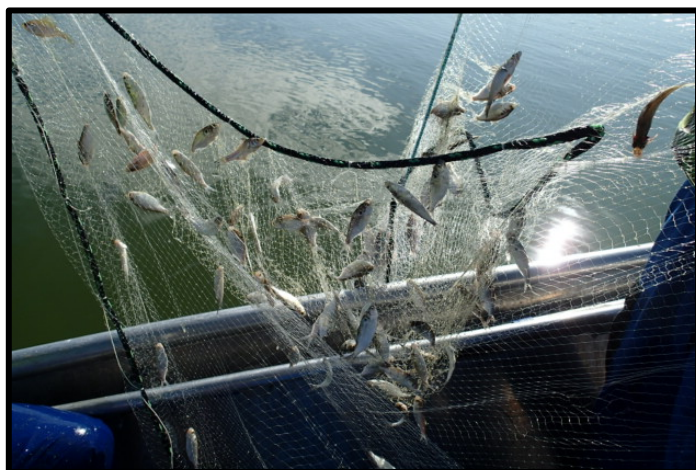




U.S. Fish and Wildlife Service

Recommended Sampling Gear Types and Standard Operating Procedures for the Early Detection of Non-native Fishes and Select Benthic Macroinvertebrates in the Great Lakes



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“Working with partners to conserve, protect, and enhance fish, wildlife, and plants and their habitats for the continuing benefit of the American people”

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1.0 Scope and Justification

This document identifies a suite of recommended sampling gear types and standard operating procedures to be used by each Great Lakes Fish and Wildlife Conservation Office (FWCO) during early aquatic invasive species detection sampling. The specific gear types deployed by each Great Lakes FWCO will be identified in their lake-specific implementation plans. Gear types will be standardized and chosen to maximize the efficiency and effectiveness for the purpose of obtaining the highest probability of detecting a new invasive species. All sampling gears deployed by the U.S. Fish and Wildlife Service's Great Lakes FWCOs should be standardized to the same dimensions and units of effort (e.g., fishing time/area/distance) for comparisons among lakes. Numerous sources were used to compile this document (e.g., The 2016 Monitoring and Response Plan for Asian Carp in the Upper Illinois River and Chicago Area Waterway System; Bonar et al. 2009). Most sample locations record GPS coordinates, temperature, and other water quality measurements.

2.0 Ichthyoplankton and Larval Fish

Listed below are a suite of gear types to be considered when sampling for ichthyoplankton and larval fish. At most sampling locations, these gear types can be used to effectively sample the entire larval fish community over all available habitat types.

2.1 Larval Light Trap

2.1.1 Targeted fishes and habitats

Light traps are used to sample larval fish in areas not easily monitored by other larval sampling gear such as in vegetation, woody debris, shallow areas, channel margins or little to low velocity areas. Additionally, Hickford (1999) found that light traps select for larger larval fishes which may be able to actively avoid net tows.

2.1.2 Specifications

Quatrefoil type larval fish light trap according to Secof and Hansbarger (1992) with cloverleaf shaped array with 30 cm (diameter) x 30 cm (length) x 25 cm (height) with 4 entry slits 2-3 mm wide. Poly carbonate construction, 7 mm fixed slot width, It has a top plate flotation block and a central container for a dive flash light or phosphorescent light/glow stick. There is a plankton net connected to the trap that is a 250 micron net with polycarbonate end bottle.

2.1.3 Deployment

Deployment depth, light wavelength, light intensity and light color can vary with water conditions and target species. Water clarity, light intensity and color, larval fish size and larval fish photoresponse must all be considered when choosing to deploy light traps (Brogan 1994). Deployment should occur in the overnight hours (at least one hour after sunset until one hour before sunrise) at depths greater than 1 meter and in areas with little to no current (Lindquist and Shaw 2005). These traps can be set from a boat or by wading depending on the waterbody and access. Put a

light source in each trap. Anchor/tie off each trap so that it remains within a 1 meter radius of the select locale (either with a block to the bottom, or to woody debris), but so it still floats at the surface. Set each trap far enough away from each other to eliminate overlapping light from the different traps in a given area.

2.1.4 *Standardized effort*

Effort is standardized to number of fish collected in a trap per unit of time

2.1.5 *Used by: (office)*

2.1.6 *Efficiency/Effectiveness/Continue to use?*

2.2 Conical and Bongo Nets

2.2.1 *Targeted fishes and habitats*

Conical nets target early life history fishes (eggs and larvae) in flowing waters.

2.2.2 *Specifications*

Conical net sampling consists of two nets being deployed with the same length, diameter, and mesh size (e.g., 0.6 m diameter, 500 μm mesh D:L= 1:3). Conical nets can either be deployed in a “Bongo” configuration with two circular frames towed from the center, or in a standard configuration with two independent frames. A flow meter is attached to the opening of the net.

2.2.3 *Deployment*

Tows should be conducted outside of the boat prop wash to avoid biasing results. When deploying nets individually, care should be taken to ensure that deployment times are standardized for the pair. Conical nets are to be fished in >1 m of water at night (at least one hour after sunset until one hour before sunrise), unless the target species is more susceptible to the technique during daylight hours. Each net will consist of a sturdy frame, flow meter, conical mesh net, and have a screened sample bucket attached to the cod. Tow duration will be 5 - 10 minutes depending on flow and debris conditions, but may vary between water bodies based on the productivity of the system. Tow time begins when the net is fully deployed and ends when it is no longer fully submerged. Tow speeds depend on net diameter and mesh size, but should be 4.6 km/h to keep the net just below the surface, and should be held consistent during the entire deployment. The track of each tow is recorded on a GPS device and starting and ending flow meter readings are recorded.

2.2.4 *Standardized effort*

Effort is standardized to the number of fish collected per volume of water taken from the flow meter.

2.3 Neuston Net

2.3.1 *Targeted fishes and habitats*

Fishes in the upper water column in shallow habitats are targeted.

2.3.2 *Specifications*

The net frame is 1.0 m wide by 0.5 m in height. The net is 3.0 m long and made of 500 μm Nitex netting that tapers down to an 8.9 cm diameter by 30.5 cm long collection bucket with a 500 μm filter. A flow meter should be placed in the middle of the net opening.

2.3.3 *Deployment*

The frame is towed outside of the prop wash alongside the boat and deployed at night (at least one hour after sunset until one hour before sunrise). Neuston netting should occur on calm nights with little wave action. Tow duration should be 5 minutes at a speed of 2.3-2.5 km/h. The net should be towed with \sim 2.5 cm of air space above the water's surface in water depths >2 m.

2.3.4 *Standardized effort*

Density is defined as the number collected/1000 m^3 . Effort can be defined as $\#/\text{volume}$ of water if a flow meter is used.

2.4 **Tucker Trawl**

2.4.1 *Targeted fishes and habitats*

Tucker trawls target smaller pelagic species in the middle of the water column.

2.4.2 *Specifications*

The tucker trawl frame is 1.0 m wide by 1.4 m in height when held vertically, which compensates for a 45° drag on the lower pipe to provide a sampling area of 1.0 m^2 when under tow. The net is 4.0 m long and made of 500 μm Nitex netting that tapers down to an 11.0 cm diameter by 17.0 cm long collection bucket with 500 μm filter.

2.4.3 *Deployment*

Trawling is conducted at night (at least one hour after sunset until one hour before sunrise) using stepped-oblique tows or targeted discrete-depth-interval tows (Beauchamp et al. 2009). Tow duration is 5 minutes at a speed of 3.2 km/h. The net is towed \sim 1.0 m off bottom at water depths ≥ 3.0 m. The distance of the trawl should be recorded

2.4.4 *Standardized effort*

Effort is defined as the volume per tow (m^3) which can be calculated as the effective cross-sectional area of the net opening times the distance traveled at target sampling depths.

2.5 **Push Net**

2.5.1 *Targeted fishes and habitats*

Eggs and larvae are the targeted fishes and targeted habitats include the river mainstem and backwater sites as well as tributary sites near the shore banks.

2.5.2 *Specifications*

A push net consists of paired square nets with flowmeters housed in the opening of nets. Specifications are 75 cm x 75 cm x 1.5 m long with 500 micron mesh.

2.5.3 *Deployment*

The net is pushed upstream using an aluminum frame mounted to the front of the boat. Boat speed will be adjusted to obtain 1.0 to 1.5 m/s water velocity through the net. A flow meter is mounted in the center of the net mouth and used to calculate the volume of water sampled. Sampling transects are conducted parallel to the bank within each study site.

2.5.4 *Standardized effort*

Effort is defined as the number of fish collected per volume of water for each net.

2.6 **Larval Seine**

2.6.1 *Targeted fishes and habitats*

Species targeted are those associated with areas of sand or gravel substrates and homogenous habitats. *Specifications*

The seine is 2.5 m wide x 1.1 m tall with 500 micron mesh.

2.6.2 *Deployment*

Two people, each holding one end, should pull the seine in a half moon along the shore.

2.6.3 *Standardized effort*

Effort is calculated as the number of fish per seine haul. Effort could also be standardized by the area seined.

2.7 **Other Effective Gear**

2.7.1 Other gears that are proven effective may be used to sample ichthyoplankton and larval fish. All non-listed gear should be proven effective at sampling target species in environments similar to those in which they will be deployed. Additional gears used should be reviewed for effectiveness and added to the gears SOP document before deployment.

3.0 **Juvenile and Adult Fishes**

In most sampling locations, a suite of three or more gear types should be used to effectively sample the entire juvenile/adult fish community over all available habitat types. Listed below is a suite of gear types that are effectively used to capture juvenile and adult fishes. Not all gears are required to be used at each location.

3.1 **Minnow Trap**

3.1.1 *Targeted fauna and habitats*

Minnow traps are intended for small fishes and crayfishes in habitats up to 10 m. Arrays are set parallel to break walls, unique features or the shoreline

3.1.2 *Specifications*

Minnow traps will be identically shaped with matching mesh sizes and opening dimensions that may vary to accommodate the targeted species (e.g., length of 42 cm, center diameter of 23 cm with 6.4 mm mesh and 31.7 mm openings on both ends).

3.1.3 *Deployment*

Minnow traps can be deployed individually or in an array. If they are deployed in an array, the array should consist of five traps attached to a mother line with a spacing of 7.6 m between each trap and each end should have an anchor and marker buoy. A 3.0 m spacing can be used as well to target more confined areas. The minnow traps can be used with or without bait. Baited minnow traps should use a consistent amount of a proper attractant, such as dogfood or cheese, for the species being targeted. Minnow traps are fished overnight (at least one hour after sunset until one hour before sunrise) for a period of 12-24 hours. In riverine environments, arrays are set parallel to current or at a 45° angle with current. In lake environments, traps are set perpendicular to shore across varied depths or along features of interest.

3.1.4 *Standardized effort*

Minnow trap effort is defined as the number of fish collected per net night.

3.2 **Seine**

3.2.1 *Targeted fauna and habitats*

Targeted fishes are small fishes (minnows and age-0 fishes) and used in standing waters at depths < 1 m. They can be set in confined areas or riverine environments where current may be a factor. Areas with steep slopes, debris or snags should be avoided.

3.2.2 *Specifications*

The standard seine is 9.1 long by at least 0.91 m tall and consists of 6.4 mm knotless mesh, also known as “ace” mesh.

3.2.3 *Deployment*

Sampling should cover an area between 139.35 – 278.71 m². The sampling area is determined by pulling a fully extended seine 15.2 – 30.5 m. Two 30.5 hauls are conducted at each site: one parallel to the shore and one perpendicular to the shore. In the event that conditions prevent the ability to pull the seine perpendicular to the shore, both pulls can be done parallel to the shore. The seine should be pulled to shore as quickly as possible to prevent escape of fishes. The leadline should always be in contact with the bottom with the float line on the surface. Deployment should

take place in the daytime and occur during late summer when many age-0 fishes are vulnerable to capture.

For lakes or slow moving river environments, a large bag or beach seine is needed to effectively sample the area. A 45.7 m long by 2 m high bag seine is used with a 2 m by 2 m bag in the center. All netting materials measure 3.17 mm stretch mesh. The bag seine is deployed perpendicular to the shoreline starting from the anchor point at the water's edge (Pope et al. 2009). Once the bag seine is fully extended it is pulled at an arc around the anchor point. The area sampled is equivalent to a quarter-circle with a radius of 45.7 m.

3.2.4 *Standardized effort*

Relative abundance is reported as CPUE (e.g., number of fish per seine haul when seine dimensions and characteristics are standardized). At least three seine hauls per site are recommended. Effort can also be defined as the number collected per area seined.

3.3 **Paired or Single Mini-fyke Net**

3.3.1 *Targeted fauna and habitats*

Targeted habitats include the shoreline or open water habitats and shallow off-channel areas

3.3.2 *Specifications*

A paired mini-fyke net unit consists of two mini-fyke nets attached to each end of a lead net that is 6 m long and 0.45 m high. Each mini-fyke net consists of two rectangular frames 0.45 m high by 0.90 m wide, followed by four circular frames (rings) 0.45 m in diameter. All netting materials are 3.17 mm stretch mesh.

3.3.3 *Deployment*

The nets are set in single or tandem configurations depending on site characteristics. Single nets are set with the lead end staked on the shoreline or another obstruction to fish movement, such as a fallen tree. Tandem nets (with leads attached end to end) will be fished in open water areas. Mini-fyke net units should be set parallel to the shoreline or along macrophytes in water depths less than 3.0 m and left over night (at least one hour after sunset until one hour before sunrise) for a period of 12-24 hours. Orientation of nets may be changed to maximize capture efficiency. Water depth should be adequate to allow fish to pass through throats sewn into each frame.

3.3.4 *Standardized effort*

Effort is standardized as number of fish collected per net night.

3.4 **Paired Modified Fyke Net**

3.4.1 *Targeted fauna and habitats*

Targeted habitats include aquatic macrophyte beds and shoreline features and should be set between 1 – 3 m water depths. Target fauna include juvenile and adult Centrarchids, Cyprinids, and Percids.

3.4.2 *Specifications*

A paired fyke net unit consists of two fyke nets tied together by their leads, each 15 m long by 0.91 m high. a fyke net attached to each end of a lead net that is 15 m long and 0.91 m high. Each fyke net consists of two rectangular frames 0.91 m high by 1.08 m wide, followed by four circular frames (rings) 0.91 m in diameter and is constructed using 13 mm bar mesh coated in a black tar-like substance.

3.4.3 *Deployment*

Fyke net units should be set parallel to the shoreline or along macrophytes in water depths less than 3.0 m and left over night (at least one hour after sunset until one hour before sunrise) for a period of 12-24 hours. Orientation of nets may be changed to maximize capture efficacy. Water depth should be adequate to allow fish to pass through throats sewn into each frame.

3.4.4 *Standardized effort*

Catch is standardized as number of fish collected per net night.

3.5 **Boat Electrofishing**

3.5.1 *Targeted fauna and habitats*

Electrofishing should target habitats in the immediate sampling area that are likely to concentrate fish (e.g., vegetation, logs, artificial structures, shoreline, substrate or depth changes, etc.) and is particularly applicable in the littoral zone (Miranda and Boxrucker 2009). Multiple fish species and sizes are susceptible to electrofishing. Electrofishing tends to be biased toward smaller individuals (Dolan and Miranda 2003).

3.5.2 *Specifications*

Electrofishing may be conducted during daylight or nighttime hours (dependent on site characteristics) in water depths between 1-2.5 m depending on the abilities of the shock unit and water conductivity. A pulsed DC (direct current) electrical current of an effective frequency will be used with sufficient power to induce taxis in fish, which depends upon the water conductivity and will be standardized for all the repeated samples at a sampling location. Miranda and Boxrucker (2009) provide an overview on standardizing electrofishing power for boat electrofishing. Electrofishing will occur with two netters. Locations for each electrofishing transect will be identified with GPS coordinates (decimal degrees).

3.5.3 *Deployment*

Electrofishing around a predetermined waypoint will occur within an area of 0.25 km² or along the shoreline for a total target sample time of 600 seconds (10 min), at an approximate speed of 2.5 - 3.0 km/h. Electrofishing should be conducted in a

downstream direction in the waterway main channels or in a counter-clockwise direction

3.5.4 *Standardized effort*

Effort is standardized to the number of fish collected/hour of electrofishing.

3.6 **Trawling**

3.6.1 *Targeted fauna and habitats*

Trawling can be conducted during daylight or nighttime hours depending on the sampling area, crew experience (safety), and vessel capabilities. Bottom trawling should be conducted in areas free of underwater obstructions.

3.6.2 *Specifications*

A Marinovich design trawl with a 4.9 m head-rope, 3.8 cm stretch mesh body and a 3.125 mm stretch mesh inside liner will be used for bottom trawling.

3.6.3 *Deployment*

Trawling should occur in water depths greater than 2 m along contours to avoid rapid depth changes that decrease sampling efficiency. Tows will have a target time of 5 min at a speed of 4 km/h to standardize catch rates.

3.6.4 *Standardized effort*

Effort should be recorded as time trawled and distance traveled. CPUE is expressed as fish per minute of trawling.

3.7 **Experimental Micromesh Gill Net**

3.7.1 *Targeted fauna and habitats*

Fishes targeted for gill nets include open-water species that are active in the day and species that exhibit crepuscular or nocturnal activities. Nets are to be fished at depths > 2 m locations.

3.7.2 *Specifications*

Each net measures 1.52 m (5 ft) high by 12.19 m (40 ft) long. The nets are made up of four 3.048 m (10 ft) panels of different size monofilament gill netting (12 mm, 16 mm, 20 mm, and 25 mm bar).

3.7.3 *Deployment*

Nets are deployed along the bottom, perpendicular to the bank, in depths of 3-8 m. The duration of net deployment can vary from 1 to 24 h depending on site specific catch rates, but is usually set overnight. A recommendation for initial deployment is 2 to 4 h.

3.7.4 *Standardized effort*

Catch per unit effort is expressed as catch per net-night or hour.

3.8 **Monofilament Experimental Gill Net**

3.8.1 *Targeted fauna and habitats*

This benthic sampling gear is used in any lentic habitat deeper than 2 m and targets Percids, Moronids, Cyprinids, Ictalurids, and Clupeids.

3.8.2 *Specifications*

1.8 m tall by 40 m long and comprised of 13 randomly ordered panels with bar-mesh sizes from 6-76 mm (6, 10, 13, 16, 19, 25, 32, 38, 44, 51, 57, 64, 76).

3.8.3 *Deployment*

Deployment is similar to the experimental micromesh gill nets

3.8.4 *Standardized effort*

Effort is defined as the number of fish collected per net night.

3.9 **Cloverleaf Trap**

3.9.1 *Targeted fauna and habitats*

Cloverleaf traps can be set in shallow or deep vegetated (submergent, emergent) habitats. They target small fishes including Centrarchids and Cyprinids.

3.9.2 *Specifications*

Cloverleaf traps are three lobed (38 cm high, 41 cm/lobe diameter) and constructed of galvanized ¼ inch wire mesh with 160 mm wide openings between lobes to allow for the entrance of juvenile fishes.

3.9.3 *Deployment*

Cloverleaf traps are set nearshore or in shallow backwater habitats and set overnight (at least one hour after sunset until one hour before sunrise). The trap should be oriented flat to the water bottom.

3.9.4 *Standardized effort*

Effort is defined as the number of fish collected per trap night.

3.10 **Combined Trammel Nets and Electrofishing Gear**

3.10.1 *Targeted fishes and habitats*

The targeted fish taxa are large-bodied fishes, specifically, grass carp (Marson et al. 2016).

3.10.2 *Specifications*

The nets are deployed in lengths of either 182.9 m or 274.3 m, with inner mesh sizes ranging from 7.62 cm to 10.16 bar mesh (15.24 cm to 20.32 cm stretch mesh sizes), and net depths of 3 m. The trammel nets differ from the tied-down gill nets in having two additional panels of netting that sandwich the inner gill net panels. The outer netting is 45.72 cm bar mesh nylon netting that works to bag large-bodied fishes in the net (those too large to be gilled in the inner monofilament gill netting; Marson et al. 2016).

3.10.3 *Deployment*

Trammel nets were set for a short amount of time (approximately 30 minutes) in order to minimize the entanglement time of fishes. The nets were deployed in a

way that would block off suitable habitat for Asian carps. The net is set to the shore and run perpendicular out from shore approximately 20-30 m, the boat is then turned and 120-214 m of net is deployed parallel to shore, and then the final 20- 30 m is deployed perpendicular back into shore. This deployment technique blocks fishes into the encircled area. Heavily vegetated areas could be sampled, but the net would have to be deployed on the outer margins of the heavy vegetation so that it would deploy properly and cover the full depth of the water column. Setting the net in very heavy vegetation would limit its effectiveness as the lead-line would not always push through the vegetation, and would be held up off bottom, allowing fishes to escape below the lead-line. Once the net is set, the crew enter the blocked off area with the boat and use its motor and modified plungers to “pound” the area. By revving the engine, banging the hull of the vessel, or pounding the water’s surface with plungers, the crew actively chases fishes in an attempt to get them to flee into the direction of the net. This method, referred to as “pounding” was developed by researchers working in the Mississippi watershed on the removal of Asian carps, which are known to be net avoidant species (ACRCC 2014). This sampling method provides several advantages over traditional gill 3 netting methods, including reduced set times (reducing stress on captured fishes), increased catch of sedentary fishes, and an increased number of sites sampled per day (Marson et al. 2016).

3.10.4 *Standardized effort*

Sampling effort was recorded as both the length of the net used and the amount of time the net was actively fished (in minutes; Marson et al. 2016).

3.11 **Other effective gear**

3.11.1 Other gears that are proven effective may be used to sample juveniles and adult fishes. All non-listed gear should be proven effective at sampling target species in environments similar to those in which they will be deployed. Additional gears used should be reviewed for effectiveness and added to the gears SOP document before deployment.

4.0 **Benthic Macroinvertebrates**

Listed below is the suite of gear types that can be used for sampling benthic macroinvertebrate such as mollusks and amphipods. In most sampling areas, a suite of two or more sampling methods should be used to effectively sample the macroinvertebrate community.

4.1 **Benthic Ichthyoplankton Sled**

4.1.1 *Targeted fishes and habitats*

The target taxa are benthic, demersal communities in shallow water with homogenous water bottom habitats. This gear allows for a benthic sample to be

collected over a much greater distance than other invertebrate collection gears, such as PONAR grabs. Additionally, the ratio of live to dead specimens can be low.

4.1.2 *Specifications*

Benthic sleds should be double sided to allow effective sampling regardless of which side it settles on. Broad runners and an adjustable cutting blade bar allow for the top layer of surface sediment to be removed and deposited into the bag (Blomqvist and Lundgren 1996). The sled measures a total length of 1125 mm, width of 700 mm, and a cutting width of 400 mm. The sled should consist of a large mesh inner net (10 mm bar) and a fine outer mesh net (2 mm bar). A flow meter is attached to the plankton net.

4.1.3 *Deployment*

Benthic sleds should be towed in areas free of underwater obstructions. The sled is deployed in neutral. A float is attached to the stern of the sled to allow for recovery should a hang-up occur. A two minute tow at a speed of 3-4 km/hour starts when the sled has settled to the bottom. Coordinates are recorded for start and end points of the tow. After retrieval, the sample is sprayed down into a bin. The sample is agitated and cleaned, then poured through a bucket-sieve.

4.1.4 *Standardized effort*

Effort for the benthic sled is defined as the number of taxa collected per volume of water.

4.2 **Colonization Samplers**

4.2.1 *Targeted fauna and habitats*

Colonization samplers passively collect invertebrates by placing a known amount of uncolonized substrate into shallow water habitats (Zales 2012). There are three types of colonization samplers: Rock bags, Hester-Dendy (HD) colonization plates and modified Hester-Dendy colonization plates.

Hester-Dendy colonization plates target limited amounts of live gastropods and amphipods.

Modified HD samplers have only 3 plates to target zebra mussels.

4.2.2 *Specifications*

Hester-Dendy: The standard HD consists of fourteen 3.2 mm thick square plates each 7.5×7.5 cm. Spacing between nine plates increases from top to bottom; eight 3.2 mm spaces, one 6.4 mm, two 9.5 mm, and two 12.7 mm. Total sampling area is 0.16 m^2 with a weight adequate to keep the sampler on the substrate.

Modified HD: Same as the HD but with only 3 plates.

Rock Bags: Rock bags are constructed by enclosing 2.5 – 7.6 cm beach stones (standardized by filling a 1 L bottle with these stones) in 13 mm nylon mesh bags that is approximately 12 x 12 inches. A 5 x 6 inch piece of 500 micron nitex mesh

is placed at the bottom of the rock bag with rocks placed on top to prevent organisms from falling out when we retrieve them.

4.2.3 *Deployment*

The HD is a type of multi-plate colonization sampler that is deployed for a specified length of time allowing organisms to colonize the areas between the plates. If deployed in unstable substrate, the HD may be rigged to float above the bottom to avoid being buried (Beckett et al. 1998). Hester-Dendys should be deployed for a standard 36 days (+/- 5 days).

Rock bags: Rock bags are deployed in shallow areas (i.e., <3 m depth) for at least 30 days to facilitate colonization.

4.2.4 *Standardized effort*

CPUE is defined as the number of individuals per sampler.

4.3 **Dredge Sampling**

4.3.1 *Targeted fauna and habitats*

Targeted fauna are gastropods and mussels. They are largely ineffective because it catches almost exclusively dead specimens. The “mini” or “petite” Ponar was chosen as the preferred dredge by the USFWS due to its ability to sample many bottom substrate types, and for its ease of cleaning, which helps mitigate crew contaminant exposure (USEPA 1999).

4.3.2 *Specifications*

The standard petite Ponar weighs 10.9 kg and samples a volume of up to 2400 mL and a surface area of 0.024 m².

4.3.3 *Deployment*

After retrieval the sample is deposited and washed into a bin. In the washdown bin, the sample is agitated and cleaned, then poured through a bucket sieve. Samples collected should be preserved in the field for later identification. Specimens such as mollusks should be washed free or hand-picked from the sediments prior to preserving.

4.3.4 *Standardized effort*

Effort is quantified as the number of specimens per ponar grab.

4.4 **Hand Collection**

4.4.1 *Targeted fauna and habitats*

Hand collection of macroinvertebrates should be used for large, slow moving or stationary species in locations with low turbidity (Haag et al. 2012). If samples are collected in deeper water, the use of a suction gun should be considered as it has been found to be easier and faster for the sampler, while decreasing unintentional damage inflicted from handling (Davies and Ramsey 1989).

4.4.2 *Specifications*

4.4.3 *Deployment*

Collecting mollusks by hand will be achieved by picking/scraping organisms off rocks, logs, debris, piers, fixed objects, and from substrate or vegetation by sight. This is a qualitative way to determine presence of mussel species. Total search time per site should be 10 min, or as outlined in a sampling plan.

4.4.4 *Standardized effort*

Total search time per site should be 10 min, or as outlined in a sampling plan. In the event multiple crew members are searching, the total search time shall equal the search time of all the individuals combined (e.g., two crew members would each search for 5 min for a combined total effort of 10 min). Effort should be standardized to the number of specimens collected per unit of time.

4.5 **Kick/Sweep Nets**

4.5.1 *Targeted fauna and habitats*

These are effective at capturing live specimens of amphipods, gastropods and mussels. Requires vegetated areas but can be problematic with organisms becoming damaged after removal from vegetation. Multiple state and federal agencies use kick net samplers to monitor benthic macroinvertebrates (Bruna et al 2011). D frame kick nets provide a comprehensive qualitative sample in a short period of time (Rabeni 1996). For triangular nets, sampling is targeted towards structural objects such as logs or vegetation in water depths ≤ 1.8 m.

4.5.2 *Specifications*

D Frame kick nets: The net has a D-shaped frame (310 mm \times 170 mm; 660 μ m mesh bag) and is mounted to a pole.

Triangular nets: Sweep net heads were triangular in shape with 305 \times 305 \times 305 mm dimensions. The bag was 152 mm deep with a heavy duty full shroud to protect the 500 μ m Nitex netting on bottom.

4.5.3 *Deployment*

4.5.4 *Standardized effort*

D Frame: A standard sample requires a minimum of 3 minutes and maximum of 5 minutes of sweeping effort in multiple vegetation beds per site.

Triangular: One unit of effort consists of five minutes total sweeping time between one or two people.

4.6 **Amphipod Trap**

4.6.1 *Targeted fauna and habitats*

Amphipods are the targeted fauna for this gear. Habitats targeted for these traps are waters in less than 1 m of water.

4.6.2 *Specifications*

Amphipod traps were modeled after the Hensler-Hutton-Stadig (HHS) trap described by Stadig (2016). The trap consists of a Gee's minnow trap (see description above) covered with 243 μ m Nitex mesh and fitted with 17 mm rubber gaskets sewn into either end to provide openings into the trap. Another trap configuration uses PVC and is 50.8 cm in length, 10.16 cm diameter polyvinyl chloride pipe with white end caps. There are 7.94 mm holes on the top for amphipod entry (Stadig 2016).

4.6.3 *Deployment*

Traps are deployed in less than 1 m of water and fished at night from one hour past sunset for six to eight hours and are baited with light (a dive flashlight is mounted in one end cap and the other end cap has foil to reflect the light). Traps are then recovered and the contents are washed down and concentrated into a 500 ml plastic sample bottle.

4.6.4 *Standardized effort*

Effort is quantified as the number of specimens collected per trap

4.7 **Modified Minnow Trap (plastic gee-style trap)**

4.7.1 *Targeted fishes and habitats*

These traps target decapods (mysids and crayfish), some gastropods and amphipods. Targeted habitats include near the shoreline. Used in 2019 by GBFWCO to target *Hemimysis*)

4.7.2 *Specifications*

Traditional round, galvanized minnow traps (length = 406 mm by 254 mm wide: 19 mm diameter throat, 500 micron mesh inside lining.

4.7.3 *Deployment*

Traps were set overnight near the shoreline for approximately 4 to 12 hours and are baited with dog food.

4.7.4 *Standardized effort*

Effort is defined as the number of specimens collected per trap night.

4.8 **Crayfish traps**

4.8.1 *Targeted fauna*

Crayfish (Specifically, Rusty and Redswamp crayfishes)

4.8.2 *Specifications*

Galvanized steel; 6 mm mesh; 420 mm long by 230 mm wide; 40 mm diameter throat, with or without conical-shaped throat restriction

4.8.3 *Deployment*

Traps are set overnight near the shoreline or in deeper marinas and baited with dog food

4.8.4 *Standardized effort*

Effort is defined as the number of specimens collected per trap night.

4.9 Pyramid Trap

4.9.1 Targeted fauna

These traps target crayfish in shallow waters.

4.9.2 Specifications

The traps are shaped like a pyramid with three funnel entrances at the base. The traps are made of 1.9 cm plastic-coated mesh and are approximately 50 cm wide at the base and 66 cm tall. At the top of the trap is a retainer collar that minimizes crayfish escape and can be used as a handle. The top is open for quick removal of crayfish.

4.9.3 Deployment

The trap is positioned upright with the top extending out of the water. This trap can be baited (cat food, dog food) and then left to soak for at least 24 hours.

4.9.4 Standardized effort

Effort is quantified as the number of specimens collected per trap.

4.10 Pillow Trap

4.10.1 Targeted fauna

The wire-mesh pillow trap is designed for capturing crayfish in backwater swamps and other natural areas

4.10.2 Specifications

The trap is shaped like a pillow that is approximately 36 cm wide and approximately 60 cm long. They have three inverted funnels at the corners that allow crayfish to enter the trap. The fourth corner can be opened and is used to insert bait and remove crayfish. The pillow trap can be baited (cat food, dog food) and left to soak for at least 24 hours (Shirley and Lutz 2009).

4.10.3 Deployment

Traps are typically set in the water by leaning them against a tree stump, stake or other object at a 45° angle.

4.10.4 Standardized effort

Effort is quantified as the number of specimens collected per trap.

4.11 Other effective gear

4.11.1 Other gears that are proven effective may be used to sample benthic macroinvertebrates. All non-listed gear should be proven effective at sampling target species in environments similar to those in which they will be deployed. Additional gears used should be reviewed for effectiveness and added to the gears SOP document before deployment.

5.0 Preservation

- 5.1** All specimens should be fixed in 95% non-denatured EtOH. If both visual and genetic ID and genetic ID are needed for egg and larval samples, it is best to split samples between preservation techniques (formalin and 95% non-denatured EtOH) so that both analysis methods can be utilized. In some cases, (e.g., eggs and larvae) samples can be preserved in formalin to better preserve such small individuals and help in the identification process. Formalin should only be used if genetic analysis is not going to be needed.
- 5.2** To properly ensure the preservation of the sample, it is important to ensure the ethanol remains at 95%. Two issues arise: first, using too little ethanol for samples with large volumes of organic content and second, the ethanol draws water out of the organic material in the sample, reducing ethanol to below 95% required for preservation. Thus, additional ethanol must be added to bring it back up to 95%. Ensure that the sample bottle has a large enough volume relative to the total organic matter in the sample because the ratio of organic matter to ethanol is important. Too little ethanol will result in sample spoilage as water drawn out of the organic matter dilutes the ethanol. A good rule-of-thumb is to preserve 1 part organic matter in 3 parts 95% ethanol. Organic matter includes algae, plankton, woody debris, and other non-target organisms. Ethanol must be changed after the first 24-48 hours, to combat dilution by water that is drawn out of the organic matter.
- 5.3** Bivalves in samples must have their adductor valves cut to prevent the animals from closing up and preventing preservation.

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Table 1. Targeted fauna, targeted organisms, targeted habitats and standardized efforts for specific gears to be used in the AIS Early Detection and Monitoring Program

Targeted Fauna	Gear	Targeted Organisms	Targeted Habitat	Standardized Effort
Ichthyoplankton and Larval Fish	Larval light trap	larval fishes	vegetation, woody debris, shallow areas, channel margins, little or no velocity areas	# fish in trap/time set
	Conical and bongo net	eggs and larval fish	flowing waters	# fish /volume of water
	Neuston net	fishes in upper water column	upper water column in shallow habitats	# collected/1000m ³ ; # collected/volume of water
	Tucker trawl	small pelagic species	mid water column	# collected/volume of tow
	Push net	eggs and larvae	river mainstem, backwater and tributary sites, near shore banks	# fish collected/volume of water for each net
	Larval seine	larval fishes associated with sand or gravel substrates	sand or gravel substrates, homogenous habitat	# fish collected/seine haul; # collected/area seined
Juvenile and adult fishes	Minnow trap	small fishes and crayfishes	up to 10 m, break walls, unique features, shoreline	# fish/crayfish collected per trap night
	Seine	small fishes (minnows and age-0 fishes)	standing waters less than 1 m. Confined areas or rivers with current	# fish/seine haul
	Paired or single mini-fyke net	Small fishes, occasionally larger fishes	shoreline or open water habitats, shallow off-channel areas	# fish/net night
	Paired fyke net	small and large fishes	shoreline or open water habitats, shallow off-channel areas	# fish/net night
	Boat electrofishing	multiple fish species, tends to be biased toward smaller individuals	vegetation, logs, artificial structures, shoreline, substrate or depth changes; litteral zone	# fish/hour of electrofishing
	Trawling	juvenile, benthic or small bodied fishes	bottom free of underwater obstructions	# fish/minute of trawling
	Experimental micromesh gill net	open water species active in day, or crepuscular or nocturnal	depths > 2 m	catch/net night/panel
	Monofilament experimental gill net		main channel habitats	catch/net night/panel
Benthic Macroinvertebrates	Benthic Ichthyoplankton sled	benthic, demersal communities	shallow water with homogenous bottom habitats	
	Colonization samplers	invertebrates; gastropods; amphipods, zebra mussels	shallow water	# individuals/sampler
	Dredge sampling	gastropods and mussels	shallow water, benthic habitats	number of specimens/PONAR grab
	Hand collection	macroinvertebrates for large, slow moving, or stationary species	low turbidity locations, shallow water	# specimens/unit of time
	Kick/sweep net	amphipods, gastropods, mussels	vegetated areas, shallow water	# specimens/unit of time
	Amphipod trap	amphipods, gastropods, mussels	waters less than 1 m	# specimens/trap
	Modified minnow trap	decapods (mysids and crayfish), gastropods, amphipods	shoreline	# of specimens/trap night
	Pyramid trap	crayfish	shallow waters	# of specimens/trap night
	Pillow trap	crayfish	backwater swamps, other natural areas	# of specimens/trap night