Forest Protection Survey Program

Survey Guideline - Aquatic Survey (V5.0)





Environment, Land, Water and Planning

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Aquatic Survey A: Fish and Spiny Crayfish

Context

The high priority species for aquatic fauna survey (excluding burrowing crayfish) are the 15 species of galaxiids in the Mountain Galaxias complex, and the various species of spiny crayfish (genus *Euastacus*), many of which in Gippsland are species complexes.

Many other species of aquatic fauna (fish, crayfish, freshwater mussels) may be detected during survey, and while not the target of the survey, these observations are to be reported. Signs of burrowing crayfish presence within upslope or riparian areas of a site may be noted as distinctive, conical soil chimneys at the entrance to their underground burrows, or they may be found walking along the ground. Further, burrowing crayfish soil chimneys may also be present within dry stream beds, and individuals of four species may be found outside of their burrows, underwater along the edges of waterways. These detections should also be noted.

The aim of the aquatic survey is the detection of the species at a location, which may involve a single individual, or multiple individuals spread over a longer distance, depending on the specifics of protection required for that species, and the length of waterways within or bounding a site.

Many of the species of galaxiids in the Mountain Galaxias complex and spiny crayfish will present identification issues. They are cryptic species and very similar in external morphology, poorly known as they are narrow range endemics and therefore infrequently found, have complex taxonomic keys, and are recently, or in the process of being, formally described and therefore not included in general field guides. However, it is a requirement to at least identify the taxa to the species complex, e.g. Mountain Galaxias, Orbost Spiny Crayfish, etc. It is therefore critical to retain a voucher of each for multiple benefits: 1) verify field based identification or provide an identification; 2) retain a specimen (curated in the Museum Victoria collection) to support the identification into the future when needed (e.g. court case, etc.); and, 3) contribute material for later taxonomic delineation of new species or comparative material for a species revision as the taxonomy is resolved.

Objectives

To detect high priority threatened fish and spiny crayfish using electrofishing and hand searching (where required) in waterways within and immediately adjacent to selected sites.

Survey effort

Surveyors are provided with the detection probabilities of the target species for each survey technique. Surveyors are to target their surveys to those species with the highest detection probabilities in each site. The species with higher detection probabilities aid in determining the target species most likely to be detected by the survey technique and will thus inform survey parameters such as preferred habitat for survey, bait type, camera settings, etc.

Sampling of a site will be undertaken during a single visit, with sampling conducted during daylight hours.

The total number of, and distance between, sampling sites per site will vary according to the size of the site and length of waterways within or bounding the site.

Specific survey effort requirements are detailed below in the methods section for electrofishing and hand searching.

Surveyors are required to take photographs, georeferenced where possible, of all fauna observations and submit this data with other survey results.

Surveyors are required to record a track log of the survey effort from the start to the end of surveying within each site. The track log is to be converted to a GIS shapefile and submitted with the shapefile attributes as outlined in the shapefile template provided.

Surveyor Requirements

A field survey team of two people qualified to be part of an electrofishing team (i.e. electrofishing training certification, appropriate medical clearance, wader training, defibrillator training, first aid level II certification)

Both team members to have a reasonably high level of physical fitness to walk long distances over rough terrain to reach sampling sites and to carry a large amount of sampling equipment.

Sound, practical knowledge and experience in all aspects of small fish and spiny crayfish electrofishing sampling methodology in small streams in forested catchments, particularly the practical limitations of electrofishing, and the

modifications required to the technique to improve detection probability based on species rarity and local catchment conditions (e.g. water permanency, stream size, stream morphology, elevation, degree of vegetation cover, in-channel disturbance, etc.).

Experience with, and expertise in accurate identification of freshwater fish and crayfish in small foothill to upland catchments (e.g. River Blackfish, Tupong, Pygmy Perch, Freshwater Eels, Flatheaded Gudgeon, etc.), and specifically with species of galaxiids (Galaxiidae), and spiny freshwater crayfish (Parastacidae). Due to the similarity of species in the *Galaxias olidus* complex, and spiny crayfish in the Gippsland area, that there are known but undescribed species and a high probability that additional undescribed species are present, it is **essential** that the common and non-threatened taxa in both groups can be accurately identified and separated from unknown specimens, so that vouchers of the 'unknown' taxa can be taken for confirmation in the laboratory.

Attention to detail to ensure that the above requirement of identifying and vouchering unknown and potentially new taxa is followed

Equipment for the technique

	Portable, commercially available backpack electrofishing unit, battery powered, suitable for low conductivity water (maximum >800V output and >2000W power), with adjustable frequency and able to operate at 80–120 Hz, with quick release safety harness	 georeferencing data, where possible, with each photo Calibrated water quality meter suitable to record water temperature, and water electrical conductivities 	batteries, spare storage card) capable of including georeferencing data, where possible, with each photo Calibrated water quality meter suitable to record
	Anode ring covered with <6 mm stretch mesh		(reported as μ S.cm ⁻¹ @ 25 °C) to guide adjustment of
	Anode pole of 1.5 m or greater in length		
	Spare complete anode pole and anode ring		total hardness, etc.)
	Electrofisher unit battery plus at least two spares (fully charged), including battery charger		Vouchering equipment for galaxiids and crayfish: follow details in attached protocol for fixation (fish) or
	Siliconized rain cover for electrofishing unit when in damp vegetation		preservation in ethanol (crayfish) of specimens for laboratory identification or verification
	Multimeter for testing batteries and electrofishing	2 x	2 x GPS units
	unit/poles		1 x hand-held compass
	Lineman's electrical gloves rated and tested at a minimum of 1,000 volts, for each team member	Ц	Appropriate spare batteries for non-electrofishing equipment
	1 x waterproof chest high waders with cleated sole boots and 1 x spare, per team member		Appropriate aquatic fauna field guides, including more specialised texts for target fauna, i.e. Coughran
	1 x polarized sunglasses per team member to reduce glare from the water surface		et al. 2015, Horwitz 1990, Morgan 1986, Morgan 1997, Raadik 2011, 2014.
	Electrofishing safety equipment (portable defibrillator, EPIRB, portable first aid kit)		Aquatic fauna datasheet/forms on waterproof paper on clipboard x 1, including HB pencils, sharpener,
	Small (aquarium) fine mesh (1 mm) dipnets (2 +) and larger (2 mm mesh x 200 mm x 200 mm) dipnet on 1.2 m long handle to collect stunned fish or crayfish		eraser, etc., or rugged waterproof laptop, tablet, PDA or mobile phone for digital data collection
п	Large, solid plastic bucket with tight fitting lid for		Waterproof field pocket-notebook plus pencils
	temporary storage of collected fauna		Large plastic bags suitable to carry voucher specimens back to the vehicle for vouchering
	Large, appropriate hiking backpack to carry non- electrofishing field equipment	_	
	Dry bags to protect smaller electronic equipment	Ш	Suitably sized (height, width, depth) plastic jars in which to place voucher specimens so that they are
	Waterproof or water resistant digital camera for		not bent, twisted or crammed.
	habitat and general images of fauna		100% ethanol for preservation of crayfish (burrowing

and spiny) voucher material

Formaldehyde solution (buffered, 35–38% w/w AR grade) for fish fixation.

Waterproof labels for use with voucher material with the following fields: Waterbody Name, Site Code,

Site preparation

The general location of the survey sites shall be pre-determined (e.g. via desktop assessment), based on aspects of the stream network which may maximise available aquatic habitat, consider distance from access tracks (e.g. avoid sites more than 1.5 km from access tracks or more than 1 hour to walk in, etc.), and the coverage of the stream network (permanent stream, intermittent stream, drainage line.

Sites may be located within or adjacent to a site, or they may also be located further downstream of the site.

Navigation

Use a smart phone, PDA, tablet or laptop with geo-referenced 1: 25,000 scale (or similar detailed) topographic raster maps (e.g. Avenza or OruxMaps smartphone applications, ESRI arcpad, Windows software, etc.), a GPS, tablet or laptop with suitably detailed vector topographic maps loaded, or paper 1: 25,000 scale topographic maps to navigate to the pre-selected survey point, or to additional survey sites established based on survey results.

Conducting the survey

Survey from late September to late Autumn, depending on streamflow/rainfall, though sampling can be undertaken during dry times from early September to early June. Avoid instream sampling during periods of higher stream flows and turbid water (usually from recent rainfall events), as this will reduce survey efficiency and decrease the probability of detection of target taxa.

Undertake surveying during daylight hours.

Ensure that the electrofishing equipment is operational before entering the site.

On arrival at a survey location, determine if water is present and whether there is a defined stream channel or a drainage line without channel.

Record if site is dry (this is a valid survey outcome and data can be entered on the VBA as 'Misc Dry'), and visually search the channel and nearby stream banks (also lift rocks, timber debris, vegetation and dry leaves) for signs of spiny crayfish and/or burrowing crayfish burrow entrances or exoskeletons (approximately 20 minutes or over a distance > 15 m, focussing on appropriate habitat). Record whether there is a defined stream channel or a drainage line without a channel.

If the site contains water, record if it is still or flowing, and note clarity (e.g. clear, tannin, turbid) and select a location to commence electrofishing and record its location (decimal latitude/longitude).

- Assess and record water electrical conductivity/temperature and adjust electrofishers settings for voltage and percent of range to maximise electrofishing efficiency for small fish and spiny crayfish (note: frequency should be set to >80 Hz). Record all these details. Reset electrofishing power-on or seconds counter to zero.
- Commence electrofishing, moving in an upstream direction, sampling areas of within-channel habitat appropriate for the target fauna to maximise detection. Sampling may be non-continuous (spot sampling) and should target the stream edges/banks and substrate, including areas of dense structural habitat. The dip netter should follow the electrofishing operator searching for missed animals, and for signs of crayfish which have been disturbed and may emerge from cover a short time following the application of electricity.
- If target taxa are located (e.g. fish and or spiny crayfish), cease sampling, unless additional sites/detections nearby are required in which case undertake sampling up to 30–45 minutes, depending on stream size and habitat attributes related to the target taxa.
- If no spiny crayfish are detected during sampling, target shallow instream areas composed of rock (boulder, cobble, pebble) for additional sampling by hand: remove rocks searching for the presence of spiny crayfish (duration 15–30 minutes per person x 2).
- Use bright (e.g. >700 lumens) head-torches (both team members) in low light conditions, such as early morning/late evening, heavy shading by vegetation, cloudy day, etc.
- For broader biodiversity benefit, also record the species and number of individuals of non-target fauna detected during sampling. This includes the presence of spiny crayfish or burrowing crayfish burrows along the water's edge,

burrowing crayfish burrows up the banks, and the presence of live or dead mussel shells instream, or dead shells along the banks (these can be placed into plastic ziplock bags for later identification).

- Record the 'time start' and 'time end' for the sampling event (= elapsed time in minutes), the electrofishing power-on time (seconds), the sample start and end locations, estimate the distance travelled (metres) and average wetted channel width, and estimate average and maximum water depth (m). Also record whether electrofishing was undertaken as 'spot' or 'continuous' sampling along the survey reach.
- Retain voucher specimens of target fauna (i.e. galaxiids in the Mountain Galaxias species complex (3 representative larger specimens), species of spiny crayfish other than Gippsland Spiny Crayfish (2–3 representative larger/mature specimens, preferably with both claws and including at least one male). Take sharp and clear digital images of the specimens before vouchering. A hand lens may be required to help in determining the sex of spiny crayfish.

Depending on initial survey results and site size, determine if additional survey sites are required before completing the sampling program for the site.

Ensure that all required information is entered onto the datasheet/form (hard copy or electronic).

Preparation of Voucher Material

Fish

The accurate identification of the cryptic galaxiid species requires examination of voucher material which retains the body morphology as close to life-like as possible. This is achieved by a process of fixing, where water is replaced in the cells with a compound (Formaldehyde) which solidifies the cells and does not cause bending, twisting or shrinkage. A 10% formalin (Formaldehyde + water) solution is used.

- 1. Select a suitably sized container to avoid bending or twisting the specimens and allow > 40% of the container volume for the liquid.
- 2. Half fill the container with freshwater and the specimens required for vouchering, including a waterproof label (details above).
- 3. Add more water until you estimate the container is 85-90% full.
- 4. Carefully fill the rest of the container with the Formaldehyde solution, seal the lid, and place into an airtight storage vessel.

In this way the specimens are in a ~10% formalin solution (1 part Formaldehyde solution : 9 parts water). This method also minimises exposure to the Formaldehyde solution.

When the voucher specimens are returned to a taxon expert (within a week of collection), they will need to be soaked in freshwater over two days to remove any traces of unfixed formalin, and then placed into 70% ethanol for longer term storage.

Protocols for the safe decanting, fixation process and washing process for formalin will be supplied.

Spiny Crayfish

Spiny Crayfish have a hard exoskeleton and are therefore not prone to shrinkage or deformation when vouchered. Consequently, laboratory grade ethanol can be used, and this will penetrate the specimen and preserve (not fix) tissue. A solution of 80% ethanol/20% clean and clear fresh water is used.

- 1. Select a suitably sized container to avoid bending or twisting the specimens and allow > 40% of the container volume for the liquid.
- 2. Place the specimens required for vouchering inside the container, including a waterproof label (details above).
- 3. Add enough of the ethanol solution to cover the specimens by approximately 40 mm.
- 4. Place the container into an airtight storage vessel, though separate from the formalin container.

Identification of Voucher Material

All voucher material to be identified/verified within two weeks of collection.

Voucher material (fish and crayfish) to be identified/verified by a taxonomic expert (Dr Tarmo Raadik, Arthur Rylah Institute for Environmental Research), before being deposited in the collection of Museum Victoria.

Data reporting requirements

Data requirements are outlined throughout this guideline and in the datasheet/forms. Complete all required fields on the datasheets/forms for each target observation.

In summary:

- the additional aquatic survey data collected (see above), not required by the survey guidelines and datasheet but valuable more broadly from a biodiversity perspective, must be included in the VBA data upload
- surveys that failed to detect the target species shall also be reported (e.g. record species, and number of each, of all other taxa sampled)
- also record if no aquatic taxa are sampled at a site with water (No Taxa)
- also record if an aquatic site was dry (Dry), as this is still an aquatic survey outcome
- record any further site identifying information that may assist in relocating the observation in the future
- record a GPS track log for all survey work on site and submit as a Track Log shapefile
- record and submit georeferenced photos

Aquatic Survey B: Burrowing Crayfish

Context

The high priority species of burrowing crayfish in forested catchments are the Narracan Burrowing Crayfish, Strzelecki Burrowing Crayfish, and the Curve-tail Burrowing Crayfish, with medium priority species being the Mallacoota Burrowing Crayfish and the Warragul Burrowing Crayfish.

Many other species of aquatic fauna burrowing crayfish may also be detected during survey, and while not the target of the survey, these observations are to be reported.

The aim of aquatic surveys is the detection of the species at a location, which may involve a single individual, or multiple individuals spread over a longer distance.

Species of burrowing crayfish can be difficult to accurately identify as they are small and morphologically similar; accurate identification in the field is very difficult. They are cryptic species, spending most of their time underground, poorly known by most aquatic biologists, rarely encountered in stream surveys, and have a complex taxonomic key (Horwitz 1990), with many important taxonomic characters too small or difficult to see in the field, or hidden, such as pores between the legs.

Consequently, a high level of taxonomic expertise and familiarity with burrowing crayfish morphological characters and taxonomy is required, as a fundamental requirement is accurate species identification. As the crayfish are not widely included in general field guides, it is essential to collect voucher material, which will have multiple benefits: 1) verification of field-based identification or provision of an identification; and 2) retention of a specimen (curated in the Museum Victoria collection) to support the identification into the future when needed (e.g. court case, etc.).

It is possible to confidently distinguish between Spiny Cray (Euastacus) and Burrowing Cray (Engaeus) burrows based on the width, shape, angle and location of burrows, in addition to the formation of a 'chimney' structure at the burrow entrance. This makes it possible to narrow down the species that dug the burrow to genera. Then, in cases where a single target species from either of those genera occur within the vicinity of the survey site, and no other non-target species are in the area as well, it is possible to determine, with an acceptable degree of confidence, that the burrows belong to a given target species (e.g. Orbost Spiny Cray). Burrow observations and geographic location may be used to make formal species identifications where there is a high level of confidence in the identification.

Objectives

To detect high and medium priority threatened burrowing crayfish using burrow tube traps and visual searching (where required) in riparian zones within sites, and along waterways within and bordering selected locations.

Survey effort

Surveyors are provided with the detection probabilities of the target species for each survey technique. Surveyors are to target their surveys to those species with the highest detection probabilities in each site. The species with higher detection probabilities aid in determining the target species most likely to be detected by the survey technique and will thus inform survey parameters such as preferred habitat for survey, bait type, camera settings, etc.

Sampling of a site will be undertaken during a single visit, with searching conducted during daylight hours and tube trapping conducted over night.

The total number of, and distance between, sampling sites per site will vary according to the size of the site and length of waterways within or bounding, and species being targeted.

Specific survey effort requirements are detailed below.

Surveyor requirements

A field survey team of two people, with at least one with previous expertise in visual surveys for signs of borrowing crayfish (crayfish tunnel opening soil mounds)

Previous expertise in the use of burrow tube traps to detect burrowing crayfish

Both team members to have a reasonably high level of physical fitness to walk long distances over rough terrain to reach sampling sites and to carry sampling equipment

Experience with, and expertise in burrowing crayfish taxonomic characteristics and accurate identification. Due to the similarity of species, their small overall size and the small size of diagnostic morphological characters, field identification will be extremely difficult. It is therefore **essential** that field identifications are validated by more detailed examination in the laboratory.

Equipment for the technique				
☐ 30 burrowing crayfish tunnel tube traps per site (maximum three sites) made to specification in Bryant et al. 2014.	 1 x hand-held compass Appropriate aquatic fauna field guides, including more specialised text, i.e. Horwitz 1990, 			
Small (aquarium) fine mesh (1 mm) dipnets (2 +) and larger (2 mm mesh x 200 mm x 200 mm) dipnet on 1.2 m long handle to collect stunned fish or crayfish	Aquatic fauna datasheet/forms on waterproof paper on clipboard x 1, including HB pencils, sharpener,			
Large, solid plastic bucket with tight fitting lid for temporary storage of collected fauna	eraser, etc., or rugged waterproof laptop, tablet, PDA or mobile phone for digital data collection			
Large, appropriate hiking backpack x 2 to carry traps and equipment	Waterproof field pocket-notebook plus pencils			
 Dry bags to protect smaller electronic equipment Waterproof or water resistant digital camera for habitat and general images of fauna 	Suitably sized (height, width, depth) plastic jars in which to place voucher specimens so that they are not bent, twisted or crammed.			
Digital or other Camera (with carry case, spare batteries, spare storage card) capable of including georeferencing data with each photo	☐ 100% ethanol for preservation of crayfish voucher material			
Vouchering equipment for crayfish: follow details in attached protocol for preservation in ethanol of specimens for laboratory identification or verification	Waterproof labels for use with voucher material with the following fields: Waterbody Name, Site Code, Date, Collector Names, and indicate if sample is 'DRY', 'Formalin' or 'Ethanol'			
2 x GPS units				

Site preparation

Specific to the three target species of burrowing crayfish is that their borrows are mainly found on the banks and floodplain of waterways, with the Curve-tail Burrowing Crayfish also found in burrows extending a short way upslope. Therefore, surveys for these taxa should be restricted to these zones.

Conducting the survey

Survey from Spring to late Autumn, with suspected higher catch rates during periods of greater crayfish activity (seasonal or local conditions such as rainfall or warm moist weather), though this is largely unknown for the target taxa.

Undertake visual searches during daylight hours.

On arrival at a survey location, search the waterway banks and floodplain for evidence of crayfish burrows by the presence of canonical soil chimneys at burrow entrances (applicable for Narracan Burrowing Crayfish and Curve-tail Borrowing Crayfish), or potential burrow openings with reduced to no soil chimneys (Warragul Burrowing Crayfish). This should also include a visual search for live crayfish or exoskeletons by searching at the base of vegetation (i.e. tree ferns), under timber debris, etc. (Approximately 30 mins over an area > 30 m in length x 5–10 m wide per person).

Using burrow presence only to record a positive identification.

Where it is possible:

- 1. to confidently distinguish between Spiny Cray (Euastacus) and Burrowing Cray (Engaeus) burrows based on the width, shape, angle and location of burrows, in addition to the formation of a 'chimney' structure at the burrow entrance, and
- 2. where a single target species from either of those genera occur within the vicinity of the survey site, and no other non-target species are in the area as well, and it is possible to identify with an acceptable degree of confidence that the burrows belong to a given target species (e.g. Orbost Spiny Cray) then
- 3. record the species using the burrows as a positive indentification to species level.

In these instances, if burrows are observed, an attempt will be made to capture a physical specimen for identification using standard techniques outlined below. The aim being to capture a voucher specimen to confirm identification to species level.

If no capture is made during the allotted time, a photo will be taken of a burrow and notes taken on aspects of its structure and position that relate to identification of the genus that dug it as well as whether the burrows are new (active) or old. This observation will then be paired with knowledge of species distributions to make an identification if possible.

If the record cannot be made with certainty (e.g. there is more than one species of Spiny Cray in the area and one of them isn't a target species, so it could be either) the generic taxon code for 'burrows' will be used and appropriate information included in the comments.

In cases where a species identification has been made based on the burrow and location, a photo of the burrow will be uploaded with the record

If necessary (e.g. the observation was made by someone not recognised as an expert), the surveyor is required to send that observation record to an expert (e.g. ARI taxonomic experts) for verification of the observers conclusion.

If a verifying expert is contacted for confirmation of an observation, their written advice must be included in the observation record comments section.

Surveying to capture burrowing crayfish specimens.

The following techniques are to be deployed where there is uncertainty as to the identification of the species potentially present, or to obtain voucher specimens to confirm identification.

Where crayfish burrows are detected, deploy 30 burrow tube traps in the afternoon, placed > 1 m apart (potentially reducing number of traps placed into a single burrow with multiple entrances). Focus on burrow entrances with evidence of recent activity (e.g. fresh soil pellets). Deploy traps overnight for >12 hours.

Depending on site size, etc. determine if additional survey sites are required and repeat the process detailed above.

The following morning collect tube traps from each sampling location and check for captured crayfish.

Record the 'time and date start' and 'time and date end' for the sampling event (= elapsed time in minutes), and the sample reach start and end location.

Record the location of each trap with a burrowing crayfish and take a digital image.

Retain voucher specimens to confirm identification in the laboratory (see Section above for method of preservation). A hand lens will be required for preliminary identification.

Ensure that all required information is entered onto the datasheet/form (hard copy or electronic).

Identification of voucher material

Procedure is outlined in the 'Aquatic Survey A: fish and spiny crayfish' section, above.

Data reporting requirements

Data requirements are outlined throughout this guideline and in the datasheet/forms. Complete all required fields on the datasheet/forms for each target observation.

In summary:

- surveys that failed to detect the target species shall also be reported (e.g. record species, and number of each, of all other taxa sampled)
- · record any further site identifying information that may assist in relocating the observation in the future
- · please use a new datasheet/form for each day
- · record and map specified target species
- record a GPS track log for all survey work conducted and submit as a Track Log shapefile.
- · record and submit georeferenced, where possible, photos
- Please enter the survey details (e.g. times and locations of the survey taking place) into the SurveyDetails page (Tab 2). Use the DataFieldsExplained page (Tab 4) to help you enter the correct details.
- Ensure the site ID is entered correctly according to the survey package and in the right format of xxx-xxx-xxxx
- Record your observations in the ObsAttributes page (Tab 3), with each observation being entered on a separate row.
- · Where burrows are used as a confirmed identification of species of a burrowing crayfish,
 - record the VBA taxon code for that species along with detailed comments indicating that the ID was made from burrows as well as details relating to pertinent aspects of the burrow.
 - If the record cannot be made with certainty (e.g. there is more than one species of Spiny Cray in the area and one of them isn't a prescribed species, so it could be either) the generic taxon code for 'burrows' will be used and appropriate information included in the comments.
 - In cases where a species ID has been made based on the burrow and location, a photo of the burrow will be uploaded with the record
 - If a verifying expert is contacted for confirmation of an observation, their written advice must be attached to the observation record.
- Ensure all mandatory fields are completed and in the correct format, failure to do so will result in an incomplete survey.
- A comprehensive list explaining the data entry fields and whether they are mandatory or optional can be found in the Lookups page (Tab 4).
- Ensure the CommonName field in ObsAttributes is entered correctly according to the TaxaIDLookup
- Please Note: Surveyors are expected to submit highest quality data. Please ensure you double check your data entry before submitting data. Submitting incorrect or incomplete information will result in a delay to reporting and may impact on the program outcomes.

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