008) collated data using questionnaires on the past distribution of *A. japonicus* in Hyogo Prefecture, western Honshu, Japan. A distribution map of *A. japonicus* was produced from the combined responses of oral interviews, answers to written questionnaires, and data from previous publications. Oral interviews were conducted with 17 people from fishermen's associations, two people from the nature conservation society in Hyogo Prefecture, and 21 people recommended by the fishermen's associations as very familiar with *A. japonicus*. The interviews were supported by information obtained through written questionnaires provided by the Boards of Education of 44 municipalities.

6. Electrofishing

Electrofishing requires a backpack voltage generator, connected to two submersible electrodes, carried by a researcher walking slowly through a stream. Amphibians and other aquatic vertebrates are first attracted to the electrical field of the electrodes and then temporarily paralyzed (Reynolds 1983).

Williams et al. (1981) considered electrofishing with seining effective for surveying *C. alleganiensis*. However, subsequent studies have not supported this conclusion (Bothner and Gottlieb 1991; Nickerson and Krysko 2003). In extensive river sections where large populations were found using other survey techniques, electrofishing failed to reveal *C. alleganiensis* (Nickerson and Krysko 2003). Electrofishing failed to locate *C. alleganiensis* during surveys on the Susquehanna drainage in New York, whereas turning rocks was successful (Soulé and Lindberg 1994). Substantial rock cover and poor water currents can result in shocked *C. alleganiensis* not moving from beneath rocks during electrofishing (Nickerson and Krysko 2003).

A two-year population study of another large aquatic salamander, the Common mudpuppy (*Necturus maculosus*), concluded that electrofishing was ineffective in surveying sites with large populations (Matson 1990). Nevertheless, there are examples of successful electrofishing for aquatic salamanders, especially when salamander abundance is being associated with other species abundance including fish. Maughan et al. (1976) used electrofishing to successfully survey the Pacific giant salamander (*Dicamptodon ensatus*), and Nakamoto (1998) exhaustively surveyed both fish and *D. ensatus* using multiple passes with backpack electrofishing. Occasionally, *C. alleganiensis* are incidentally captured with electrofishing by fisheries biologists during late summer/early autumn.

Because of its potential to harm salamander health and reproduction the use of electrofishing for surveys is not generally recommended, and should be confined to

occupancy surveys of special conservation significance where other techniques are not effective. Electrofishing is well known for causing spinal injuries and mortality in fish (Cho et al. 2002; Wang et al. 2004), and there is potential for electric shock to reduce salamander reproductive success (particularly during the breeding season) and to damage the immune system (Nickerson and Krysko 2003). Electrofishing can seriously affect the health of critically endangered fish such as the Chuanshan taimen (*Hucho bleekeri*), and electrofishing is banned in the range of *H. bleekeri* in Taibai, Shannxi Province, China (W. Zhenguan, pers. comm.)

Nevertheless, electrofishing may be the best technique for occupancy surveys in some difficult habitats where the detection of threatened salamanders is of major conservation significance (Nickerson and Krysko 2003). Wang et al. (2004) reported the capture of eight *A. davidianus* with electrofishing, whereas nocturnal surveys revealed none and bow-hooks only one (Zhang and Wang 2001).

7. Underwater camera systems

The use of waterproof video systems for surveys minimizes habitat disturbance, and video systems can locate den sites, record reproduction and behavior, and provide other valuable information on Cryptobranchid biology. Waterproof video systems are very effective where Cryptobranchids utilize heavy large rocks or bedrock crevices for shelter.

Black and white cameras have been used successfully. However, suitably small underwater color cameras are now available. Although color cameras are less light sensitive than black and white, the use of color is more efficient at revealing salamanders and eggs. We are not aware of an "off the shelf" video camera system optimal for surveying all Cryptobranchid species, or one that incorporates all features needed for efficient aquatic surveys. However, there are two relatively inexpensive systems available suitable for surveys of aquatic salamanders: 1) fishing video systems, and 2) inspection cameras.

Fishing video systems (12 volt) can easily be modified for surveys of Cryptobranchids. However the waterproof charged couple device (CCD) cameras associated with these systems are too large to access many crevices. These cameras are also relatively bulky and better suited to use from a small boat or canoe. Inspection cameras are very lightweight, and with small camera heads, have proven effective for surveying *C. alleganiensis*. A limitation of both systems is that standard monitors are relatively small and are not waterproof.

Video systems are being developed by researchers that are waterproof, lightweight, and incorporate a wireless camera system, digital recorder, and video goggles.

The video recorder, battery pack, and wireless components are placed inside waterproof bags and worn in a backpack. Improved waterproofing of video goggles and some components of wireless inspection cameras would provide greater flexibility in using these systems.

In addition to utilizing video camera systems for active surveying, cameras may be left in the field as a passive survey technique, if connected to a 12 V (volt) surveillance digital recorder. Batteries for the recorder need replacement, and data must be retrieved approximately once a week, depending on battery size and data storage capabilities of the recorder. Batteries are heavy and transport for recharging is arduous, but solar panels could be used to provide electricity in remote but secure locations.

8. Passive integrated transponders (PIT) and mark-recapture

PIT tags are small, waterproof, glass-encased capsules containing an alphanumeric code read with a portable reader. PIT tags are generally inserted sub-dermally with a syringe and needle, have life spans of at least 10 years, and are relatively inexpensive. PIT tags are available as read-only tags containing unique factory-set alphanumeric codes or as read-write tags that can be changed to any value. The new read/write PIT tags enable details to be recorded, retrieved or changed using the receiver, including the GPS location, habitat, tagger's name, and contact information. Gorsky et al. (2009) used 23 mm read/write PIT tags to assess Atlantic salmon (*Salmo salar*) migratory path selection. Although the size of PIT tags has steadily decreased, the detection range increases with PIT tag size. The standard reader ranges for read-only PIT tags are 3-8 cm for the smallest microchips (1.5 × 7 mm) and 15-45 cm for the largest (34 mm). Fish less than 55 mm have been successfully tagged using 11.5 mm PIT tags that weigh 0.1 g, and the smallest PIT tags now available should be suitable for all but the smallest Caudata.

A promising new technique, for surveying and locating salamanders in shallow water habitats is the use of submersible antennae and larger PIT tags that have been detected up to 90 cm through water (Hill et al. 2006) and detection range should further increase through improvements in antenna technology (Hamed et al. 2008). Cucherousset et al. (2008) showed that detecting Pyrenean brook salamanders (*Calotriton asper*) using PIT telemetry was 30% more efficient for individual sampling, and four times as efficient in sampling over time, than direct sampling through visual searching and rock turning. The efficiency of PIT telemetry was negatively correlated with the presence of large stones that blocked the PIT signal, and positively correlated with the number of easily sampled spring inlets and undercut banks (Cucherousset et al. 2008).

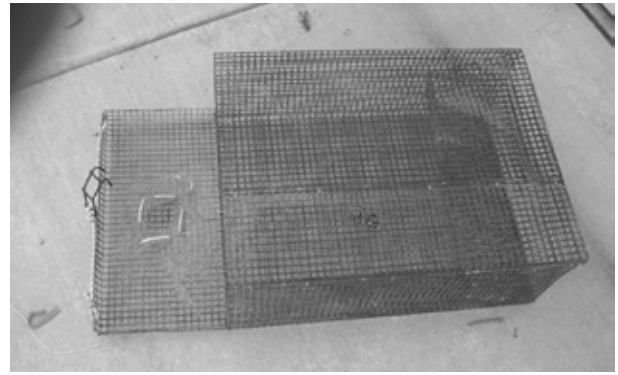


Figure 9. Trap used to capture *C. alleganiensis* in the Allegheny River drainage during the summers of 2004 and 2005. Bait (White sucker, *Catostomus commersonii*) was attached to the inside of the hinged door of a wire mesh cage. The bait cage was later removed and replaced using plastic zip ties. From Foster et al. 2008. Used with permission from *Herpetological Review*.

Bub et al. (2002) showed that when PIT tags were hidden within different stream microhabitats, more than 80% were subsequently located with portable antennas. Hill et al. (2006) tested specialized “PIT pack” antenna systems and found that design modifications and reduced equipment weight made PIT packs easy to use. The read range of optimized PIT packs approached 90 cm when the PIT tag was submerged in water. Breen et al. (2009) found a detection efficiency of 76% with PIT-tagged fish using a portable antenna investigating displacement, mean movement distance, and home range of Mottled sculpins (*Cottus bairdii*).

Prior to PIT tagging, photographs of head or tail spotting patterns were used to identify post metamorphic individual *A. japonicus* for mark-recapture studies (Kawamichi and Ueda 1998; Tochimoto 1991; Tochimoto et al. 2005). PIT tagging is the most common technique for mark-recapture studies. For example, Tochimoto et al. (2005) recorded 1204 individual salamanders in the Ichi River, Hyogo Prefecture, between 1975 and 2004, with 588 of these PIT tagged between 1998 and 2004. Okada (2006) tagged more than 500 individuals in Tottori Prefecture between 2001 and 2008.

Wheeler (2007) used the BioMark® submersible antenna with a detection distance of up to 30.5 cm to survey for previously PIT tagged *C. alleganiensis*. Of six *C. alleganiensis* marked using PIT tags, surveyors were able to detect only two the following day. A search of the area with rock turning did not detect any additional *C. alleganiensis*. The four undetected *C. alleganiensis* had either moved into water deeper than the reach of the detector wand antenna (two meters) or moved under the cobble substrate (Wheeler 2007).

Automatic systems to survey movement have been used with PIT tags in fisheries research. These consist of remote antenna arrays spanning water bodies. Meynecke et al. (2008) successfully used remote PIT technology to monitor fish movement for 104 days in a mangrove

Survey techniques for giant salamanders

Table 1. The advantages and disadvantages of survey techniques.

Survey technique	Advantages	Disadvantages
1. Wading, turning substrate, netting, and snorkeling.	Low equipment costs. Simple and rapid surveying. Snorkeling provides better vision and a closer proximity to exposed <i>C. alleganiensis</i> . Rocks can be tilted more easily due to buoyancy and water currents can provide “lift” of rocks.	Cannot sample deep water, surveyor strain and fatigue are high, and there is considerable habitat disturbance. Risks of blind searches include bites and cuts and rock turning can result in being held under water by a trapped arm. Some institutions will not allow surveying alone due to risk of injury. Costs for wetsuits, mask, snorkel, dive boots, and other equipment. Transporting heavy equipment (along shallow mountain streams) and working in high velocity areas can produce increased surveyor strain and fatigue.
2. Scuba/hookah diving	Deeper water habitats can be surveyed that are not accessible to other methods besides traps and trot-lines. Diving enables prolonged submergence, with less fatigue than snorkeling, at depths of one to two meters. Systematic checking of all cover and ensuring the capture of all exposed Caudata.	Surveying multiple sites requires the transport and handling of many air cylinders. Refilling air cylinders when at remote survey sites requires extensive transportation time. Requires substantial equipment costs including scuba or hookah equipment and sometimes boats, and extensive training time and costs. Diving is more dangerous than other surveying methods. It is time consuming to sanitize snorkeling, scuba and hookah diving equipment.
3. Nocturnal spotlighting	Nocturnal lighting creates little habitat disturbance, and enables the simultaneous survey of other nocturnal amphibians.	Potential costs of equipment (lights and boats), limited visibility through poor water clarity, and increased safety concerns.
4. Bow-hooks/trot-lines	Efficient for the detecting of the presence/absence and population assessment of Cryptobranchids at low population densities.	Bow-hooks (using fishing hooks) can cause injuries to salamanders, increase salamander stress over hand collecting, and increase predation risk. Bow-hook lines should be made too short to reach the esophagus and possibly cause injuries.
5. Questionnaires	Regional assessment of occupancy.	Relies on credibility of respondents.
6. Electrofishing	Presence/absence and population surveys in difficult habitats of major conservation significance.	Electrofishing for surveys is not generally recommended because of its potential to harm salamander health and reproduction and its use should be confined to occupancy surveys of special conservation significance where other techniques are not effective. Electrofishing has high equipment costs, a number of particular safety concerns, and requires several surveyors working together.
7. Underwater camera systems	Minimal habitat disturbance, location of den sites, recording of reproduction and behavior, and provision of other information on Cryptobranchid biology. Video camera systems can provide a passive survey technique in combination with a digital recorder.	Problems with waterproofing, battery charging and supply, limited water depth, and viewing monitors in bright sunlight. Costs can be high with this method for camera, recorder, and monitor, and only a single site can be monitored per camera.
8. Passive integrated transponders (PIT) and mark recapture	Recorded information can be retrieved from tagged salamanders (with limited habitat disturbance) enabling calculation of movement and dispersal. Allows tracking of confiscated animals.	Only previously tagged animals are detectable, a relatively short detection range, the workable water depth being limited by wand length, and detection range limited by shelter type and depth. PIT tag surveys using hand readers are economical; however, optimized antenna systems are costly. PIT tags can be lost.
9. Radiotelemetry	Monitoring of individuals to study movements, habitat use, and survival. Smaller, lighter, longer-lived, and more reliable units have increased the efficacy of radio-tracking with increasingly smaller individuals.	Surveys can be costly due to the initial expense of transmitters, antennas and receiver. Surgical implant is required for attaching transmitters to salamanders.
10. Modular artificial spawning dens and rock placement	Modular artificial spawning dens provide efficient means to support critical spawning habitat, enable monitoring of egg and larval survival, and survey male and female occupancy and movement. Further development of the capacity to provide camera surveillance will increase all the above.	Modular artificial spawning dens are relatively easy to construct but there are material and labor costs. They are heavy and require vehicular transport and a team to place in selected locations. Their stability under exceptionally high stream velocities, in comparison to natural rock dens, is untested.
11. Wire mesh baited traps	Trap surveying is not hampered by deep, turbid, or cold water. There are low levels of habitat disturbance, and sites with very heavy rocks and ledges can be surveyed.	Material and labor costs for trap construction, and supplying a large amount of fresh bait. Setting traps is labor intensive and transporting traps to remote areas may be prohibitive. Trapping should not be performed during the breeding season because females may spawn in the traps, and trapped males cannot guard dens. Flooding may carry away traps. Lost traps may be a hazard to wildlife. As with all unguarded equipment, theft or vandalism may be a problem.
12. Population genetic techniques	Minor tissue sampling enables ongoing studies of the number and significance of genetic subpopulations, loss of genetic variation, migration and dispersal, effective population size, and parentage. Samples can be subdivided and provide material indefinitely for future work and comparison.	Contamination and poor storage of samples limits analysis. Cryptobranchids and some other Caudata have low genetic variation, which can limit the use of techniques. More sophisticated genetic techniques are expensive.
13. Environmental DNA (eDNA) detection	Inexpensive, no habitat disturbance, can be used in streams difficult to monitor by other methods, shows occupancy.	Targeted primers need to be designed to amplify a species-specific short DNA fragment. Laboratory costs per sample and the need for several samples to exclude false positives or negatives. Efficiency depends on DNA shedding rates, population demography, water temperature, and thermal properties, to estimate population size.

stream and recorded more than 5000 detections with a recapture rate of 40%. River monitoring systems for fish commonly use four different types of antennas: pass-through, flat plate, crump weir, and circular culvert antennas. Flat plate detectors appear ideal for salamanders as they can be up to six meters in size, are buried slightly in the streambed, and can detect salamanders up to 45 cm above the plate.

The problem of PIT tag loss can be substantially reduced by careful application and sealing of the insertion site (Christy 1996). A coincidental value of PIT tagging to conservation is that resource managers and international border inspectors can utilize PIT tags to identify home locations of confiscated salamanders.

9. Radiotelemetry

Radiotelemetry can consistently be used to monitor individual animals and has been used to study movements, habitat use, and survival of many vertebrate species (Kenward 2001). Radio transmission can be received in turbid waters, stream flows, or depths that preclude traditional survey techniques (e.g., rock turning and visual searches). Surveys using radio-telemetry with *C. alleganiensis* have investigated dispersal (Gates et al. 1985b), site fidelity, and frequency and timing of movements (Coatney 1982; Blais 1996; Ball 2001). These surveys have revealed the use of unique microhabitats including bedrock ledges, root masses, and bank crevices (Blais 1996) as well as the location of den sites and causes of mortality (C. Bodinof, pers. comm.).

Monitoring by radiotelemetry requires attachment of a very high frequency (VHF) radio transmitter to the target salamander. Each transmitter is tuned to a unique frequency and emits a pulsed radio signal allowing an observer to locate individual salamanders. Optional sensors to detect motion, pressure, depth, or temperature can be incorporated into radio transmitters. To extend battery life, microcontrollers have been developed to turn transmitters on and off at preset times (Rodgers 2001). Technological advances have resulted in smaller, lighter, longer-lived, and more reliable units. Such advances have increased the efficacy of radio-tracking in increasingly smaller organisms while minimizing concern for adverse effects of transmitter attachment.

Several methods of transmitter attachment have been used with varying success for Cryptobranchids, including 1) coelomic implant (Blais 1996), 2) subcutaneous implant (Blais 1996), 3) force-feeding (J. Briggler, pers. comm.), 4) neck collar (Wheeler 2007), and 5) suturing through the tail (Okada et al. 2006; Wheeler 2007; Blais 1996).

Wheeler (2007) observed poor retention with external tail attachments, as well as collars fastened around the neck of *C. alleganiensis*. However, Okada et al. (2006) reported that transmitters attached externally (su-

tered through the tail) to large *A. japonicus* were retained for two to four months and caused minimal injuries. Radio transmitters were force fed and retained for 18 to 30 days (Coatney 1982), and 16 to 25 days (Blais 1996), in *C. alleganiensis* with no harm. Force-feeding transmitters may be useful for detecting untagged Cryptobranchids, which aggregate during a relatively short breeding season. Surgical implantation of transmitters should be performed by an experienced veterinarian or biologist (Fuller et al. 2005), and amphibians should be given ample recovery time from effects of anesthesia and surgery before release (Byram and Nickerson 2008).

A recommendation to minimize the effect of transmitter attachment is the use of the smallest possible tag. Transmitters also should not exceed 3-5% body mass and researchers should use the least conspicuous attachment technique (Withey et al. 2001). Jehle and Arntzen (2000) used very small transmitters of 0.5 g to track individual *Triturus* spp. above a minimum acceptable body mass of 8.0 g. PIT tag tracking may be useful for salamanders smaller than 8.0 g, but radio tracking antenna systems are cheaper, and radio tracking has a much greater range than PIT tags. Different sizes, battery life, outputs, and ranges of these and various other transmitter models have been used for radio-tracking Caudata. While trade-offs exist among unit weight, detection range, and battery life, many small units offer \geq six months of battery life. Resources providing an overview of radio-tracking technology and study design include Fuller et al. (2005), Millspaugh and Marzluff (2001), and White and Garrott (1990).

Radiotelemetry studies of Caudata include *T. cristatus*, *T. marmoratus* (Jehle and Arntzen 2000), *Ambystoma maculatum* (Madison 1997; Faccio 2003), *A. jeffersonianum* (Faccio 2003), *A. californiense* (Trenham 2001), *C. a. alleganiensis* (Gates et al. 1985a; Blais 1996; Ball 2001), *C. a. bishopi* (Coatney 1982), and *A. japonicus* (Okada et al. 2006).

10. Modular artificial spawning dens and rock substrate placement

A recent innovation in survey techniques for Cryptobranchids is development of modular artificial spawning dens. Bankside artificial dens have been used for *A. japonicus* in channelized habitat (where suitable sites were lacking), and in artificial streams for reproduction during farming of *A. davidianus*. The Ozark Hellbender Working Group developed modular spawning dens for *C. alleganiensis* that proved highly successful in attracting *C. alleganiensis* and providing spawning sites. Dens made of ferrocement are light, simple, and economical to construct. Artificial dens offer the possibility of incorporating underwater video systems giving discrete and continuous monitoring of occupancy and activity. Rocks

have been placed in streams to similarly provide habitat and increase survey efficiency for *C. alleganiensis*.

11. Wire mesh baited traps

Cryptobranchus alleganiensis have been surveyed over several years using baited traps in deep water habitat of some larger (7th order) rivers (including the Gasconade River, Missouri, USA). Such habitats have proved difficult to survey without trapping due to their depth (> 5 m maximum) and often very turbid waters (lateral Secchi Disk < 1.0 meters visibility). The efficiency of baited traps varies with water temperature (Nickerson 1980); trapped *C. alleganiensis* in deep rivers in Missouri were greatest during the peak foraging period in spring and very low during the summer breeding season. When water temperatures reached above 22 °C, capture rates were very low. Besides seasonal effects, trapping is highly dependent on how the trap is set. Foster et al. (2008) had greatest success when bait was fresh and the trap was flush with the substrate.

Wire mesh baited traps have been widely used to survey Cryptobranchids using a variety of baits. *Cryptobranchus alleganiensis* can detect baits from considerable distances (Townsend 1882; Nickerson and Mays 1973), and smelly, fresh baits are most successful in trapping. Traps baited with chicken livers proved unsuccessful with *C. alleganiensis* (Soulé and Lindberg 1994). Foster et al. (2008) used similar traps successfully when baited each day with fresh fish; fresh meat bait proved unsuccessful. Kern (1984) successfully captured *C. alleganiensis* using hoop-nets baited with fresh sucker fish (*Carpoides* sp.). Trapping with crab traps baited with strong smelling saltwater baits (such as sardine, mackerel, or squid) was effective for catching adult *A. japonicas* (S. Okada, pers. comm.). When surveying Cryptobranchids, the bait bags should be strong enough to resist tearing from salamander bites and the possible ingestion of bag material. Trapping should not be performed during the breeding season because females may spawn in the traps, and trapping can prevent males from guarding nests.

The Missouri Department of Conservation, USA, has a major survey program for *C. a. alleganiensis* using traps in habitats unsuitable for other methods. Trap design was modified from those used by Foster et al. (2008; Figure 8) by placing a funnel on both ends and making the traps collapsible to reduce storage space. Numerous bait types (chicken liver, crayfish, carp, and Gizzard shad) were used as bait, but fresh Gizzard shad (*Dorosoma cepedianum*) was the most successful bait. Besides the bait used, the general success of trapping is also highly dependent upon how the trap is set.

Trapping is a valuable sampling technique used for *C. alleganiensis*. In a comparative study, Foster et al. (2008) reported on three techniques of surveying Hell-

benders: rock turning, bank searches, and trapping. Rock turning had the highest capture efficiency but damaged the habitat; bank searches were effective at finding juveniles. Besides its use in habitat accessible to other techniques, trapping was useful for water slightly exceeding the maximum depth possible with other techniques and in areas with unmovable rocks or difficult-to-access ledges. Trapping may be more effective for capturing the largest size classes (Figure 10; Foster et al. 2008). Trapping is similarly effective for catching adult *A. japonicus* (S. Okada, pers. comm.). Snorkeling, scuba, or hookah diving combined with trapping would enable better trap placement, especially at greater depths.

12. Population genetic techniques

Genetic information can guide conservation breeding programs determining the number and significance of genetic subpopulations. Using increasingly sophisticated genetic techniques, evolutionary phylogeny, paleogeography, species status, migration, effective population size, parentage, and population bottlenecks can be ascertained. Surveys using molecular techniques to assess population genetic structure, variation, and migration patterns have rapidly progressed over the last 10 years. This progress has been largely driven by improved sequencing and computer analysis, Information Technology systems, and a growing bank of genetic techniques and resources (GenBank Database 2009).

Mitochondrial techniques are useful for understanding relationships among and historical changes within populations (Sabatino and Routman 2009), however, mitochondria are maternally inherited and only track female lineage.

Genomic microsatellite markers, together with mitochondrial DNA information, may provide the most informative phylogenetic information. Microsatellite markers have the advantage of requiring very little tissue (even less than used in mitochondrial sequencing techniques) and this allows for noninvasive sampling such as buccal swabs. Polymorphic microsatellite markers have very recently been published for *C. a. bishopi* (Johnson et al. 2009) and *C. a. alleganiensis* (Unger et al. 2010).

13. Environmental DNA (eDNA) detection

Environmental DNA (eDNA) has recently been confirmed as a sensitive and efficient tool for inventorying aquatic vertebrates in lotic and lentic aquatic habitats. Under the Amphibian Research and Monitoring Initiative, U.S. Geological Survey scientists and their partners developed an efficient protocol for detecting eDNA from two amphibian species that occur in low density, fast-moving stream water; the Idaho giant salamander (*Dicamptodon aterrimus*) and the Rocky Mountain tailed

frog (*Ascaphus montanus*). Environmental DNA analysis costs approximately US\$30. Sampling efficiency increases in comparison with fieldwork, for example, by 20 times for *D. aterrimus* and 11 times for *A. montanus* (direct survey population estimates of 0.16 and 0.04 individuals per m², respectively). With Asian carp, sampling cost efficiencies increase from 16 to 100 times when compared to field searches. The sensitivity of an eDNA test depends on the sampling of five to 10 litres of water, the amount of DNA shed by the target species, and the thermal and chemical properties of the water. False negative rates can be estimated using repeated sampling, and the probability of false positives can be excluded by careful primer design and protocol testing using related non-target species (Goldberg et al. 2011).

Conclusion

Cryptobranchids are iconic amphibians that provide a range of conservation challenges. Of all the aquatic amphibians, Cryptobranchids appear to offer the greatest potential to link amphibian conservation with watershed management. They also offer the greatest potential to apply a suite of modern and innovative techniques to conservation strategies. Their long-term survival is highly dependent on the effectiveness of these survey techniques to elucidate population structure and demography, bottlenecks in recruitment, threats, and critical habitat components.

There is a wide variety of survey techniques to detect, capture, and track Cryptobranchids and other aquatic Caudata. However, these techniques vary widely in

efficacy, and a combination of several techniques will prove most effective at providing critical information on occupancy and status. Each survey technique has advantages, disadvantages, and biases depending on survey objectives (Nickerson and Krysko 2003).

When choosing survey techniques, a primary concern is animal welfare. The preservation of nest sites and other critical habitat is essential, as is limiting the spread of pathogens. Suitable *C. alleganiensis* nesting sites are increasingly scarce in many locations, and in some locations siltation is destroying the sites that remain. Underwater camera systems are the only survey techniques that do not disturb habitat, especially when used with artificial spawning dens. Only radiotelemetry, PIT tagging with long-range detection, and environmental DNA (eDNA) detection enable ongoing sampling without further habitat disturbance (Nickerson and Krysko 2003).

Wading shallow water and turning substrate, including leaves and gravel, is a simple way to survey Cryptobranchids and may be efficiently combined with surveys of larvae and juveniles. Survey efficiency for adult and larval Cryptobranchids, and other Caudata through rock turning, is improved by the use of downstream seines. Scuba or hookah diving are the only techniques that detect all sizes of gilled larvae and multiple age groups of non-gilled and adult Cryptobranchids within short survey periods, but they are one of the most expensive and training-intensive methods. The use of eDNA promises the most rapid and cost effective survey technique for the inventory of Caudata.

Final remarks: Cryptobranchids are one of the most endangered groups of Caudata, having highly specialized habitat requirements at different life stages. Various sur-

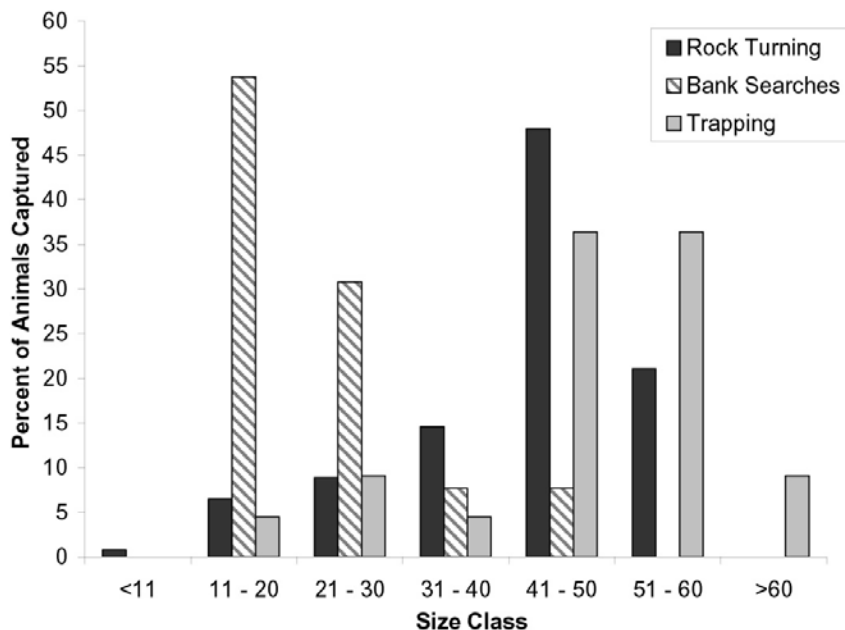


Figure 10. The relative success of three capture techniques in locating various size classes of *C. alleganiensis*. From Foster et al. 2008. Used with permission from *Herpetological Review*.

vey techniques offer a range of advantages and disadvantages, and surveys should include several techniques to reduce bias. Cryptobranchids' high site fidelity and reliance on easily damaged critical habitat components make them vulnerable to survey techniques that require disturbing habitat structure. Therefore, the choice of survey technique should always include minimum habitat disturbance and potential to affect salamander health. Equipment must be sanitized when moving among sites to limit the spread of pathogens.

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