

**United States Fish and Wildlife Service**  
**Section 10(a)(1)(A) Scientific Permit Requirements**  
**For Federally Listed *Eurycea* Salamanders**

**INTRODUCTION**

This document identifies the minimum requirements for conducting and reporting on presence/absence surveys and related permitted activities for the following federally listed species, collectively referred to as “*Eurycea* salamanders” in this document:

- Barton Springs salamander (*Eurycea sosorum*)
- Austin blind salamander (*Eurycea waterlooensis*)
- Salado salamander (*Eurycea chisholmensis*)
- Georgetown salamander (*Eurycea naufragia*)
- Jollyville Plateau salamander (*Eurycea tonkawae*)
- San Marcos salamander (*Eurycea nana*)
- Texas blind salamander (*Eurycea rathbuni*)

Section 10(a)(1)(A) permits issued by the U.S. Fish and Wildlife Service (Service), also known as recovery, scientific, or enhancement of survival permits, allow for “take” of listed species that may or will occur while conducting research or other activities to enhance the survival of the species. Take is defined as to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture or collect, or to attempt to engage in any such conduct. Harm is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing essential behavioral patterns, including breeding, feeding, or sheltering. Harass is defined by the Service as intentional or negligent actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to, breeding, feeding or sheltering.

We encourage permittees to use the current species range information available through the Service’s Environmental Conservation Online System (<https://ecos.fws.gov>) as well as recommendations from the appropriate Austin Ecological Services Field Office (Austin ESFO) species lead or consultations biologists to help determine when and where surveys are necessary or recommended.

The purpose of this document is to identify the minimum requirements for conducting presence/absence surveys and to serve as guidelines for related activities covered under section 10(a)(1)(A) permits, such as tail clipping and specimen preservation. We recognize survey and research methodology will differ between *Eurycea* salamander populations, site-specific situations, and objectives of permit holders. We have therefore attempted to include general, overarching guidelines that must be applied in all or the majority of circumstances and identify when additional coordination with our office is necessary for case-by-case approval.

Please note this document supersedes any previous guidance from the Service on conducting surveys for the Northern Edwards salamanders or other federally listed *Eurycea* salamander

species. It is essential that all section 10(A)(1)(a) permit holders refer to their current permits regularly, as some may have additional or more detailed terms and conditions that pertain the specific activities covered by their permit. For example, permit holders with covered activities that include more research and management activities may have different terms and conditions than those for the presence/absence survey requirements outlined in this document or may be subject to approved study or management plans. All permit holders should discuss questions regarding permit compliance and implementation feasibility with the appropriate Austin ESFO species lead as soon as questions arise. Information that relates to the effectiveness or feasibility of these survey guidelines in conserving the *Eurycea* salamanders is welcome and appreciated at any time. We will consider modifications or alternatives to these methods as new information becomes available.

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## SURVEYS

All surveys proposed to be conducted at **known salamander locations** must be approved in writing by the appropriate Austin ESFO species lead prior to initiation<sup>1</sup>. The following procedures must be used to confirm presence or infer absence of *Eurycea* salamanders (also refer to the “Inferring Absence” section of this document):

- Surveys should be conducted in a defined area by turning over rocks and searching under other cover structures, such as leaf litter, woody debris and aquatic vegetation within that defined area.
- Individual salamanders observed during count surveys must be classified as adults or juveniles using methods described in the corresponding annual report or be categorized by the following size classes based on visual estimates of total length (tip of snout to tip of tail): 0-1 inch, 1-2 inches, and greater than 2 inches.
- During surveys, base flow in the surface habitat or cave pools in the subsurface habitat must be present. When conducting drift net or bottle trap surveys in the subsurface habitat, base or storm flow must be present.
- All other research or survey techniques designed to elicit information on *Eurycea* salamander populations, such as abundance, survivorship, reproduction, and movement, should follow the minimization measures found throughout this document as much as possible.

Other survey methods may be employed by section 10(A)(1)(a) permit holders only if specified in their permit. Surveys for research or monitoring purposes typically necessitate approval of a study or monitoring plan in writing by the appropriate Austin ESFO lead biologist(s) prior to implementation. Surface count surveys must adhere to the following conditions unless otherwise approved in writing by the appropriate Austin ESFO lead biologist(s):

- Visual count surveys in surface habitat should include an area-constrained survey technique, such as the drive method<sup>2</sup> (Bendik et al. 2014, p. 208) when possible to avoid double counting individual salamanders.
- Surface count surveys do not necessarily equate to population estimates for *Eurycea* salamander species. Furthermore, surface count surveys may not be adequate for estimating “take” of individuals for federally issued incidental take permits, as other

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<sup>1</sup> This will help us monitor which locations are being studied and prevent possible over-disturbance due to multiple concurrent studies. For assistance in identifying known species specific locations or the appropriate species lead biologist, please contact the Austin ESFO.

<sup>2</sup> In the drive method, a consistent search area is established by marking the ends of a stream segment of any length. Observers start at the downstream end of the segment and systematically remove cover objects to create an open area stretching across the width of the stream, bank-to-bank. This open area is continuously backfilled with upstream cover objects as more area is searched with the observer moving upstream toward the end of the segment. Only salamanders that move downstream to the observer and cross the open area of substrate created are counted. All moveable cover objects within the search area must be searched by the end of the survey.

information, such as project footprint size and types and levels of impact also should be taken into consideration.

### **Minimization Measures**

- All field gear (including footwear) that comes into contact with animals, water, or soils must be cleaned and disinfected between survey sites to prevent inadvertent spread of disease or parasitic organisms among sites. We recommend mixing 4 ounces of bleach in 1 gallon of clean water and using the solution to rinse off all field gear prior to visiting another potential *Eurycea* salamander site. The bleach solution must be entirely rinsed off the equipment after a minimum of 15 minutes soaking time and must evaporate from the gear before re-use. Alternative disinfectants may also be used instead of bleach according to national protocols developed by the Declining Amphibian Task Force (p. 1) or the White Nose Syndrome Decontamination Team (2016, p. 5)<sup>3</sup>. Disinfection must occur away from salamander habitat. Disinfection between sites is not required if the sampling sites are along the same tributary.
- Efforts must be taken to minimize disturbance to potential habitat areas.
- Survey activities should be limited to the top 3-4 inches of gravel once the first salamander is detected at a site to minimize habitat disturbance.
- Surveying for salamanders in caves must also follow the requirements listed above, as appropriate for cave environments. Surveyors should take care not to pull or touch cave walls.

### **INFERRING ABSENCE**

A site will be considered “occupied” once a *Eurycea* salamander is detected at that site, but absence cannot be implied simply by failing to detect a salamander (Mazerolle et al. 2007, p. 679). To infer that surface habitat is not occupied by salamanders, we require permittees follow all of the survey guidelines to infer absence listed above in addition to the following:

- At least **15 consecutive visual surveys** must be conducted **at least 24 hours apart under suitable sampling conditions** described in this document (e.g., conducting half of the surveys in the spring or summer, adequate flow conditions, absence of recent floods).
- Surface count surveys may be conducted any time of year as long as at least half of the 15 surveys (i.e., 8 surveys) are conducted during the spring or summer of the same calendar year (February 1 through September 1) to account for seasonal variability and detection probabilities for some *Eurycea* species. We believe this may be more important for species with seasonal patterns, such as Jollyville Plateau and Georgetown salamanders (Bowles et al. 2006, p. 118; Pierce et al. 2010, p. 293, Bendik 2017, pp. 5010-5012).

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<sup>3</sup> We recommend commercially available disinfectants, such as Virkon<sup>®</sup> Aquatic Disinfectant, as a 1 percent solution with a minimum of 10-minute contact time for wetsuits and waders and a 5-second submersion for field gear (e.g., nets, coolers).

Exceptions to this may be approved by the appropriate Austin ESFO species lead on a case-by-case basis.

- Inferring absence may not be possible during drought or low flow conditions, as it is more difficult to detect the presence of salamanders. Permit holders should contact the appropriate lead biologist for guidance regarding the applicability of the above requirements during drought or low flow conditions at a project/survey site.
- If a site is dry, surveys cannot be performed for inferring absence. Flow must be adequate to provide useable habitat for salamanders, as determined by the permittee's best professional judgement.
- Surveys to infer absence must not take place following extensive rainfall (greater than 1-inch within the watershed) or local flooding that may have temporarily displaced salamanders or removed cover objects from the site.
- Each survey must be conducted at least 24 hours apart from one another.
- An inference of absence expires 3 years after surveys are completed within a defined area. Additional presence/absence surveys following these guidelines will be required after this time.
- Surveyors must take care to search as many cover objects as is feasible within the survey area. This includes aquatic vegetation, cobble, leaf litter, and woody debris.
- Care must be taken to minimize disturbance within the surface habitat area being surveyed.
- Each survey effort must be a minimum of 10 person-minutes for each square meter of habitat surveyed (i.e., 10 total minutes surveyed per site area, such as two observers searching for 5 minutes each).
- Inference of absence will only apply to the specific area surveyed. For example, if the absence of salamanders in a 100 meter (m) (328 foot (ft)) stream stretch needs to be confirmed, all 100 m must be searched in each of the 15 surveys. The extent of the survey area must remain constant between consecutive surveys.
- If salamanders are not detected by the **eleventh** visual surface count survey, trapping must also be used to test whether salamanders are occupying the subsurface environment of the spring or stream segment. Surveyors must adhere to trapping methods, as described below. If there is no feasible or effective way to set a trap at the site, this must be noted in the annual report.

Please note that Austin ESFO consultations biologists may require additional project-specific survey requirements (e.g., surveys immediately prior to initiating construction activities, surveying several times throughout the duration of extended construction activities) as minimization measures for projects. Changes to habitat conditions of the specific survey area may also necessitate additional searches after the fifteenth survey.

## SALAMANDER TRAPPING AND CAPTURING METHODS

Permittees should use their best professional judgement when deciding which trapping or capture methods to use in different habitats or for different study designs. Acceptable trapping methods within subsurface habitats<sup>4</sup> to help infer absence include the following:

### 1) Drift Nets

Drift nets may be placed over spring outlets to capture salamanders from surface and subsurface habitats. They should be deployed for no less than 2 weeks and must be checked a minimum of two times per week (SMARC 2020, p. 9). If the site is located in a public area without restricted access, the net must be checked every 24 hours to prevent possible vandalism.

### 2) Bottle Traps

A type of aquatic funnel trap constructed from a plastic soda pop bottle (Adams et al. 1997 p. 49). At sites where the spring outlet is not obvious or where placing a drift net is not physically possible, bottle traps may be used to increase the chances of capturing salamanders. Bottle traps must be deployed for a period of no less than 7 days (Willson and Dorcas 2003, p. 128) and checked at least once every 7 days to minimize mortality. Permittees should use their best professional judgement to determine if traps should be checked more frequently at specific sites. For cave and borehole locations, traps must be deployed for at least 2 weeks and checked at least twice per week<sup>5</sup>.

Other guidelines for trapping and capturing salamanders include the following:

- All traps must be designed in such a way as to allow water to flow through them.
- All traps must be secured in a way that allows them to be retrieved. Strings and knots used to deploy traps should be inspected for loosening or deterioration during each visit to minimize the risk of losing a trap.
- Flagging or marking sites where traps are deployed is also encouraged to prevent trap loss but may be conducted in a manner to be less obvious to potential vandals.
- Traps must be removed before periods of forecasted heavy rainfall and high flow events.
- If flow at trapping sites is measured at greater than 2 feet per second, traps should be modified to minimize injury and mortality to salamanders.

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<sup>4</sup> These methods may also be used for surface habitat trapping for other purposes than inferring absence if allowed under a permit's covered activities.

<sup>5</sup> Traps may require more frequent checks on the surface compared to subsurface sites due to the relatively greater potential for disturbance by humans and wildlife. In addition, cave-adapted salamanders have been shown to have much lower metabolic rates than surface-dwelling salamanders in order to withstand long periods of starvation in their low-energy environment (Hervant et al. 2000, pp. 1428-1429; Hervant et al. 2001, pp. 271-272).

- All animals must be removed from nets and traps at each visit.
- Any captured salamander must be returned to its habitat as soon as possible. If it cannot be safely returned to its habitat after its capture, permittees should transport the salamander to a Service approved captive facility for that species or another facility approved in writing by the appropriate Austin ESFO species lead.
- If salamanders are captured with noticeable health conditions, injuries, or reduced chance of survival in the wild, they should be transported to a Service approved captive facility for that species or another facility approved in writing by the appropriate Austin ESFO species lead.
- If a trap is found with a predator of *Eurycea* salamanders within it, absence cannot be inferred.
- Absence cannot be inferred from trapping in wells (McDermid et al. 2015, pp. 556-557).
- If exploration is possible within subsurface locations (e.g., caves), at least 15 successive visual surveys according to the methodology described above must also be performed at these sites.
- The trapping methods listed above may be used for purposes other than inferring absence as part of research or management activities conducted under a section 10(a)(1)(A) scientific permit.
- Some types of traps, such as leaf litter bags<sup>6</sup>, mop heads, minnow traps, and other methods approved in writing by the appropriate Austin ESFO lead biologist may be used to capture salamanders for increasing detectability but **cannot be used to infer absence**. Austin ESFO lead biologists will consider additional trapping methods (those not mentioned in this document) for approval on a case-by-case basis to increase detectability.
- Gravel may not be used in leaf litter bags without first being approved by the Austin ESFO in writing on a case-by-case basis, as rocks may harm salamanders when the bags are checked.
- Minnow traps must be checked a minimum of two times per week to minimize mortality. They must be checked at least every 48 hours when predators of *Eurycea* salamanders are known to be present.

## SPECIMEN/TISSUE COLLECTION AND PRESERVATION

Not all permitted biologists are authorized for specimen collection and preservation activities. Permittees should refer to their own section 10(a)(1)(A) to verify they are authorized to collect salamanders as voucher specimens. Guidelines for specimen collection and preservation are provided below.

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<sup>6</sup> Permittees may follow procedures detailed by Jung and Pauley (2003, entire) or Waldron et al. (2003, pp. 25-26) to construct and deploy leaf litter bags.



- No more than three adult voucher specimens per species annually per site (for positive identification) from previously **undocumented** locations may be collected.
- One voucher specimen per year may be collected from known locations if the individual salamander appears to be morphologically different than other salamanders normally observed at a particular site for genetic analysis and/or specimen preservation if it is suspected that the individual represents a previously undescribed species or a species not previously known to occur at that location. Collection of additional voucher specimens must be approved in writing by the appropriate Austin ESFO species lead. If a voucher specimen is collected from a known *Eurycea* salamander site for this purpose, an Austin ESFO salamander biologist must be notified within 3 business days.
- Voucher specimens must be delivered with precise locality and observer information within 30 days of collection to a researcher-accessible natural history collection at a public facility or other facilities approved in writing by an Austin ESFO salamander biologist.
- Prior to preservation, all voucher salamanders must be humanely euthanized using any of the non-physical methods approved by the American Veterinary Medical Association (AMVA) (2020, pp. 22-23). Tricaine methanesulfonate, commonly referred to as MS-222, is an anesthetic agent recommended by the AMVA for the humane, temporary immobilization and euthanasia of amphibians and other aquatic, cold-blooded animals (AMVA 2020, pp. 37-38).
- A small tail clip (e.g., 5 mm) may be excised after a specimen is collected for voucher purposes.
- Voucher specimens must be maintained or preserved in ethanol (70 to 100 percent) or in a tissue storage reagent to protect genetic material (e.g., RNAlater) unless otherwise approved by the appropriate Austin ESFO lead biologist.
- Individuals also may be maintained alive in aquaria at an established, permitted captive facility for *Eurycea* species over the short-term (one or two weeks unless otherwise approved by the Austin ESFO in writing) until disposition of tissue samples and the voucher specimen can be arranged.
- All voucher specimens must be photographed with a ruler for scale and provided in the permit holder's annual report.
- Tail-clipping living salamanders for the purpose of obtaining genetic information is authorized only after a study plan submitted to and approved in writing by the appropriate species lead at the Austin ESFO.<sup>6</sup> The study plan must describe (1) the number of individuals from each site that will be tail-clipped and (2) the aseptic techniques that will be used to minimize the risk of infection. No more than 20 percent or 3 mm (0.12 in) of the tail (whichever is less) may be clipped (Cantu et al. 2020, in review). In cases where

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<sup>6</sup> If a permittee is authorized for tail-clipping activities, it will be specifically state in their 10(a)(1)(A) permit. Permittees should refer to their own 10(a)(1)(A) permits to verify they are authorized to conduct tail-clipping activities.

more of the tail tissue is needed, this amount and the need for it should be specified within an associated study plan that has been approved in writing by the appropriate Austin EFSO species lead.

## MARKING ACTIVITIES

Not all permitted biologists are authorized for specimen collection and preservation activities. Permittees should refer to their own section 10(a)(1)(A) to verify they are authorized to collect salamanders as voucher specimens. The following requirements apply when conducting marking activities:

- Marking methods, such as the use of visible implant elastomers (VIE), may be used for research purposes only on a case-by-case basis and **must be approved in writing through the submission of a study plan to the appropriate Austin ESFO lead biologist prior to their use.**
- Permittees may use digital photographs of *Eurycea* salamander pigment patterns as a substitute for traditional salamander marking methods (e.g., VIE), for example, by following the methods presented by Bendik et al. (2013, pp. 2-5).
- Salamanders may be captured using either small aquarium nets, other larger nets, seines, or any of the capture or trapping methods described above (see “Salamander Trapping and Capturing Methods” in this document).
- Efforts must be made to minimize handling time, stress, and risk of infection to salamanders during marking activities. These include the following: (1) disinfecting needles with ethanol after each injection, (2) keeping salamanders shaded and at or close to their aquatic habitat temperatures at all times, (3) replacing their water with clean spring water as frequently as possible, (4) avoiding lights shining into the salamanders’ eyes, (5) providing temporary cover, such as a mesh material during holding, (6) shielding or providing protective cover from potential predators (e.g., birds) during holding, and (7) conducting VIE processing procedures as quickly as possible. Other minimization measures are presented by O’Donnell et al. (2008, p. 17).
- Any salamanders with visible gas bubbles must be left in a dip net and placed in moving water within their aquatic habitat to allow them to crawl to the substrate rather than floating downstream.
- Efforts must be taken to aid in the survival of injured salamanders by contacting a captive holding facility for transfer. Injured salamanders not expected to survive their injury may be collected as voucher specimens. Voucher specimens collected as a result of injury must be reported to the appropriate Austin ESFO species lead within 3 business days of collection.
- The length of individual salamanders must be measured (either snout to vent or snout to hind limb insertion) or size-class estimated to determine the relative proportion of juveniles and adults.
- Captured salamanders must be released as close to where they were captured as possible.

## ANNUAL REPORTING REQUIREMENTS<sup>7</sup>

The following information must be included in each annual report unless stated otherwise.

### 1) Personnel

- Names of each person involved in survey efforts on each date activities were conducted
- The scientific permit (TE or PER) number

### 2) Location

- Location of all sites surveyed (i.e., presence and absence) in a Geographic Information System (GIS) layer with georeferenced survey location data using global positioning system (GPS) including references, such as road names and political boundaries<sup>8</sup>
  - Coordinates of each spring outlet, cave opening, and other potential habitat features surveyed in a spreadsheet and included on a GIS layer (each set of coordinates should be labeled as a spring, stream, cave, or well along with their provided coordinates)
  - All GIS files should be in North American Horizontal Data (NAD 83), latitude/longitude (decimal degrees)
- Survey area of each site described or mapped in relation to a spring outlet or cave opening surveyed, where applicable
- A general description of the land use immediately surrounding each area surveyed including signs of human use or vandalism or possible sources of water quality contamination or significant habitat alterations (e.g., sewer lines, drainage pipes, impoundments)
- A general description of the geology of each area surveyed (appreciated, but not required)

### 3) Methods

- Description of survey methods unless otherwise provided in an approved study or monitoring plan
- Dates and times of each survey conducted
- Notable weather conditions on the survey day and previous week (e.g., rainfall amounts)
- Estimated survey area and total time spent searching for salamanders (i.e., in person-hours) at each site if absence was inferred

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<sup>7</sup> Permittees should refer to their 10(a)(1)(A) permit for annual reporting requirements for other authorized activities involving *Eurycea* salamanders that may not be included in this document.

<sup>8</sup> If GIS is not available, permittees may report locations and detection results of all sites surveyed (i.e., presence and absence) on a U.S. Geological Survey quad map (7.5 minute or larger scale) but coordinates must also be provided.

- Description of efforts used to minimize handling time and stress on captured salamanders, if applicable

#### **4) Survey Results**

- The number and lengths (i.e., size class estimates or measurements) of all observed salamanders sorted by site and survey date, presented in an Excel spreadsheet
- All analyzed data and descriptions of how the values were calculated
- Both positive and negative survey results for each survey conducted in each survey area
- Notable observations on salamander behavior or body condition when surveyed (e.g., presence of gravid females, injuries, deformities, other obvious anomalies) including any dead salamanders found
- Results of any species identification performed by a taxonomist or geneticist and the method used to delineate that individual to species with the report from the taxonomist/geneticist if available
- Number of voucher specimens collected per site
- Location and accession numbers of all specimens collected as vouchers
- Photographs of voucher specimens

#### **5) Trapping Methods and Results**

- Dates, duration, and locations of trap deployment
- Trapping methods including type of trap used, mesh size used (if applicable), how often traps were checked, and type of bait used
- Identification of any area(s) where trapping could not be conducted
- If applicable:
  - Notable observations on salamander behavior or body condition when contained in a trap including any dead salamanders that were found
  - Evidence of any salamander deaths that occurred in a trap (i.e., presence of salamander remains)
  - Any human tampering or vandalism of traps
  - Any lost or irretrievable traps

#### **6) Habitat Characteristics**

The following habitat information is **required** in each annual report:

- A brief description of the substrate within the site where surface counts are being conducted (e.g., estimates of cobble, gravel, or boulder size; presence of leaf litter, silt, and aquatic vegetation; substrate depth)

- Measurement or description of water quantity (e.g., discharge, flow rate)
- Detection of a noticeable spring orifice
- Brief site description (e.g., cave, spring, stream, spring-fed pools)
- Presence and proximity to potential threats within an near habitat if observed during survey efforts (e.g., impoundments, feral hog activity, major roadways, septic tanks, potential predators)
- Habitat photos (particularly important at sites where absence is inferred)

The following site condition data is **not required**, but may be requested on a case-by-case basis and are dependent on the purposes of the study or monitoring efforts:

- Embeddedness
- Dissolved oxygen
- Specific conductance
- pH
- Turbidity
- Water temperature
- Water depth

## 7) Caves and Karst Features

The following cave and karst feature information is **required** in each annual report:

- Brief description of the interior of each cave or feature surveyed including:
  - Principle formations and whether there is standing water or water flowing through them
  - Approximate area for any water and approximate width, length, depth, and flow rate if collecting this data is feasible
  - Depth from the surface or distance from the cave entrance at which salamanders were observed, if applicable
- Report of any indications of high CO<sub>2</sub> levels or noxious gas and reference to labeled locations on cave map

Information on the following is **preferred but not required**:

- Other stygobitic species observed
- Water temperature, pH, dissolved oxygen, specific conductance
- Air temperature (to the nearest 0.2°Celsius (°C))
- Relative humidity (to the nearest 1 percent).

- Indicate the brand and model of the equipment used and the equipment's degree of accuracy.
- Air temperature and relative humidity should be taken at a minimum just inside the entrance and at the farthest humanly accessible part of the cave or feature.
- Several GIS locations are preferred, particularly for large caves or those with multiple rooms, and should be referenced to labeled locations on the cave map
- Report any indications of high CO<sub>2</sub> levels or any noxious gas and reference to labeled locations on the cave map
- A description of each newly discovered cave or feature surveyed and include a detailed, scaled cave map with plan and profile views including the following:
  - The approximate passable length of the cave or feature
  - The approximate heights and widths of passages
  - Locations of any standing or flowing water and direction of any water movement

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