

Instructions for monitoring testfishing of eels with fykenets and dissections. – English version

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Instructions for monitoring testfishing of eels with fykenets and dissections *Provfiskeinstruktion för Ålprovfiske med ryssjor och dissektion*

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1. Introduction and background

Annual testfishing for eels with fykenets have been carried out in lake Mälaren outside the institute of freshwater research in Drottningholm since the 1970ies.

The aim of the study is foremost to follow the development of an eel stock that was introduced 1980. The monitoring was standardised in association with a later restocking of eel fry marked with Alizarin in 1997, to be able to follow the development of these eels in the local area.

The parameters that is studied is foremost catch per unit effort (CPUE), size and growth, gender ratio, age at maturation, etc. The spread of the restocked eels has also been studied by performing monitoring testfishing outside the royal palace in Drottningholm.

Starting 2010 some eels have been chosen to be marked with PIT-tags as well as with data collecting tags (DST) to gather information from individual eels regarding growth, migration and habitat preferences (depth and temperature). The long-term goal of this study is to verify the low natural mortality of stocked eel fries that we have seen indications of from other lakes.

The monitoring is performed annually from the beginning of May until the middle of July, depending on water temperature. The monitoring starts when the surface water temperature reaches 10° C since that is the temperature where the eels starts to be more active after the more dormant winter period. The monitoring stops around 10^{th} of July mainly due to logistical reasons.

2. Equipment

2.1. Preparations before test fishing

- Working clothes: Rubber boots, Grundén oilskins.
- Solas certified life jacket (checked out from the testfising depot).
- Boat (make sure the boat "Silverfisken" is booked in outlook)
- Water resistant labels for the netbags. 10/20? Labels/bag
- 2 bigger buoyes with correct contact information on them to mark the outer test fishing area.
- Dedicated storing box for Sotholmen eels in the Grovlab feezer.

2.2. Preparations of fykenets

- Fykenets * 40 (8 fykenet pairs in each set).
- Rope/string to make loops to fasten the fykenet to each other (approximately 1 meter long, see fig 2-9)
- Buoys *5 with approximately 20 meters of line attached
- Mushroom anchors * 10 (five with attached 10 meters lines and five with shorter lines).
- Plastic badges with the letter s A-E (five in total). And badges with the uneven numbers from 3- 15 in five sets (35 in total)
- Temperature trackers * 2 (placed in cod end "C7" and "D7")
- Plastic barrels * 5 (to pack the sets of fykenets in)

2.3. Field equipment found in pumphuset

- Keys to the boat
- Oars
- Wooden Tray to stack the fykenets on in the boat when taking care of the catchNet-bags for eels *10 with water resistant labels on them
- Barrel with a lid to keep the eels in
- Buckets (a couple of them)
- Grapnel with a long line
- Extra lines and buoys for the fykenets (in case some are missing)
- Eel fishing box (see chapter 2.4)
- Sinker

2.4. Equipment found in the eel fishing box

- Testfishing permit (make sure to bring it with you in the boat)
- Water resistant protocols (two of them; one for eels and one for crayfish)
- Writing pad with a floater and pencil attached
- Extra water resistant labels for the eel net bags
- Thermometer (digital) for surface temperature measurements
- GPS (only needed when deploying the fykenets for the first time each season)
- Thread and needle and plastic stripes for mending the fykenets
- Knife
- Extra plastic badges for numbering the cod ends of the fykenet
- Pit-tag reader "HPR light BIOMARK reader" (bring it with you in the boat).

- Markers
- Pencils
- Measure board (small for crayfish)
- Streamer tags (for tagging crayfish)
- Scissors
- Pliers
- Safe box for collecting waste including sharp objects
- List of recently tagged eels (if there are any) that should be released directly from the boat

2.5. Equipent used in lab

- Benzocaine (dosage 2,4 grams per 20 liter of water) (kept in the ice cream box with the red lid in the chemical locker in the aquarium)
- Protective gear (long plastic glows, protective googles etc. can be found on the shelfs in Grovlab)
- Plastic barrel to anaesthetizes eels in
- Pit-tag reader "HPR light BIOMARK reader"
- Plastic bags of different sizes to be used when freezing the eels. (make sure to mark the samples with lake, local, species, cod end number, number of eels and date)

2.6. Equipment used after the field season

- Pressure washer
- Thread and needle to mend the fykenets
- Plastic barrels (*5, one for each set of fykenets)
- Soft soap

Note that the handling of the fykenets require heavy lifts, try to avoid back injuries.

3. Preparations

Before you are to go out and put fykenets into the water they have to be prepared. Begin with examine 5*8 pair of fykenets (a total of 40 paired fykenets with 80 cod ends). Check so that they are fairly whole and clean. It is especially important that the mesh of the last cod end in the fykenet is completely without holes. The fykenet cod ends are numbered with plastic badges. The first cod end (number 1) is the one the furthest out in the lake. That cod end is labelled with the plastic badge with the letter corresponding to the local (see the map figure 11). All the uneven cod ends are then labelled with the numbers 3-15. The temperature recorders are fastened inside cod end C7 and D7.

Before the fykenets are tied together the cod ends of the fykenets are tied to prevent them from opening in the water. The fykenets are connected to each other by a loop of string that is tied around the knots of the fykenet cod ends (figure 2-9). Pull hard in the lines to check that the loops are connected securely but do not tie the knot of the cod end to tight since that complicates the opening of them in the boat. A mushroom anchor is attached to each end of the fykenet sets using a 1 meter tow line. A buoy with a tow line of about 10 meters is then attached at the top ring of the anchor connected to the first cod end of the set. The tow line between the anchors and the fykenets is used to stretch the sets of fykenets and must be attached to the bottom part of the mushroom anchor, i.e. not in the top ring. As the fykenets is considered a weighed down fishing gear and not fixed fishing gear, the fykenet sets must be marked with a bouye at the outer end of the set. The bouye should be clearly marked with "SLU AQUA", "Test fishing" and an up to date mobile phone number to the data collection field telephone. When all 8 pairs of fyknets are adequately connected they form a set of fykenets with 16 cod ends numbered from 1-16. Each set of fykenets are placed in a plastic barrel. Begin with the bouye and the mushroom anchor in the bottom of the barrel and then place the connected fykenets on top. Make sure the nets do not get twisted when put in the barrel. Mark the barrel with the letter of the set so that the right fykenet set is put in the right local.

Repeat above for all sets of fykenets (five sets in total).



Figure 1. Each fykenet has two cod ends. Several fykenets attached to each other makes a set of fykenets.



Figure 2-9. How to attach the fykenets together with the loop string.

4. Boat "Silverfisken"

The boat "Silverfisken" is a Linder 440 Fishing aluminium rowing boat. It is 4.3 m long and weighs 94 kg. Aluminium oars and a key for the padlock are in the outer storage in the pump housing. When fishing with fykenets outside the institute of freshwater research at Drottningholm a special wooden tray is used which is mounted on Silverfisken. The wooden tray is located in the pump housing and is specially adapted to Silverfisken and thus do not fit other boats. No motor is used during the eel fishing at Sotholmen.

Never leave shore without a life jacket! There must always be at least two people on board the boat.

5. Putting out and taking up the fishing gear and handling of the catch

5.1. Protocols

The following protocols must always be brought in the boat:

- Catch journal2 (Appendix 1)
- Protocol_marked_ crayfish (Appendix 2)
- Protocol release of temperature recorders (only necessary to bring when initiating and finishing the test fishing) (Appendix 3)

Waterproof protocols and a writing board with a floater is located in the plastic box in the outer storage at pump housing. New protocols can be printed from <u>\\storage-dh.slu.se\restricted\$\Rederier\Rederi} Provfiske\Instruktioner vid</u> <u>fältarbete\Protokoll_fält\Å1</u>..

All catches except perch, roach, ruffe, bream and white bream are recorded on the protocol. In case of abnormal amounts of these species that could affect the fykenets catchability, this is also noted. Keep the eels separated so that they can be traced to the correct cod end of the fykenet. To keep them separated, put all eels from one cod end in one net bag and mark the tag on bag with corresponding fykenet number and date.

5.2. Putting the fykenets in the water

The testfishing usually begin when the water reaches a surface temperature of 10 degrees. This usually happens in early or mid-May, and the testfishing is then continued until about mid-July when the catches usually begin to level out or decrease. Normally the test fishing lasts for 9-10 weeks, with two efforts a week, the fykenets will be emptied between 18-20 times in total.

Take a maximum of two barrels into the boat at the same time. There should always be two people in the boat because of security. And it is also required for the work; one person rowing the boat and one person in charge of the fykenets.

The fykenet sets are laid at five different locations in the Sotholmen bay shown in Figure 11. Place the fykenet sets starting very close to the shoreline. Use the GPS coordinates in Table 1. Also take a new GPS point both at the outer and at the inner end of each fykenet set, as well as at cod end C7 and D7 where the temperature recorders are. Make a note of the waypoint number together with the GPS number on the capture protocol. Lay the mushroom anchor first, and then row the boat in a straight line while the fykenets are fed out of the barrel. Remember to register the depth with a sinker at each fykenet sets inner and outer end as well as at cod end C7 and D7. Note the depths on the catch protocol. For fykenet set E and A you aim for the "yellow sea marking" (see map below). For fykenet set B and C you aim for the yellow house on the opposite shore (behind Sotholmen) and for fykenet set D you aim for the grove of small trees at the shore side of the Freshwater Laboratory (here it is usually only possible to lay out the fykenets in one way because the abundant reed vegetation otherwise prevents the fykenets from sinking to the bottom). Make sure that the fykenets are not twisted. The floaters on the fykenets guide arms should be placed up in the water column. Also make sure that the knots do not get lose. The fykenets should always be kept quite stretched, from time to time you can hold on and stretch the nets a little extra. Do not forget to take another GPS waypoint on the outer part of the fykenet set as well, and write down the waypoint number on the protocol. Release the last mushroom anchor, and before releasing the buoy, stretch the fykenet set one last time (Fig. 4). Release the buoy. Sometimes abundant aquatic vegetation and reeds can make it difficult to get the fykenets to sink properly, in the worst case you may be forced to go in and correct them with an oar after the fykenet set have been put out. Repeat the same procedure for all five fykenet sets.

Two larger buoys should be anchored at each end of the testfishing area. The buoys should be clearly marked with "SLU Aqua - Test fishing in progress" also mark the buoys with the telephone number to the data collections field telephone.

Starting point for the fykenet sets	Sweref99 N	Sweref99 E
Α	6580821	663598
В	6580733	663629
С	6580712	663679
D	6580630	663720
Е	6580902	663553

Tabell. 1. GPS coordinates for the starting point of each fykenet set.

5.3. Emptying the fykenets

The whole procedure of emptying of the fykenets (perperations+on the lake+work after) usually takes about 4 hours. The fykenets are usually emptied twice a week, preferably Mondays and Fridays, with exceptions for holidays and so on. However, there should never be more than 4 days between each time of emptying.



Figure 10. Emptying Fykenets on the wooden fykenet tray.



Pack the boat according to the equipment list in section 2. Attach the wooden tray to the stern of the boat as seen in Figure 10. There is a hook on the underside of the tray to fasten it to the boat.

The five sets of fykenets A-E are placed according to Figure 11. Row out to the buoy of the first set and start at the outer end of the line. Pick up the buoy and the mushroom anchor. (If the buoy has come loose from the line or if the line has come off, you need to dredge after the fykenets.) Pull up all the line into the boat and feed it down on the flooring as you go. Take care not to tangle your feet when moving. Take the entire first pair of fykenets on the wooden tray table before opening the first cod end, then the fykenet will not be pulled back into the water while you empty it. Continue with the next fykenet and so on. If possible, avoid lifting the innermost mushroom anchor since it keeps the fykenet set at the same starting position throughout the entire testfishing. The cod ends are emptied one by one by opening the knot at the far end of the cod end. If there are eels inside the cod end, the eel is poured into a net bag which is then placed in the lidded barrel you have brought with you. NOTE! soak the net bag first before putting in the eel. And do not poor water in the barrel since that makes it worse for the eel.

After a fykenets cod end is emptied, it is tied together again and fastened together with the cod end on the fykenet next to it, with the help of the string loop (see chapter 3 pictures 1-9). Continue to empty the cod ends until the whole set of fykenets have been inspected, be careful how you place fykenets on the wooden tray table so that they are not twisted and can be fed out straight when the fykenet set is to be laid again. If occasional mesh breaks are detected in the outermost cod end of the fykenet, these holes must be repaired immediately. Larger holes must be laced together until you can replace the fykenet. If you feel unsure of how to mend the fykenets properly, you can instead use plastic stripes that can be found in the eel fishing box. Note the catch for each cod end in the protocol and also note with pencil the number of the cod end and the date on the waterproof label that hangs on the net bag so that each eel can be traced to the correct cod end afterwards. Example; eels caught in line B fykenet 11 on the 1st of july you write "B 11 1/7". It is appropriate that the one manning the oars also handles the protocol. Each caught eel is scanned for pit tags already in the boat (use HPR light BIOMARK reader), so that eels that should be released again (see eventual separate list of marked eels in the eel fishing box) are handled as little as possible.

By-catch is noted in the protocol under "remarks" for each cod end of the fykenets. Note the species and the quantity of all but the most common species (perch, roach, ruff, bream and white bream). These are excluded, as it would involve a lot of extra work without providing a measure of prevalence. Note, however, in the remarks column on the protocol, whether the by-catch may have affected the eel catch (for example, large pike that blocked the entrance to the fykenet or large quantities of common species). Caught fish is then emptied overboard. If the cod end of the fykenet contains only single fish of species that are not noted, these cod end do not need to be emptied at each inspection. In the case of a lot of fish, on the other hand (which is often the case in connection to roach and perch spawning), the guide arms also need to be shaken or picked clean. In the long run it is best if you empty as much as possible, as often as possible, preferably each time you see to the fykenets. If a catch is recorded in the protocol, it is important that it is also taken out of the cod end, otherwise there is a risk that the catch is recorded twice. If crayfish are caught, these are also taken out of fykenet. Measure the crayfish and note on the crayfish protocol; length, moulting phase, tag number (if they are already tagged) and any injuries or disease symptoms. Crayfish that are over 7 cm should be tagged (see instructions in section 6 below). Then release the crayfish back into the lake in the same immediate area as they were caught. This is important because the tagging makes it possible to study the crayfish's movement patterns based on catch and recapture sites. When you have gone through the whole set of fykenets it is time to lay it out in the water again. The fykenets should be placed in the same way they were at the beginning see figure 11. Make sure that the inner mushroom anchor is still placed at the same starting point it had before and then row the boat out in a straight line while the fykenets are fed out from the wooden tray table. For fykenet set E and A aim at the "yellow sea marking". For fykenet set B and C aim towards the yellow house on the opposite shore (behind Sotholmen) and for fykenet set D aim at the grove of small trees at the shoreline on the same side as the Freshwater Laboratory is situated (in this place you can usually only lay out the fykenets in one way because the abundant reed vegetation otherwise prevents the fykenets from sinking to the bottom).

The floaters on the fykenets guide arms should come upwards in the water column. Make sure that the knots between the cod ends do not go up. The fykenets should always be kept stretched, from time to time you can hold on and stretch a little extra when putting them out. Release the mushroom anchor, and before releasing the buoy, stretch the fykenet set one last time (Figure 12).

The surface temperature is measured with the digital thermometer and noted in the protocol, preferably the measurement is made in the middle of the strait between fykenet set B and C.



Figure 12. Laying and stretching of fykenets

5.4. Completion of the protocol in the boat

Fill in the protocol with the names of the two test fishermens, date and water temperature. If eels are present in the cod end, put a line in the "eel column", one line for each eel in case of several eels in the same cod end. Be sure to note the correct cod end number and fykenet set. Also note here whether the eel was marked, and what PIT tag number it had. The remark column notes the presence of deviating fish species or whether the fykenet cod end contained exceptional amounts of fish that could affect the catch. Also note if there were any damage or holes in the fykenet, or if there are any other things worth to note. Don't forget that all crayfish are measured and all of over 7 cm are tagged (see Treatment of crayfish during eel sample fishing). Also note the date and cod end number on the label that hangs on the net bag in which the eel is placed so that the individual eel can be traced back to the correct cod end of the fykenets.

5.5. To take care of the catch at the end of the day

Take in the barrel with eel into "Grovlab". Pour 20 liters of water into the eel barrel and then add a ready-mixed double dose of Benzocaine (2.4 g mixed in 95% alcohol) which is in the ice cream box with a red lid in the chemical storage in the aquarium house. Mix around so that the solution is distributed throughout the barrel with the eels and put on the lid. NOTE! Be sure to wear long plastic gloves and goggles! It can take a few minutes for the eels to become sedated. A tip is to gently squeeze the eel's waist, if no muscle movements can be felt the eel is sufficiently sedated. All tagged eels that are to be frozen must first (after sedation) be photographed (together with a note showing the number of the tag), measured and weighed. For the eels that are tagged with data collecting tags (DST) that is not to be released again, the eel must be anesthetized, put to death and the mark must be operated out and saved before the eel can be frozen. Eels with standard PIT tag markings are frozen in the same way as the untagged eels. If some eels are chosen to be saved for later tagging and release, these eels are placed in a net corf in one of the water tanks in the aquarium house. In order to keep the eels apart and to know which eel is which, the catch protocol at the water tank must always be filled out. Also mark each net corf with date and cod end number. The other sedated eels are individually packaged in small plastic bags together with a note made with pencil stating the cod end number and date (use the one form the net bag). Then put all the collected eels in a plastic bag that is well marked with information about the location, date, number of eels and fishermen's initials. The bag is then stored in the box dedicated to the eels from Sotholmen in the freezer in Grovlab. Also fill out the list posted on the freezer door, be sure to fill in the correct information and which freezer shelf the samples are stored. After the days field work is done make a copy of the protocols for eels and crayfish catches (check the readability), the copies are then inserted in a folder marked "Eel testfishing".

5.6. Data entry into Sötebasen

The data is entered into a database called Sötebasen. Enter as much information as you can in each step so that all information collected is entered into Sötebasen. The single fishing occasion is considered a collections (insamling) and each cod end is considered an effort. This means that for every fishing occasion you end up with a total of 80 efforts. Go to <u>\\storage-dh.slu.se\restricted\$\Sötebasen</u> and save a copy of Sötebasen to your own computer. Open Sötebasen and click the button "sök/redigera/registrera data". If you already have made a data collection you enter the insamlingsID and then click the button "gå vidare". If you do not have a InsamlingsID you just click "gå vidare". Then you enter water "vatten", starting year ("start år"), ending year ("slutår"), method ("metod"), responsible organization ("ansvarig (org)), performing organization ("utförare (org)), Purpose of the collection ("syfte") and then you sign the collection with your own name ("inmatat av"). Any other notes can be filled in under remarks ("anmärkningar"). Then you go to effort ("ansträngningar"). Here you enter all the cod ends of the fykenets starting with A1 then A2 and so on all the way to E16 and register all the recorded catches during the collection. Catches are noted by clicking the button "registrera summerade fångster".

5.7. Completion of the testfishing

At the last day of the test fishing, take one or two blue barrels out into the boat. The fykenets are picked up and gone through as usual, but instead of putting the fykenets out again, all the cod ends are tied up, taken apart and rinsed so that algae, dead fish

and such come out, as good at it is possible in the boat. Remove the temperature loggers. The fykenets are then placed in the blue barrels. Put one set of fykenets in each barrel but take at most two sets of fykenets at a time as there will be a lack of space in the boat otherwise. Once back on land, use the wheeled chart to pull the barrels to the car washing drain by the small red tool shed. Try to avoid back injuries since handling the fykenet barrels involves heavy lifting. Fill the barrels with water so that it covers all of the fykenets. Also mix in soft soap or detergent. Then let the fykenets soak for a few days or weeks.

5.8. Temperature recorders

The temperature logger is removed and read if possible with the mobile app HOBOmobile. If the temperature logger is of an older model or requires other equipment to be read, the temperature logger is handed in to the responsible person within the data collection. The temperature files are saved as Excel files, one original file and one file that is edited so that it fits the temperature database frame. Remember to edit away any irrelevant measurements not made in the field. Save files the in а suitably named folder under \\storagedh.slu.se\restricted\$\Temperaturdata\Mätningar i sjöar\Rådata. Temperature data will then be entered and saved in the temperature database.

5.9. Cleaning of the fishing gear (after the fishing season)

When the field season is over, ropes, buoys and mushroom anchors are rinsed off. The ropes are allowed to dry and then wound up on the shafts of the mushroom anchors. You do not need to untie the ropes from buoys and anchors. Collect everything in a plastic box and store them behind the red fence at the recycling until next field season.

After the end of the test fishing, empty and rinse the fykenets and place them in barrels with soap and water for a few days. Then hang the fykenets and use the pressure washer to clean the fykenets. Keep the nozzle at some distance of approx. 30 cm from the fykenets, otherwise there is a risk that the mesh will break. Check each fykenet for holes or other damages and repair them if possible. Any holes in the fykenets outer cod ends are fixed with repair yarn and a needle. Let the fykenets dry and then pack them in the barrels again and set in the designated place until the next eel fishing season.

6. Treatment of crayfish caught during the eel fishing

All crayfish caught is measured and examined. Unmarked signal crayfish that is over 70 mm long must be tagged with a yellow streamer tag. Start by sexing and measuring the length of the crayfish and inspect the crayfish for injuries, determine the molting phase the crayfish is in and look for signs of plague or other remarks.

6.1. Measuring the length

The length of the crayfish is measured from the tip of the nose (Rostrum) to the middle tail flap (telson). Place the crayfish on its back with the head end against the edge of the measuring board and stretch out the tail. Measure the length to the end of the middle part of the tail, not including the small hairs on the tip of the tail, see Figure 13. The easiest way is to hold the crayfish's claws together with one hand and use the other hand to press down the tail against the measuring board.



Figure 13. Length measurement of a signal crayfish

6.2. Determine the molting phase of the crayfish

The molting phase of the crayfish is determined by gently pinching the crayfish shell between your thumb and your index finger. If the shell feels hard and do not budge, the molting phase is determined as Hard "Hård". If the shell is soft or do give in when pressure is applied it is either on its way to molt "på väg att omsa" or it has recently molted "Nyömsad". This is determined by looking at the shell of the crayfish to see if it looks new fresh and clean and is a bit slippery then it is recently molted, Or if it is an older shell it gives a darker and more dirtier impression then it is on its way to molt "På väg att ömsa".

6.3. Determine the gender of crayfish

The female signal crayfish has a wider tail than the male. The male has larger claws and the first pair of swimmerets is instead longer, forward-facing mating organs, which the female lacks (figure 15).



Figure 15. Gender assessment of crayfish is a relatively simple and fast process. The crayfish in the picture on the right is a male, it has the first pair of swimmerets pointing forward. The left picture shows a female, they lacks this morph. Females have a wider tail and usually smaller claws than the male.

6.4. Tagged crayfish

When catching a crayfish tagged with a yellow streamer tag (Figure 16) note the crayfish's tag number and tick the box that indicates that the crayfish has been recaptured in the crayfish protocol. Then the tagged crayfish is treated as a regular crayfish, i.e. it is measured, sex and moulting phase is determined and any injuries or symptoms of disease are noted in the protocol.



Figure 16. Crayfish tagged with a streamertag.

6.5. Tagging of crayfish

Take out a streamertag from the envelope and note the tag number in the crayfish protocol. The tag should be placed in a small window in the space between the crayfish's dorsal shield and the tail (Figure 17). Carefully hold the tail out to the side so that the soft parts of the crayfish are exposed and insert the tags needle so that it comes out directly on the opposite side of the crayfish's hind body (Figure 18). Use a plier to pull the needle through so that the indentation in the brand's yellow plastic strip ends up where it should be in the middle of the crayfish (Figure. 19). Then cut off the needle a bit into the yellow plastic strip. The crayfish is now tagged (Figure 20) and ready to be released in the same area as it was caught.





Figure 17-20. Tagging a crayfish with a streamertag.

7. Safety and first aid as well as deviations and incidents - abbreviated version for field work during the day and at Sölab in Drottningholm

The employer, SLU, has the main responsibility and must ensure that risks are prevented and that a satisfactory working environment is achieved. The safety instructions that are available must be followed. Never work alone in a boat! Always wear a life jacket with a crotch strap! We use inflatable life jackets with a buoyancy of 216 N and automatic release. Use your good judgement when driving boat and car. If an accident occurs or if someone becomes ill, the employer must be contacted and, if necessary, consult a doctor. If the field workers decides that it is dangerous to go out on the lake, for example due to bad weather or substandard boat / equipment, they have the right to cancel the test fishing. Incident reporting is mandatory, which means that all accidents but also near accidents (i.e. when an accident almost happens) must be reported. SLU Safety needs knowledge of incidents and actual events connected to the safety aspects of SLU's various operations. Therefore, incident reporting mandatory, since it is also important for us to be able to plan and design future field work so that accidents are minimized. See information https://internt.slu.se/stod-service/adminmore at stod/sakerhet/kris-och-incidenthantering/rapportering/rapporteringsformular/.

For Aqua, it specifically applies that a deviation or disturbance must always be reported to the project manager and to the field manager, when the work has deviated from instructions and routines, when set goals have not been reached, when an accident has occurred or when another serious event has occurred that affected the assignment. A disturbance can be, for example, theft or loss of equipment, bad weather or illness. A deviation can be an effect of a disturbance which means that the work cannot be carried out according to plan, such as that the correct number of samples could not be collected or that a fieldwork could not be performed within the given time frame. The purpose of the reporting is to enable improvements in the planning of the field work. Reporting is done through the Form for disturbance and deviation:

https://arbetsplats.slu.se/sites/MiljoCert/Akvatiska%20resurser/SitePages/Miljoar enden_formular.aspx.

We recommend all field workers to be vaccinated against tick-borne virus, TBE, which is paid for by the employer.

Here are instructions on how to use the template. After reading the instructions, you can delete all text in the template and start to write. If you need the instructions again you can always open a new template document or you can go directly to the template instructions on Canvas: <u>https://slu-se.instructure.com/courses/1097</u>. We recommend all field workers to be vaccinated against tick-borne virus, TBE, which is paid for by the employer.

7.1. First aid

Also se information posted at the entrances to the laboratory

Revive with the rhythm 30: 2.

Press 30 times. Place the palm of one hand in the middle of the sternum, place the other hand on top so that the fingers overlap. Press down with straight arms so that the chest sinks down 5-6 cm. The frequency should average 100 compressions per minute, no more. Let the chest rise back. Count high.

Blow 2 times.

Open the airways. Place your mouth tightly over the patient's mouth. Squeeze the nose with your thumb and forefinger. Blow air into the lungs of the person in need. While blowing, check that the patient's chest is rising (moving). Repeat the exhalation. Two blows should take 5 sec.

Continue to revive continuously with a rhythm of 30: 2

8. Environment and environmental certification

The Department of Aquatic Resources and the entire SLU are environmentally certified, with the aim of reducing environmental impact and increasing knowledge and environmental commitment. This means that we try to work in as environmentally conscious a way as possible. When we work in the field and in the laboratory, we therefore have environmental routines for field work and other parts that must be taken into account. We would like you to follow these routines as much as possible to minimize the department's negative impact on the environment. Test fishermen should be familiar with SLU's environmental policy and goals and

the actual or potential environmental impact associated with the field work. You should also contribute to a well-functioning environmental management system by following the routines and strive to improve environmental performance, report deviations and submit improvement proposals (see links below).

https://internt.slu.se/stod-service/admin-stod/miljo/

https://internt.slu.se/riktat/lasta-interna-sidor/institutionen-for-akvatiska-resurser/institutionens-miljocertifiering-enligt-iso-14001/.

9. Dissection and sampling manual and data entry in Sötebasen



Figure 21. Eel on measuring board. photo John Persson.

9.1. Preparations (To do before dissection)

- 1. Take out eels from the freezer no later than the afternoon of the day before sampling. The size and number of eels in the package are decisive for how fast the eels thaws. Large bags can take a long time to thaw, longer than one night. In the winter, you may need to bring out sacks two days before dissecting, but it depends on how many eels there are and how you lay them out to taw. If you have a big pack of eels you can put them in the fridge over a weekend and they will not be totally thawed on Monday anyway. It is also important to note the removal of frozen samples from the freezer in the list posted on the freezer door.
- 2. An estimate of how many eels you can dissect in a day is about 20 eels / day, depending on how much routine you have and how many types of measurements and samples should be taken on each eel. If all samples are to be taken, one should initially limit oneself to about 15 eels per day.

- 3. Bring out a cutting board, knife, tweezers, digital calliper, otolith bowl, one pair of crude scissors and a smaller pair of scissors, scalpel, Wettex dishcloth, measuring board and a small plastic bucket or tub for the scale.
- 4. Go to the excel file "dissektionsprotokoll total.exe" in the folder <u>\\storage-dh.slu.se\restricted\$\Ålen\Dissektioner\Aktuell total</u> and "book" new individual numbers. Be sure to keep the file up to date and if you do not use all of your booked numbers you should unbook them as soon as you are finished with your eels. Print individual numbers and cut them into small separate labels. These are placed on the individual eels, which reduces the risk of mixing them together. It is also used to identify the eel when photographing.

The following two points only apply if DNA, fat and / or poison samples are to be taken (se section 9.5)

- 5. If DNA samples are to be taken. Fill eppendorf tubes with 95% ethanol and label them with sample numbers NOTE! Use a pen that can withstand alcohol. Print out the special protocol for genetic testing.
- If tissue samples ought to be taken for analysis of toxins or fat content. Write individual numbers on the plastic bags, four pieces per eel. (NOTE! Only two bags / eels unless samples are to be taken to the Swedish Food Agency (SLV)).
- 7. Tear large enough pieces of aluminium foil, one piece per eel. (Only if samples are to be taken to SLV).

9.2. Dissection

Start by going to \\storage-dh.slu.se\restricted\$\Sötebasen and download the latest version of the access database "Sötebasen". Save it to your own computer. Start up Sötebasen, it is the database where all information from the dissection is filled in and stored. Make sure that the computer is always in contact with the network when you work in Sötebasen otherwise problems can occur since Sötebasen constantly saves every change you make to the server. At the main page of Sötebasen, click on "Search / edit / register data". If background data regarding the collection has already been entered, fill in the collection ID that was received when entering the data into the search field and then click on "proceed" ("gå vidare"). If Collection Data has not been entered before, then just click on "proceed" directly. Fill in information regarding the collection of the eels. Water body and starting year are mandatory fields, but try to fill in as much information as possible about the collection. Sometimes it can be good to go back to previous collections and see how these have been filled in. Then click on "efforts". Here you fill in, among other things, "effort number", "effort location", "type of effort" and "end date". You shall of course try to fill in as many fields as you can. Now you click on "to individuals". Here is where you fill in data from the dissection of your eels. Start by choosing the customized entry form "Eel dissection". Fill in the correct "Effort number", "Individual number", which is the same as the eel's individual number and also fill in "Catch date" for the eel. Fill in today's date under "Dissection date", fill in Eel under the category "Species". Fill in "Yes" under the category "age test". Last but not least, fill in the state of the eel you are dissecting under the category "QualityMeasure1", for example if the eel is "Fresh" or "Frozen & thawed". Now you are finally ready to start with the eels. Remember to also fill in any measurements made on the eel while still living. This can be done under the category "Quality Measure2".

- Take an eel out of the bag. Weigh (grams) it without removing mucus, remove only any plant material and such not belonging. Then wash the eel under running water to get of most of the mucus. (The mucus sticks harder on frozen or drier eels compared to "perfect" thawed eels. Mucus that sticks hard is easier to remove if the eel is soaked in luke warm water for a few minutes.) Measure the length of the eel from nose tip to the tail tip (mm). The eel is then assigned an individual number from pre-printed labels that are attached to the eel's skin.
- 2. Note the developmental stage on the eel. The stage of the eel is determined to be either "Yellow eel", "Semi Silver eel" or "Silver eel" based on the overall impression that the eel gives in terms of eye and fin size, colour and skin texture and firmness in the body. At a later point, the gonadal size can also be weighed into the assessment (Table 1). For the "beginner", the one

who has not dissected eels before, you should initially let the project manager or another experienced person look at the eels to assess the developmental stage, sexing and look for any oddities.

Yellow eel	Semi silver eel	Silver eel
The eel has small	The eel is in a	The eel has large
eyes and fins in	development phase	eyes and fins in
relation to its body	somewhere	relation to its body
size.	between yellow eel	size.
The eel feels	and silver eel and	The eels flesh feels
slightly looser in	does not meet one	firm.
the flesh than	or more criteria for	The eel has a silvery
those in more	being classified as	or rust coloured tone,
mature stages.	either yellow eel or	often with lighter
The eel often has a	white eel.	streaks on the
yellow-green		abdomen
colour.		The eel has well-
The eel has few or		developed
no neuromasts		neuromasts
The eel's gonads		The eel's gonads are
are small and not		large and well
very well		developed
developed		

Table 1. Criteria for classification of the eel's developmental phase.

3. Look along the lateral line of the eel and note if the eel has neuromasts (a type of sensory organ that detects water flows). They are often seen as pigmented, slightly raised dots along the lateral line and can sort of look like pimples (Figures 22 and 23). If the eel has one or more Neuromasts, fill in "Yes" otherwise you fill in "No" under the category "Neuromasts".



Figure 22. Eel without neuromasts. Photo Niklas Sjöberg.



Figure 23. Eel with neuromasts. Photo Niklas Sjöberg.

- 4. Note and photograph any damage, disease symptoms or other abnormalities that the eel has. Injuries are noted under the category "skada" (which means injury).
- 5. Photograph the eel. Take a picture of the eel's head where the left eye and pectoral fin are visible together with the eel's individual number. Then photograph the eel so that its full size is visible together with the measuring board and the individual number. Put the pictures in the folder <u>\\storage-dh.slu.se\restricted\$\Sötebasen\Bildarkiv</u>. Then the pictures has to be linked to the right individual in Sötebasen. This is done by pressing the button "registrera bild kopplad till individ" and then you fill in the full picture name and the individuals ID number. The handling of pictures are usually done after all the dissections are done and not during the dissection process.
- 6. Then place the eels next to each other in a plastic tray together with the individual number. It is easiest if you perform steps 1-6 on all individuals before going to the next step.
- 7. Start by measuring the eel's eyes with the calliper (millimetres with two decimals). Two measurements are taken on each eye, one horizontal and one vertical. Do not measure the eye itself, but the "eye socket", i.e. the "window in the skin" (see Figures 24 and 25). Left eye is noted horizontally under "VäH" Left eye is noted vertically under "VäV" and so on.
- 8. Then measure both pectoral fins. Measure from the base of the pectoral fin to the tip of the fin (Figure 26). Note the value for the left pectoral fin under "VäBr" and the right pectoral fin under the category "HöBr".



Figure 24 and 25. Measurement of the eels eyes.



Figure 26 Measurement of pectoral fin, from fin base to outer tip of the fin. Photo Caroline Durif.

9. Then cut open the eel abdomen. Start at the anal opening and cut forward as far as possible. Lock at the gonads and note the gender. In figure 27 you can see the male gonads in the top of the image and in the bottom you see the female gonads. The female's gonads are clearly cohesive and broad, like "draperies". If the eel is over 55 cm and if the weight is over 3 hectograms, you can be fairly sure that it is a female. A male has significantly smaller gonads than the female, where the male's gonads consist of lobes that can be folded up or down without the next lobe being stirred. If in doubt, ask the project manager or another experienced person. Fill in gender under the category "Gender". Females are referred to as "f" and males are referred to as "m".



Figure 27. Determine the gender of eels. Male gonads at the top and female gonads at the bottom of the picture.

10. Use the small scissor to open the swim bladder and check for Anguillicola (swim bladder parasite, black worm, see figure 28). If it contains the parasite, count them all and note how many there are in the "NAnguillicola" category.



Figure 28. Anguillicola in the eel swim bladder.

- 11. If the eel's stomach does not seem completely empty, you can also cut it up, and if it is easy to see what the eel has eaten, for example fish, or something else, this is noted under the category "Stomach".
- 12. Use the scalpel to split the skull in the middle with a straight cut starting at the caudal part of the scull and cut down and continue in rostral direction between the eyes, and through the jaw. Keep in mind that the skull is hard, and that you should preferably go through the head with a single incision. If you make several incisions, the risk increases significantly that you also split or lose the otoliths. If you lay the incision too far to one side, there is also a great risk of crushing the otolith on that side. Using the tweezer, pick out the otoliths on both sides of the incision, they are located in the auditory organ, just behind the brain (see Figures 29-31). The otolith that is to be taken out and saved for age determination is sagitta, which in the eel is the largest of the otoliths. Wipe off blood and tissue debris by gently rubbing the otolith against a damp dishcloth, between your fingers or on the top of your hand, then place them in a bowl of water. Make sure they are clean. Finally, place the otoliths in an otolith bag well marked with the eel's individual number. If you find two otoliths, fill in 2 under the category "NOtolites". If you only find one, type 1, if an otolith breaks, type 1.5. Also fill in "Yes" under the category "Age sample" and "Otoliths" under the category "Tot tissue". Also, do not forget to finally sign the individual dissection with your name under the category "Signindivid".



Figures 29 and 30. Section through the eel skull, and the place where the otoliths lie.



Figure 31. Figure showing how the otoliths are placed in the eel skull

- 13. After all the eels in the particular collection have been dissected, you can use the special labelprinter stored in Grovlab to print labels for the sample bags. Press the "Print labels for eel" button. There you write from and to which individual numbers you want to print. Then paste the labels on the sample bags with the corresponding individual numbers you wrote on the bags during dissection.
- 14. Throw the eel and take the next one.

9.3. Data entry

Dissection data is put into and stored in the access database "Sötebasen".

Photos from the dissection are entered in the folder <u>\\storage-</u> <u>dh.slu.se\restricted</u><u>\Sötebasen\Bildarkiv</u>.

9.3.1. Explanations to the form for eel individuals "Ål-dissektion"

IndividID (Individual ID). Digits. The unique number that Sötebasen assigned to the eel individual

AnsträngningsID (Effort ID). Digits. The unique number that Sötebasen assigns to the single catch effort.

InsamlingsID (Collection ID). Digits. The unique number that Sötebasen assigns to the Collection.

AnsträngningNr (Effort No). Digits. The number you choose to give a specific effort

DissektDatum (Dissection Date). Date (yyyy-mm-dd). The date when the eel is dissected

Fångstdatum (Catch date). The date on which the eel was caught

FångstDatumOS (Catch Date Uncertainty). Drop-down menu. If any uncertainty prevails around the catch date, fill it in here

Art (Species). Drop-down menu. Here you fill in which species is dissected. In this case, select "Eel".

IndividNr (Individual No). Digits. Here you fill in the eel's individual number. NOTE! Check the number against the dissection file total_2017-01-03.xls on restricted \\storage-dh.slu.se\restricted\$\Ålen\Dissektioner\Aktuell total

Behandling (Treatment). Drop-down menu. When dissecting you fill in "Landad / avlivad / död" (landed/euthanized/dead) unless the eel has been tagged and recaptured then there is also the option "Återfångad&avlivad" (recaptured/euthanized).

KvalitetMätn1 (Quality Measurement 1). Drop-down menu. Here you fill in the condition of the eel at the dissection, for example "fryst och tinad" (Frozen and thawed) or "Färsk" (Fresh).

Vikt1 (Weight1). Digits. Fill in the eel's weight in grams to one decimal place.

Längd1 (Length1). Digits. Here you fill in the length of the eel in millimetres.

Neuromaster (Neuromasts). Drop-down menu. Here you fill in "Yes" if the eel has one or more neuromasts or "No" if the eel lacks neuromasts.

Stadium (Stadium). Drop-down menu. Here you classify the eel either as "Yellow eel", "Semi-silver eel" or "Silver eel".

Skada (Injury). Free text. Here you describe any injury or damage to the eel.

VäH (Left Eye horizontally). Digits. Here you enter the measurement of the eel's left eye horizontally in millimetres with two decimals.

VäV (Left eye vertically). Digits. Here you enter the measurement of the eel's left eye vertically in millimetres with two decimal places.

VäBr (Left pectoral fin). Digits. Here you enter the measurement of the eel's left pectoral fin in millimetres to two decimal places.

HöBr (Right pectoral fin). Digits. Here you enter the measurement of the eel's right pectoral fin in millimetres to two decimal places.

HöH (Right eye horizontally). Digits. Here you enter the measurement of the eel's right eye horizontally in millimetres with two decimal places.

HöV (Right eye vertically). Digits. Here you enter the measurement of the eel's right eye vertically in millimetres with two decimals.

Kön (Gender). Drop-down menu. Here you enter the gender of the eel. "F" for female and "m" for male.

NAnguillicola (No Anguillicola). Digits. Here you enter the number of Anguillicola worms that you find in the eel's swim bladder.

Mage (Stomach content). Drop-down menu. Here you fill in if the eel's stomach is "Tom" (empty) or contains "Fisk" (Fish), "Kräfta" (Crayfish) or "Annat" (Other).

NOtoliter (No Otoliths). Drop-down menu. Here you enter the number of otoliths that you manage to dissect out of the eel. If an otolith breaks, it is stated as a half otolith, for example if you have a whole otolith and a broken otolith, enter the number "1.5".

Åldersprov (Age Sample). Drop-down menu. Here you enter "Yes" if you have taken a sample for age determination of the eel. Otherwise, enter "No".

TotVävnad (Total Tissue). Drop down menu. Here you fill in which tissue was taken for age analysis. Usually "Otoliths" are taken from Eels

SignIndivid (Sign Individual). Drop-down menu. Here you fill in your name and thus sign the dissection and entries.

MärkeNr (Tag No). Digits. If the eel has been tagged, the number of the mark is stated here.

Märkning1 (Tag Type1). Drop-down menu. If the eel was tagged, it is stated here what kind of mark the eel was marked with, for example, "pit tag" or "DST".

Märke2Nr (Tag No2). Digits. If the eel had several markings, the number of the other tag is stated here.

Märkning 2 (Tag Type2). Drop-down menu. If the eel has been marked with two different types of marks, it is stated here which type of eel's other tag was, for example, "pit tag" or "DST".

KemMärkning (Chemical Tag). Drop-down menu. Here it is stated whether the eel has been chemically tagged with, for example, "Alizarin" or "Strontium".

AnmIndivid (Remarks Individual). Free text. Here you can write other remarks concerning the individual.

9.4. Eel Fry dissection

9.4.1. Equipment list



9.4.2. Eel fry dissection

When dissecting eel fry, most of the steps that apply to adults are skipped. In the case of eels below 300 mm, no examination is made regarding sex. Otherwise, the same procedure applies with regard to the input in "Sötebasen".

For sampled locals with only a few captured individuals (up to about 10), all eels are sampled.

For other sampled locals, a selection of eels is made for sampling otoliths and Anguillicola. Project manager announces the number of eels and the size ranges that should be sampled. Eels not sampled are only measured and weighed (you do not have to count the number of Anguillicola, no otoliths are taken and the eels do not receive an individual numbers).

If there is a shortage of time, due to planning, you can first select the eels to that you are going to collect the otoliths from and deliver them for casting and future analysis. This is relatively easy as frozen eels are individually packaged. For the eels stored in ethanol, you can easily go through the eels and pick out the individuals to be sampled and leave the other eels in the ethanol.

1. The eels are thawed. Frozen eels are usually frozen one by one in separate bags, so it goes quick to thaw them. If the eels are in ethanol - leave them until you dissect them, otherwise they dry out very quickly.

- 2. The eels are measured and weighed. A scale with two decimals is required. Frozen eels often have a fresh length and sometimes even weight noted on the bags. Also register these values in "Sötebasen".
- 3. Record any damage or anomalies on the eel under the category "skador" in Sötebasen. Also try to take pictures of injuries together with the eel individual number. Enter the pictures into Sötebasen by placing them in the folder <u>\\storage-dh.slu.se\restricted\$\Sötebasen\Bildarkiv</u> and linked to the correct individual in Sötebasen by pressing the button "registrera bild kopplad till individ".
- 4. Use a narrow scalpel and split the skull. Be sure to place the incision right in the centre of the skull. Use magnifying lamp. Pick out the otoliths, put them in small eppendorf tubes (in principle always applies when the otoliths are very small). There are special racks where the pipes can stand. Leave the lids open for at least 24 hours so that liquid can evaporate. Be sure to bring two otoliths from all eels.
- 5. Cut up the abdomen. Take out (pinch with tweezers or cut) the entire swim bladder, place on a white / light surface and open it there. Look under the magnifying lamp and count the swim bladder parasite *Anguillicola crassus*.
- 6. For individuals over 300 mm, try to determine the sex. Use a loupe if necessary. Sometimes it helps to drip on a little ethanol which makes the gonads white and thus easier to see.
- 7. Discard the eel and take the next one. In some cases, the project manager may want to save the eels that are not sampled on otoliths and Anguillicola, then you refreeze the eels or place them in alcohol again.
- 8. 7. Print labels with the label printer by clicking on "skriv ut etiketter ål" in Sötebasen and paste these on sample bags. Close the eppendorf tubes with the dry otoliths and put the right tube in the right sample bag.

In some cases, project managers may want to save the eels that are not sampled for otoliths and Anguillicola, then you freeze the eels or place them in alcohol again. Time required: Sampling at the individual level is relatively time consuming as it is a tedious job. Count on a maximum of 20 pcs / day, start with 10 pcs. but try out what is suitable for you. The eels that are only to be measured and weighed are goes fast.

9.5. Special dissections

9.5.1. DNA sampling

Adults: Cut off the outermost tip of the right pectoral fin and place it in a labelled Eppendorf tube filled with 95% ethanol. The fin must be undamaged from the beginning. The fin clip should be at least 3x3 mm, and not larger than about 10x10 mm. Use scissors and tweezers for sampling, shake in water between each cut and wipe with a clean paper towel. Use the tweezers to put the piece of fin in the tube. Be careful not to get mucus, scales and foreign tissue into the tube. Then submit these samples together with a list of where the samples come from, individual number, length, weight and DNA sample number (see template and instructions for gene samples under \\storage-dh.slu.se\restricted\$\Rederier\Rederi Provfiske\Instruktioner vid fältarbete\Genprovtagning) to the person in charge at the Gen lab.

Fry: The genetic samples do not necessarily have to be from the same eels sampled for ageing. The smallest eels are sampled, i.e. part of the tail between the tip and the anal opening. At least 0.15 g should be taken, preferably 0.5-1.0 g. The minimum size of eel is 0.4 g total weight, then you have to take the whole tail up to the anal opening and it will be 0.15 - 0.17 g.

Pre-numbered test tubes with 95% ethanol solution were delivered. Ideally, only 10% of the volume in the test tube should constitute of eel, but it did not work. There was quite little alcohol in the tubes so the tail had to be adjusted in size by cutting into several parts (often two pieces). The outermost tip of the tail was not included.

This part is not so time consuming, maybe a maximum of 0.5-1 hour extra / local. The genetic sample number on the test tubes is then entered in "Sötebasen".

9.5.2. To take sample for fat and toxin analysis

Start with cutting a piece from the eel for fat weight. Cut the eel about five centimetres behind the anal opening. Then cut off a piece of about five centimetres of the tail, weigh it, note the weight and place the piece in the other marked plastic bag. See picture below. Put the samples in the Freezer in a box marked with year and "eel sausage".



Figure 32 preparation of fat and toxin tissue samples.

Then cut another piece, about 25 grams, behind, and 25 grams in front of the anal opening. See red lines in figure 32 above. Divide this piece into two halves, cut along the backbone so that it ends up in one of the two halves. Place the backbone piece in the third marked plastic bag. The boneless piece is wrapped in foil and placed in the fourth marked plastic bag. These two bags are frozen in a separate box marked with "SLV samples". Be sure to write down the eel's individual number on all the bags.