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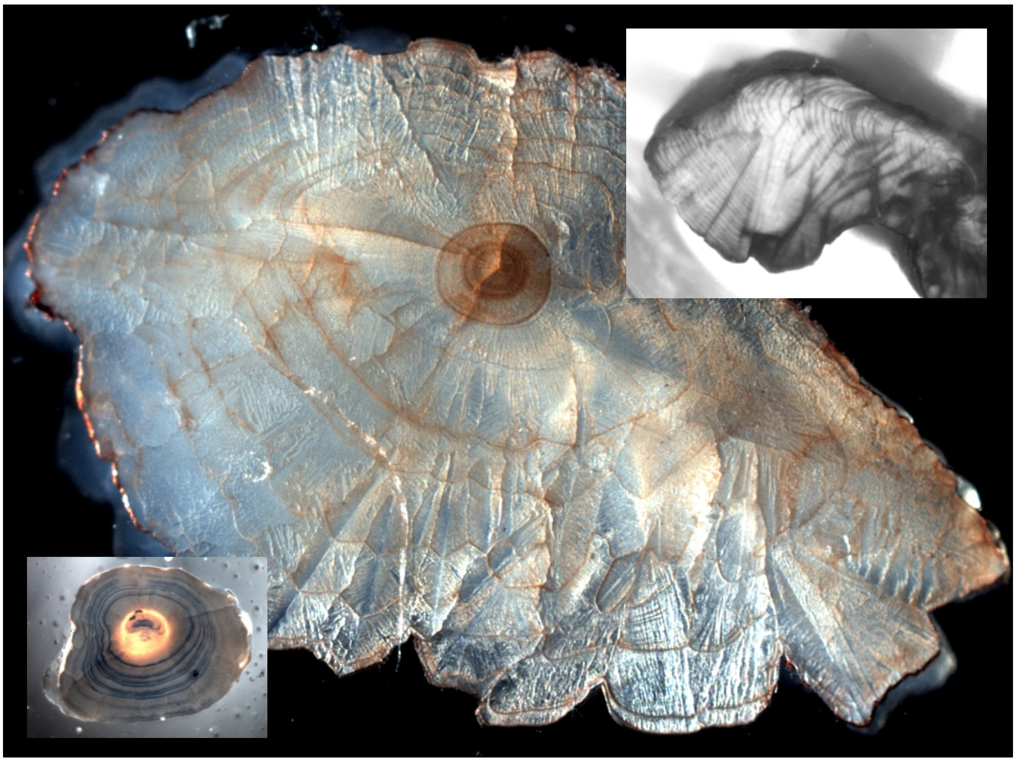
Annex5: Manual for the Ageing of Atlantic Eel

Otolith preparation methodologies, age interpretation and image storage

Produced by the participants of the ICES Workshop on Age Reading for European and American Eel

Version 2: April 2011

2011



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Preparation of this document

This publication was prepared by the ICES Workshop on Age Reading of European and American Eel, held in CEMAGREF, Bordeaux, France from 23-25 March 2011 and chaired by Francoise Daverat (Fr).

This illustrated manual of best practice and otolith images has been designed as a stand-alone document and further information can be found in both the EIFAC 1988 publication on eel age determination (Vollestad, Lecomte-Finiger and Steinmetz, 1988) and the report of the ICES Workshop on Age Reading of European and American Eel, ICES WKAREA, 2009.

The 2011 Version (Vers 2) updates the manual written in 2009 and should be used in conjunction with the reference collection of otolith images compiled by the 2011 workshop. Improvements to some of the preparation techniques, such as cutting and burning and the Swedish polishing and staining protocol are included in Version 2.

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1 Introduction

Eel age determination for Atlantic eel has long been problematic with much debate on both the techniques and the interpretation with relatively few validation studies. Validation is difficult given the terminal nature of ageing with otoliths and also the relatively slow growth and long life cycle often involving different habitats. Ageing using sagittal otoliths, rather than other structures such as scales and opercular bones, appears to be the only viable option for eel. The extraction of eel otoliths was described by Moriarty (1973).

The results obtained using different preparation methods may vary considerably (Moriarty & Steinmetz, 1979; Moriarty, 1983; Berg, 1985; Vøllestad, 1985; Vøllestad & Næsje, 1988; Fontenelle, 1991; Poole & Reynolds, 1996) but few have been validated. The ageing of slow growing eels and the occurrence of supernumerary zones has caused much confusion (Dahl, 1967; Moriarty, 1972, 1983; Deelder, 1981; Poole *et al*. 1992) although subsequently, the 'burning and cracking' method was validated in some situations (Moriarty & Steinmetz, 1979; Moriarty, 1983; Vøllestad & Næsje, 1988; Poole & Reynolds, 1996). Burning and cracking was then recommended by an EIFAC eel age workshop in 1987 as the best option for ageing eels (Vøllestad, Lecomte-Finiger & Steinmetz, 1988), particularly for the slow growing and older specimens (e.g. Vøllestad & Næsje, 1988). There have been many developments since 1988, both in improved otolith preparation techniques, imaging and validations along with the use of eels of known age and chemical marking of otoliths.

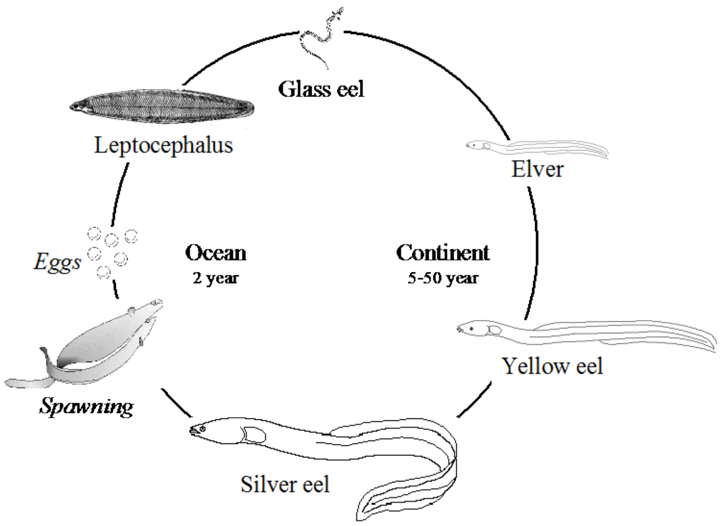
Considerable recent improvements have been made to both the technology of otolith preparation, viewing and imagery of otoliths which has led to a better understanding of the determination of age for both species of Atlantic eel. Validation studies have looked at eels of known age from Sweden (Svedang, *et al*. 1998), marked otoliths, examples of otoliths from eels of known and unknown age prepared by burning and cracking and by grinding and polishing. Studies have also been carried out on a range of other otoliths including burnt and cracked glass eel otoliths for identifying the zero band, from where the continental phase ageing commences.

This manual, while designed as a standalone document, should be read in conjunction with the reports from the ICES Workshop on Age Reading for European and American Eel, 2009 and 2011, held in Bordeaux.

2 Glossary

2.1 Eel Terms

Eels are quite unlike other fish and consequently come with a specialised jargon (WGEEL, 2008). This section provides a quick introduction and is not intended to be exhaustive.



The life cycle of the European eel. The names of the major life stages are indicated. Spawning and eggs of *Anguilla anguilla* and *A. rostrata* have never been observed in the wild (supplied by Dekker).

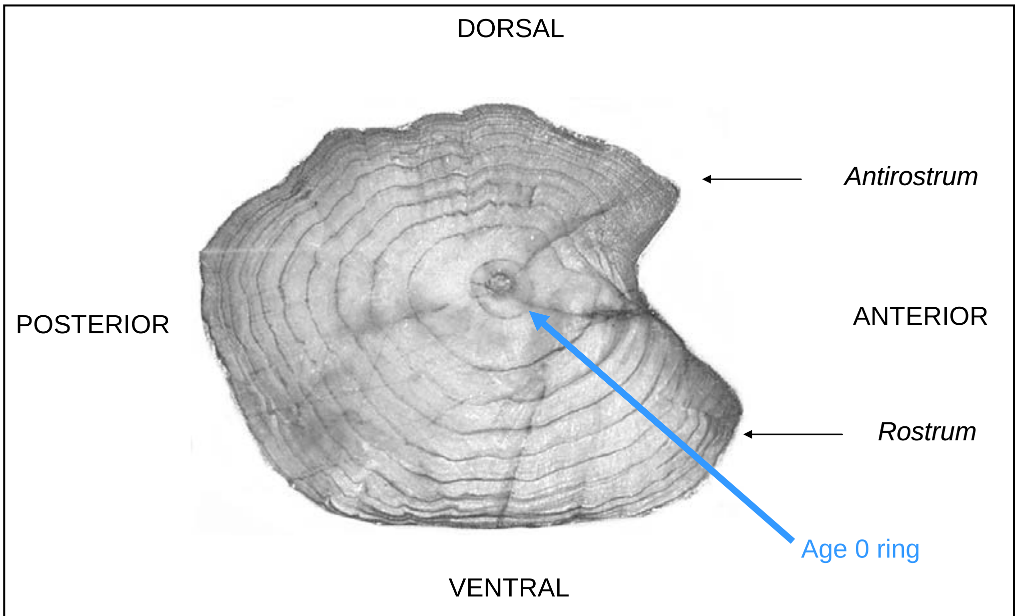
Atlantic Eel	The collective term Atlantic eel will be used in this report to cover both *A. anguilla* and *A. rostrata* where there are no differences.Differences between the species will be indicated by their specific names.
Glass eel	Young, unpigmented eel, recruiting from the sea into continental waters
Elver	Young eel, in its 1st year following recruitment from the ocean. The elver stage is sometimes considered to exclude the glass eel stage, but not by everyone. Thus, it is a confusing term.
Bootlace, fingerling	Intermediate sized eels, approx. 10-25 cm in length. These terms are most often used in relation to stocking.The exact size of the eels may vary considerably. Thus, it is a confusing term.
Yellow eel (Brown eel)	Life stage resident in continental waters. Often defined as a sedentary phase, but migration within and between rivers, and to and from coastal waters occurs. This phase encompasses the elver and bootlace stages.
Silver eel	Migratory phase following the yellow eel phase. Eel characterised by darkened back, silvery belly with a clearly contrasting black lateral line and enlarged eyes. Downstream migrating phase, moving towards the sea,. This phase mainly occurs in the second half of calendar years, though some are observed throughout the winter and following spring.
Eel River Basin	"Member States shall identify and define the individual river basins lying within their national territory that constitute natural habitats for the European eel (eel river basins) which may include maritime waters. If appropriate justification is provided, a Member State may designate the whole of its national territory or an existing regional administrative unit as one eel river basin. In defining eel river basins, Member States shall have the maximum possible regard for the administrative arrangements referred to in Article 3 of Directive 2000/60/EC [i.e. River Basin Districts of the Water Framework Directive]." EC No1100/2007.

River Basin District	The area of land and sea, made up of one or more neighbouring river basins together with their associated surface and groundwaters, transitional and coastal waters, which is identified under Article 3(1) of the Water Framework Directive as the main unit for management of river basins.
Stocking	Stocking is the practice of adding fish [eels] to a water body from another source, to supplement existing populations or to create a population where none exists.

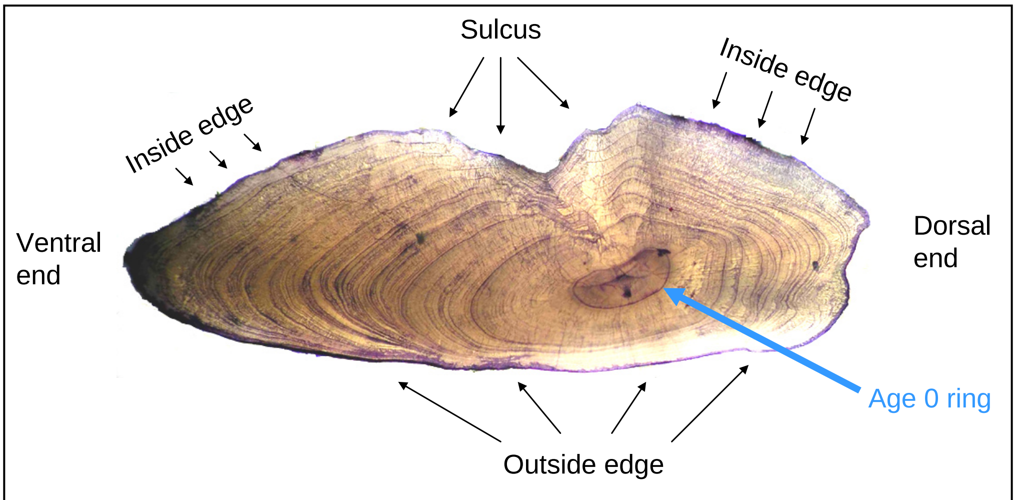
2.2 Otolith Terms

(updated from Vøllestad, Lecomte-Finiger & Steinmetz, 1988) and see also Figure 2.1

Annual zone:	Structural feature of the otolith corresponding to the growth during a complete year of life
Annulus:	The theoretical boundary between two successive annual zones
Burning & cracking:	The traditional otolith preparation of burning and cracking has been improved by cutting the otolith before burning. Both methods are covered in this manual by the term "Burning and cracking".
Frontal Plane:	The flat cut, or cracked, face of a transverse section of an otolith
Growth Check:	A boundary between two growth zones, not necessarily annual (also see supernumerary)
Hyaline:	See translucent.
Nucleus:	The hypothetical or real origin of the otolith; synonymous with focus or core
Opaque zone:	A zone that inhibits the passage of light. In transmitted light opaque zones appear dark and in reflected light they appear bright (white)
Radius:	A determined measurement from a focus to a specific point
Sagittal Plane:	The view of the otolith when lying flat, convex side up. Most grinding takes place on the sagittal plane.
Supernumerary:	A growth mark or check not accepted for annual age determination, also referred to as a growth check or false annulus.
Translucent zone:	Previously known as the hyaline zone. A zone that allows the passage of light. In transmitted light translucent zones appear bright, in reflected light they appear dark.
Validation:	The confirmation of the temporal meaning of a growth increment. Analogous to determining the accuracy of age determination; used in reference to true age.
Verification:	Determining the precision (reproducibility) of age determination, used in reference to the precision of estimated age.
Zero band:	The first growth check outside the nucleus from where continental age determination commences (~170µm radius from centre).



European eel Otolith, cut on the sagittal plane



American eel from USA, cut on the frontal plane

Figure 2.1. Illustrations of sagittal and frontal plane views of otoliths indicating the glossary terminology. The Age 0 ring is equivalent to the zero band referred to in this report. The anterior and posterior regions of the otolith above are in accordance with the orientation of the eel (from Christine Gazeau).

Also useful: http://www.cmima.csic.es/aforo/index.jsp

3 Otolith Extraction from Anguillid Species

The method described here is an adaptation of that described by Moriarty (1973) and is just one approach, with many researchers using different variations. This technique minimises loss and damage to otoliths and is a quick, clean and efficient method.

A primary transverse incision is made behind the eye in two phases using a scissors; first cutting the skin and flesh and second penetrating the cranium through to the roof of the mouth and providing access to the cranial cavity (Figure 3.1). This reduces cranial compression whilst also lowering the risk of otolith shearing observed in other methods.

Figure 3.1: Transverse cuts behind the eyes through the skin and flesh (top) and through the skull (bottom) leaving the lower jaw still intact.

Gripping the eel's lower jaw in the left hand, three secondary longitudinal cuts are then made with a sharp pointed scissors along the dorsal surface of the cranium, perpendicular to the primary transverse cut, (Figure 3.2). Each cut extends further along the length of the cranium, the extent of which depending upon the individual skull length. The first cut severs the skin, the second cuts through the upper portion of the skull and brain cavity and the third through the lower portion of the skull and brain cavity and roof of the mouth.

Figure 3.2: Longitudinal cuts through the skin and flesh (top) and through the upper and lower skull (bottom) leaving the lower jaw still intact.

The exposed cranial sections can be moved aside using the thumb on the left hand to reveal the otolith lying within the sacculus, one located on each side, (Figure 3.3 & 3.4). Each otolith is found lying behind the brain, one on each side of the base of the cranium. Once extracted, the otolith can be cleaned by rubbing on the back of the left hand with the right forefinger (unless the otolith is to be used for genetics).This extraction method allows sufficient control over the placing of specific cuts thereby avoiding damage and loss of otoliths and may also be performed single-handedly where required keeping the right hand clean for writing, labelling and cleaning otoliths.

Figure 3.3: The brain case separated and showing the location of one of the otoliths.

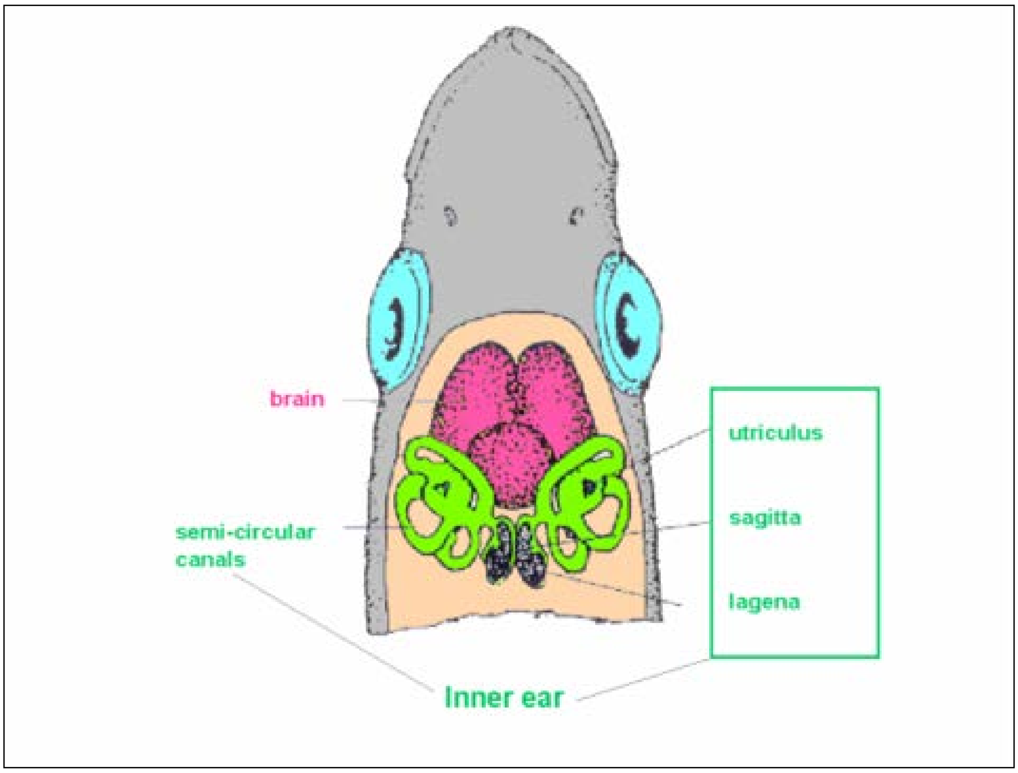


Figure 3.4: Showing location of otoliths in the head of an eel; the sagittae are extracted for age determination (adapted from Secor *et al.*, 1992; (www.cmima.csic.es/aforo/oto-wat.jsp)

4 Field Data for Age Determination

The following field data, where possible should be recorded for each eel otolith and be available along with the image (the image should be saved with the eel unique ID number attached):

Unique Eel Identification Number

Location of Capture

Date of Capture

Length and weight if available

Sex

Habitat type

Other features – e.g. wild, stocked, pre-grown in culture

It is recommended that age is estimated from each otolith by at least two readers, and preferably three. There are, therefore, two main approaches to providing vital information on the otoliths being processed:

- **Vital information fully available to all readers.**

  In most cases, given the relationship between stress and metabolism and the creation of annual and false check bands within otoliths, it is advisable that the readers have vital information to assist with correct age interpretation – this could save much time and effort if it prevents erroneous interpretation.

- **Selective vital information withheld from all readers.**

  There may be situations when a priori information might bias a reader's approach to age interpretation and a blind test is strongly recommended (Nielson, 1992). If you have background knowledge of information likely to influence your decision on age you should not partake in the blind test. For example, when ageing otoliths of eels sampled in contaminated versus reference sites, site information should be withheld and otoliths should be processed randomly; otherwise, readers may bias their interpretation of age/growth in accordance with their expectations of results.

5 Otolith Storage and Preparation Protocols

5.1 Introduction

Before extracting and storing otoliths, it is important from the outset to establish the nature of the research being undertaken, and whether or not long-term storage and archiving of material will form a significant part of the work. Where it is not necessary for the material, including otoliths, to be preserved this should be avoided. Where chemical storage of tissue is required, the otoliths should be removed first. The issue of cross contamination between specimens when sampling otoliths is more serious when DNA extraction is envisaged because it is easy to carry small amounts of soft tissue or mucous between specimens unless precautions are taken (instruments are cleaned (wiped) thoroughly between samples). It is also highly likely that sample processing stations will be contaminated by slime and tissues from a number of fish.

Best practice is to extract both sagittal otoliths from each fish. One can then be archived for future use, such as otolith microchemistry, stable isotope analysis or genetics, whilst the other can be used for immediate ageing.

There are two main otolith preparation protocols for the Atlantic species of eel, *A. anguilla* and *A. rostrata*, currently in use. Those are, with slight variations between institutes, the burning and cracking (including cutting before burning), and the grinding and polishing (and in most cases staining) protocols. Clearing whole otoliths "in toto" has limited use for small eels of young age.

5.2 Storage Media

The WKAREA 2009 concluded that dry storage is the best medium in which to retain otoliths. Advantages and disadvantages of dry storage and alternative methods considered can be referred to in the workshop report. Consequently, it is recommended that paper envelopes are utilized in burn and crack (*cut and burn) techniques, whilst microtubules such as eppendorfs are used for grinding and polishing, where microchemistry or genetic analysis is anticipated. It is important that otoliths are fully dry before storing in eppendorfs to avoid deterioration due to damp.

Care should be taken to avoid physically damaging otoliths due to stacking of envelopes and binding tightly with rubber bands. Ensure eppendorfs are not damages in, for example, postage.

Care should be taken to avoid using open micro-titre type trays for storage of eel otoliths. There is a danger of individual otoliths dropping into the wrong cell, or inadvertently migrating between cells during transit.

5.3 Grinding & Polishing Methodology

This procedure provides a safe and reliable method for processing eel otoliths and assessing the age of the eel by counting the annuli illuminated as a result of the grinding and polishing. Figure 5.1 gives an outline of a possible approach. The right otolith is chosen for consistency, although for eel this is not critical.

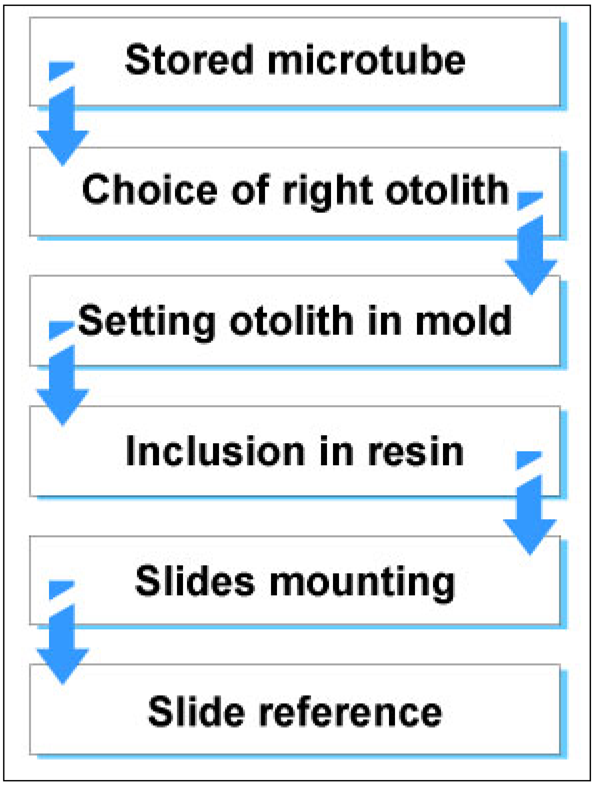


Figure 5.1: An approach to grinding and polishing

5.3.1 Reagents

A variety of chemical reagents are used during the different phases of preparation and reading. Most commonly wax, epoxy resin, Crystal bond or Loctite Branded Super glue are used as the adhesive.

EDTA, Hydrochloric acid and Acetic acid are used in the etching process, whilst Toluidene Blue or Neutral red stains are used in the staining process. Alumina and diamond powder pastes may be used to polish the otolith following grinding.

5.3.2 Equipment

Stereo dissecting microscope (with mounted camera and image capture and analysis software), glass microscope slides, fine point forceps, mounted needles, slide container box, self adhesive grinding papers (600, 800, and 1200 grade) (Figure 5.1).

Fig. 5.2: Equipment and arrangement of the grinding papers, alumina powder paste on laboratory bench.

5.3.3 Methodology

This preparation method involves the initial mounting/embedding of an otolith in a convex position onto the end of a glass slide (Figure 5.3). The bottom and sides of the otolith are affixed to the slide using some form of high strength adhesive which is then lightly drawn over the dorsal surface of the otolith to fill in tiny crevices on the surface of the structure and produce a fuller bond to the slide.

The otolith is examined under a stereo dissecting microscope, at as low a magnification as possible, using a variety of light types including transmitted, reflected or polarized. Annuli are rarely sufficiently visualized at this point and as such the otolith is ground along the sagittal or transverse plane depending on the orientation during embedding. The otolith is ground until the centre of the nucleus is reached. This can be performed manually, or by using a grinding wheel in conjunction with silicon carbide wet-dry sandpapers, after which the otolith is then polished using a decreasing range in coarseness (1200-4000 grit) of silicon carbide wet-dry sandpapers, jewellery cloths or pastes made from aluminium or diamond powder (Figure 5.4).

From this point onwards a variety of different techniques involving etching and staining are employed to help improve visualization of annuli which are then counted, bearing in mind that translucent zones (winter) are bright, and opaque zones (summer) are dark, when the otolith is viewed with transmitted light whilst opaque zones (summer) are bright, and translucent zones (winter) are dark when viewed with reflected light.

Six main protocols exist for the preparation of otoliths by grinding and polishing, and are used in institutes in Belgium, Canada, France, Germany, Italy, United Kingdom, Sweden and the USA (Table 5.1). The general principle involves the mounting/embedding an otolith concave side down on a glass slide. The bottom and sides of the otolith are affixed to the slide and the adhesive is then lightly drawn over the top surface of the otolith which fills in tiny crevices on the surface of the structure.

The otolith is examined under a dissecting microscope using a variety of light types including transmitted, reflected and polarized. Annuli are rarely sufficiently visualized at this point and as such the dorsal surface is lightly ground in the sagittal plane and then polished using a variety of silicon carbide wet-dry sandpapers. From this point onwards a variety of different techniques involving etching or staining is employed to help improve visualization of annuli. It is these different techniques which account for most of the variations between laboratories.

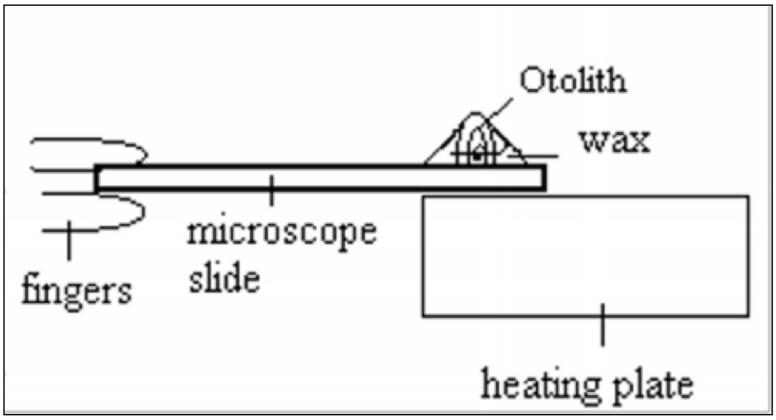


Figure 5.3: Mounting/embedding of the otolith on the glass slide.

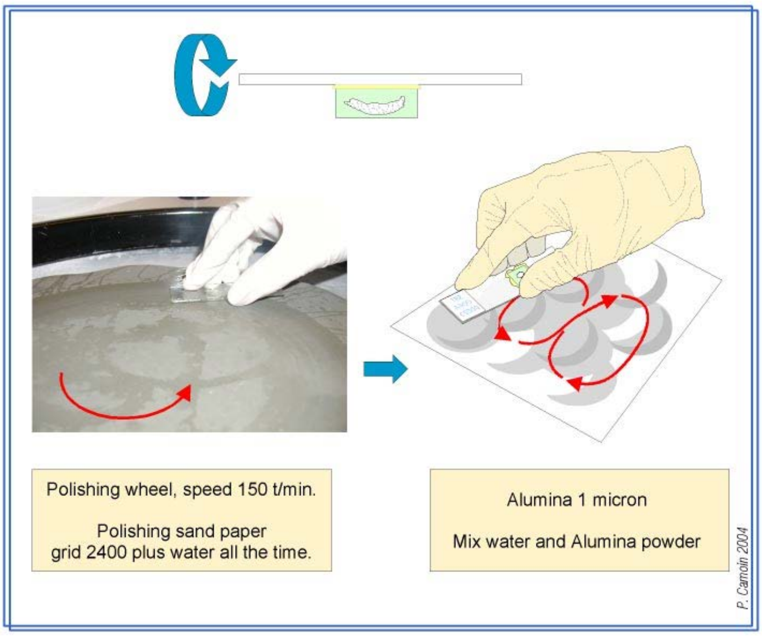


Figure 5.4: The grinding technique.

Table 5.1. Summary protocols for the preparation of otoliths by grinding and polishing for each country.

Preparation Stage	France	Japan	Sweden	N.Ireland	Germany	USA/ Canada
Otolith Alignment	Concave side down Sagittal grind	Transverse Transverse grind	Concave side down Sagittal grind	Concave side down Sagittal grind	Transverse Transverse grind	Concave side down Sagittal grind
Embed	Epoxy resin	SpeciFix + SpeciFix-20 curing agent (5.2:1)	N/A	N/A	Clear mount wax	In a gelatine mould with a mixture of epoxy resin (18g of MIA-100) and hardener (5g of MIA-95)
Mount	Crystal bond	Double-side tape (flip upside down for a “double trim”)	Crystal bond	Loctite Brand super glue	Crystal bond	Dried mixture of Epoxy resin /Hardener in a mould of gelatine
Grinding	Grinding wheel (150 t/min, 1200 grit with water until otolith is reached; switch to 2400 grit. Check every 20 secs under a m’scope until the nucleus is reached	Grind with “Discoplan” using 70µm grinding paper to “some degree” and then switch to 13 µm grinding paper. Both sides are ground	Grind manually with 1200 grit until centre and edge are visible; water is added constantly	Grind manually with graded sand paper in a figure-8 pattern. Check slide often to see when centre is exposed.	Grind manually with a series of grinding paper: 600, 800 and 1200 grit down to 0.1–0.2 mm .	800-1200 grit paper
Polishing	A paste with aluminium powder 1µm and water. Polish manually.	Polishing disk with DP-Lubricant Blue (wet) and DP-Paste (diamond paste) *very specific details for the polishing machine.	3 µm Lapping Film. Add water constantly	Jewellery polishing cloth	Alumina powder slurry (0.3 µm, mixed 1 : 10 with distilled water) on the plastic slide, then clean with distilled water and on a wet fibre polish.	Polishing disk with Alumina powder 1µm and water (paste). Polish manually on the disc.
Etching	5% EDTA for 3 mins	1% HCl for 1 min. or 3-5% EDTA for 3-5 mins.	1% HCl for 1 min	N/A	N/A	5% EDTA for 3 mins
Washing	Distilled water	Distilled water	N/A	N/A	N/A	Distilled water
Staining	5% Toluidine blue (smeared), dry over night	1% Toluidine blue If HCl stained 30-120 mins If EDTA stained 5-30 mins.	10 % Neutral red 10 minutes	N/A	N/A (crack and burn is done first to highlight the rings)	5% Toluidine blue (smeared): Toludine Blue O [CAS No. 92-31-9] dry over night
Rinse	Distilled water	Distilled water	Water	N/A	N/A	Distilled H_2O

5.3.3.1 Slide preparation and Etching – Swedish method

Slide preparation

The slide is labelled with the following details: area, fishing year, individual number, eel life stage.

Place the slide on a heating plate (100˚C) and melt the crystal bond on the surface. Mount the otolith in the crystal bond in a convex position on the microscope slide.

The procedure must be handled in a ventilation (fume) cupboard.

Grind on a wheel beginning with 1200 grits silicon-carbide sanding papers until the centre and edge are visible. Add water constantly during the grinding process. NOTE; It is important to grind at an angle that reveals the outer annuli at the edge without grinding through and loosing the nucleus. Regular checks should be made during the grinding at the nucleus or the edge.

Polish with 3µm Lapping Film until surface is smooth. Add water constantly during the polishing process.

Etching

Etch the otolith by immersing the whole slide for 1 min in 1% HCl acid

Rinse in water.

5.3.3.2 Etching – CEMAGREF method

A summary protocol for etching is given here, as developed by CEMAGREF (Figure 5.5).

The whole slide preparation is immersed in:-

5% EDTA for 3 mins

or

1% HCl for 1 min.

The otoliths are then ready for staining.

5.3.3.3 Staining – Swedish method

Prepare the neutral red solution 24 hours before staining.

> 0,5g neutral red powder (SIGMA- ALDRICH Product reference: N2880-25G)
>
> 0,5g Sodium chloride.
>
> 50 ml distilled water.
>
> 0.25ml of Acetic acid (100%)

Put a drop of the solution upon the otolith and stain for approximately 10 minutes (Can differ between samples),

Rinse in water.

5.3.3.4 Staining – CEMAGREF method

A summary protocol for etching is given here, as developed by CEMAGREF (Figure 5.5).

The whole slide preparation is immersed in:-

5% Toluidene blue (smeared), dry over night

or

1% Toluidene blue:-if HCl stained, 30-120 mins.

if EDTA stained, 5-30 mins.

or

10% neutral red for 10 minutes.

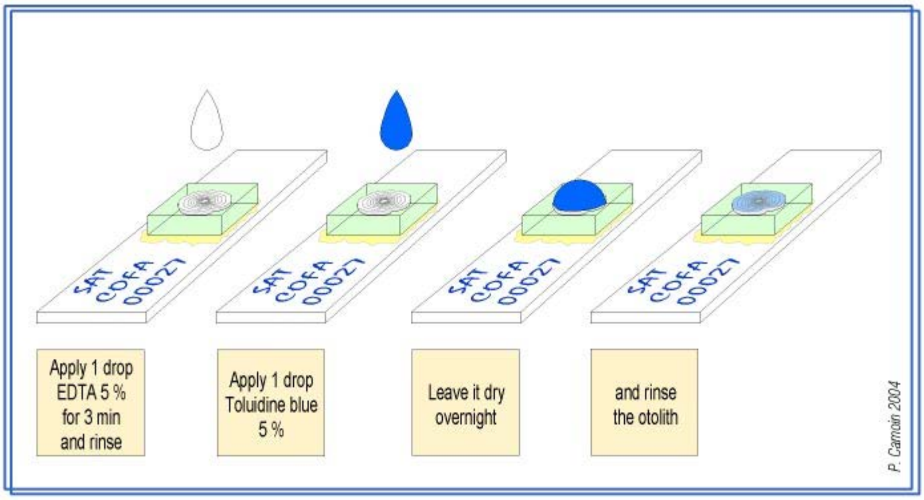


Figure 5.5: Protocol for etching and staining in toluidene blue (source: CEMAGREF)

5.3.4 Canadian method (from R. Tardif and G. Verreault, MRNF; and V. Tremblay, AECOM)

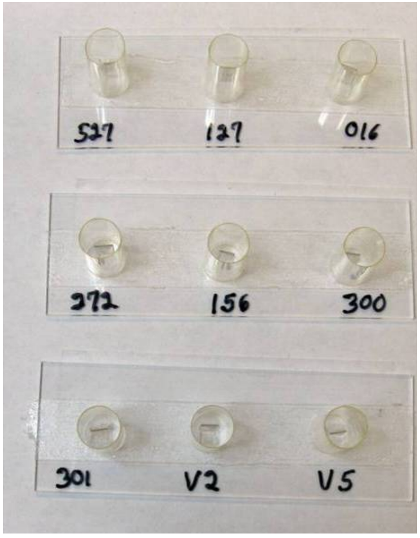
5.3.4.1 Otolith Preparation

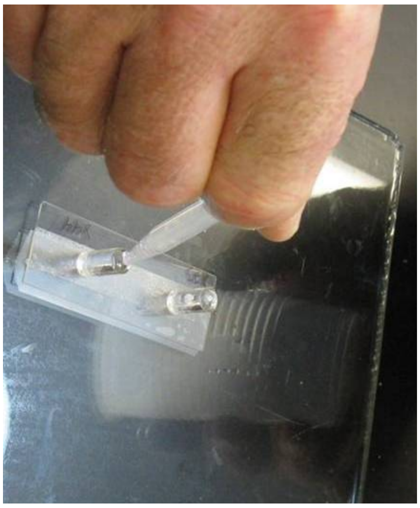
The dried right otolith is cleaned with successive baths of bleach (50%diluted), distilled water and ethyl alcohol (70%) solutions (10 minutes each).

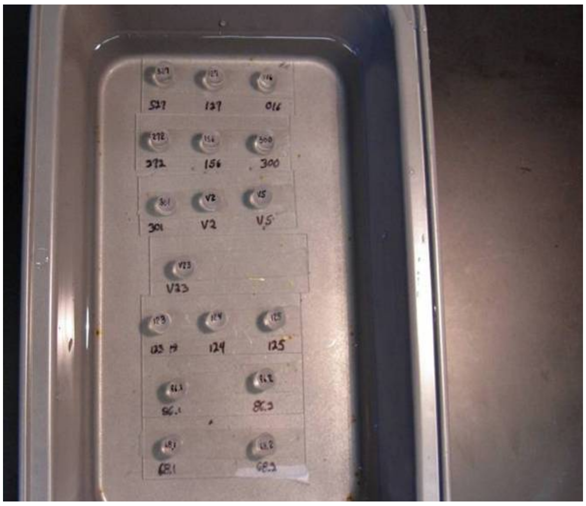
5.3.4.2 Moulding and Embedding

Once dried, the otolith is put concave side down on a slide, and then embedded in a gelatine mould with a mixture of epoxy resin (18g of MIA-100) and hardener (5g of MIA-95) (8-10 mm mixture on the otolith). The mixture was previously heated for 4 minutes at 50°C.

This procedure should be handled in a ventilation (fume) cupboard.







5.3.4.3 Grinding

After 18 to 24 hours of embedding, the embedded otolith is labelled and removed from the gelatine mould simply by dissolving gelatine with warm water for 30 minutes.

The individual structure is then grounded manually to the core on its sagittal plane with 800 to 1200 grit paper and distilled water.

Add water constantly during the grinding process.

NOTE; it is important to grind at an angle that reveals the outer annuli at the edge without grinding through and loosing the nucleus. Regular checks should be made during the grinding at the nucleus or the edge.

5.3.4.4 Polishing

Structure is then polished on a polishing disc (PRESI) with a paste made of alumina powder (1µm) and distilled water.

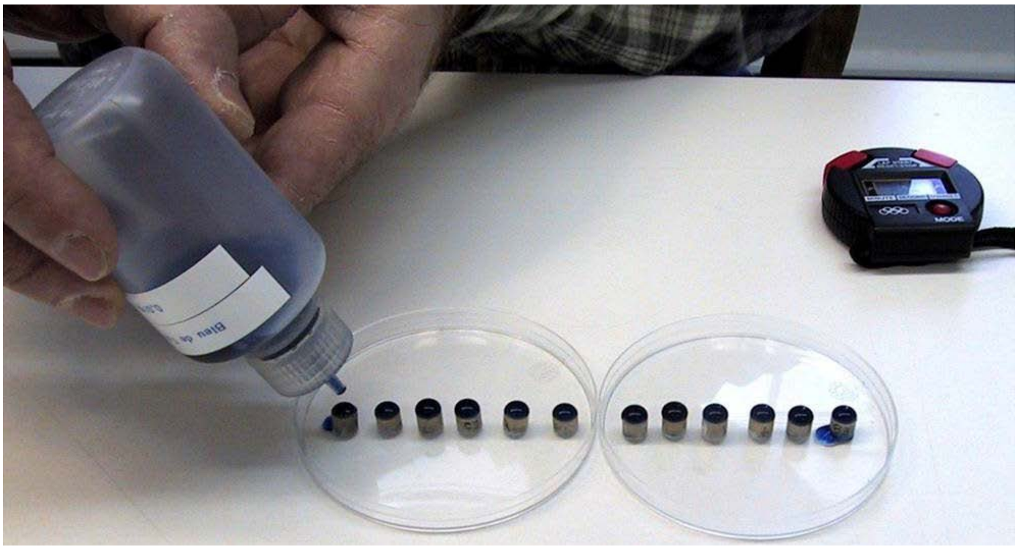
Add water and powder if needed during the polishing process.

5.3.4.5 Etching

Each prepared section is then etched for 3 minutes with a solution of 5% Ethylenediaminetetraacetic acid (EDTA) to enhance the annuli for aging (2.0g of powder in 38 ml demineralised water). This solution has to be changed monthly (or every 2 weeks for best results). The otolith is then rinsed with distilled water. The otolith is then ready for staining.

5.3.4.6 Staining

After decalcification, put a drop of the staining solution upon the otolith and stain for 3 minutes. Solution is made of 0.01% Toluidine blue O [CAS No. 92-31-9] (0.1g powder in 50 ml demineralised water (0.02% solution). In other bottle, use 1 part of this primer solution in 19 parts of demineralised water. For example, use 5 ml of the primer solution in 95 ml of demineralised water.



5.3.4.7 Image acquisition

Before grabbing the image structure under a stereomicroscope equipped with a camera, put again a drop of the coloration solution on the top for 1 minute, rinse it and take the image before it dries off.

Rinse in water.

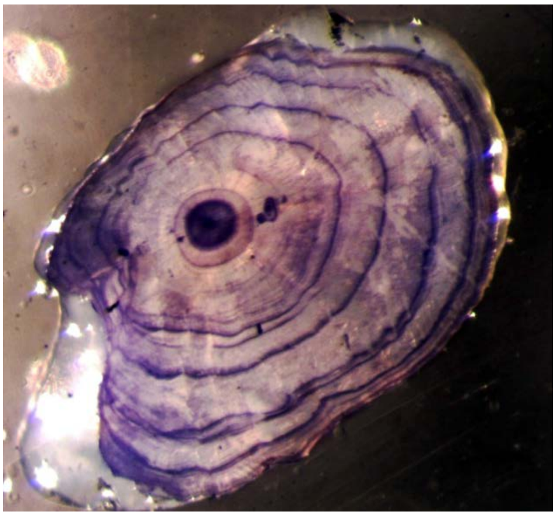
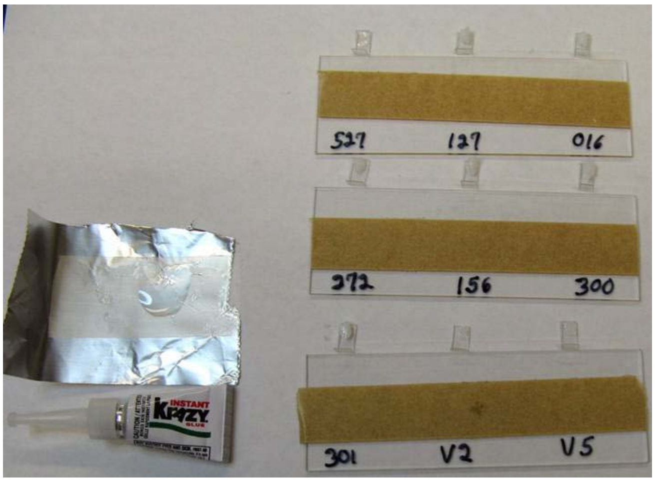


Figure X.1: A ground, polished and stained otolith (sagittal plane) from *A. rostrata*

5.3.4.8 Transversal cut alternative

Since grinding and polishing has a tendency towards underestimating the age, especially in older slower growing eels, a cut can be performed with a precise low speed isometric saw (Beuhler IsoMet) in the transverse plane. This cut is made after dissolving gelatine mould in water. To be grinded in the transverse plane, the otolith has to be mounted on a plastic brace before insertion in gelatine mould. Before cutting, the otolith core is adjusted to the Isomet saw, as follows:



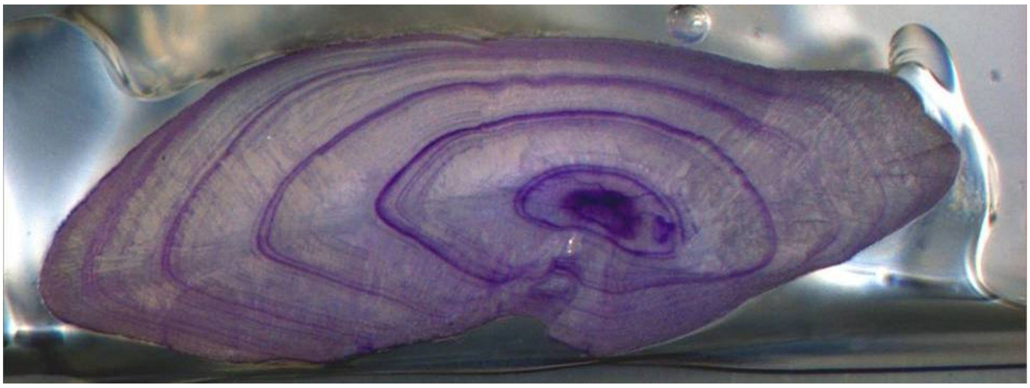


Figure 5.6: A ground, polished and stained otolith (transverse plane) from *A. rostrata*

5.3.5 Advantages

The grinding and polishing method (grinding in the sagittal plane) gives a safe and reliable method for preparation of otoliths from eels that are not too old or slow growing. It is the only method that allows marks inserted in the otolith using OTC, strontium or alizarin to be viewed (burning and cracking destroys these marks).

5.3.6 Disadvantages

Grinding and polishing has a tendency towards underestimating the age, especially in older slower growing eels. This may be due to loss of outer rings by over-grinding or because of the shape of older larger otoliths. This could be overcome by grinding in the transverse plane, although this variation is time consuming.

5.4 Burning and Cracking Protocol

5.4.1 Standard Operating Procedure for burning and cracking of Eel Otoliths

Christensen (1964) first reported that eel otoliths could be successfully prepared by burning in a flame and cracking to reveal the annuli on the broken face of the otolith. The preparation of eel otoliths was described by Moriarty (1973) and developed by Hu & Todd (1981) with a more permanent mount made by mounting the otolith in silicone sealant against a glass slide.

The otolith is burnt either before or after cracking. Traditional burning and cracking was used but with the advent of better digital imaging processes, the workshop of 2009 recommended replacing the burning and cracking method by first cutting and then burning the otolith. The cutting and burning method is described here and the traditional technique is described as a variation.

5.4.2 Purpose and Scope

This procedure provides a safe and reliable method for processing and preserving eel otoliths and assessing the age of the eel by counting the annuli highlighted as a result of the burning process.

5.4.3 Principle of the Method

Cutting the otolith in half across the short axis - on the frontal plane, through the nucleus which should be visible in the dry otolith, helps to produce a flat surface for examination. After cutting, the otolith is burned which highlights the winter bands so they can be differentiated and counted when examined under reflected light.

5.4.4 Equipment

Glass microscope slides, fine forceps, fine pointers, heavy scalpel with insulated handle for burning, second scalpel with sharp blades for cutting, Bunsen burner/spirit burner, slide container box, syringe and clear silicone sealant for glazing, a light box or light projector for illuminating the nucleus of the otolith when cutting.

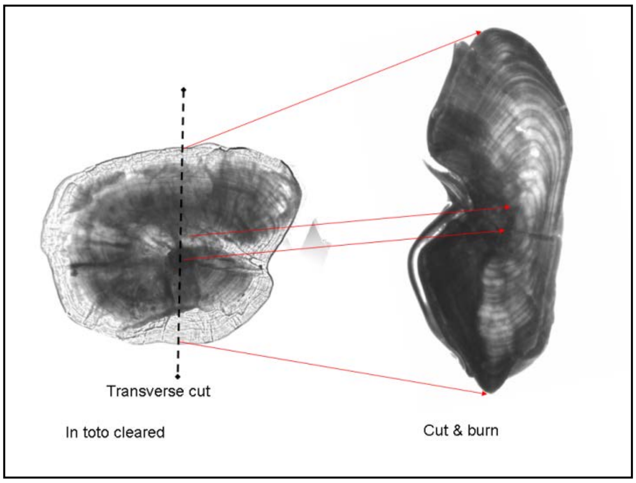
5.4.5 Procedure – cutting & burning

Eel otoliths, once removed from fish heads, should be cleaned by rubbing gently and placed into your storage medium (scale envelopes, unsealed eppendorf tubes etc.) and left in a warm dry place for several days to dry.

Otoliths are removed from the envelope etc. using a fine forceps and placed on a glass microscope slide convex side down. The otoliths are then stuck to the slide with clear Sellotape. A sharp scalpel is then used to cut the otoliths in half along the transverse axis, through the nucleus (Figure 5.7). Care must be taken to cut through the nucleus of the otolith. Illuminating the otolith from below, using a light box or modified projector, helps identify the nucleus (Figure 5.7).

After cutting, the otolith halves are placed on a scalpel blade and placed into the upper blue part of a moderate Bunsen flame for up to 30-60 seconds, by which time they have darkened to a slate grey colour (Figure 5.7). This will follow initial darkening from light brown to black. It is important that the flame is not too hot as incomplete, or patchy, incineration can occur.

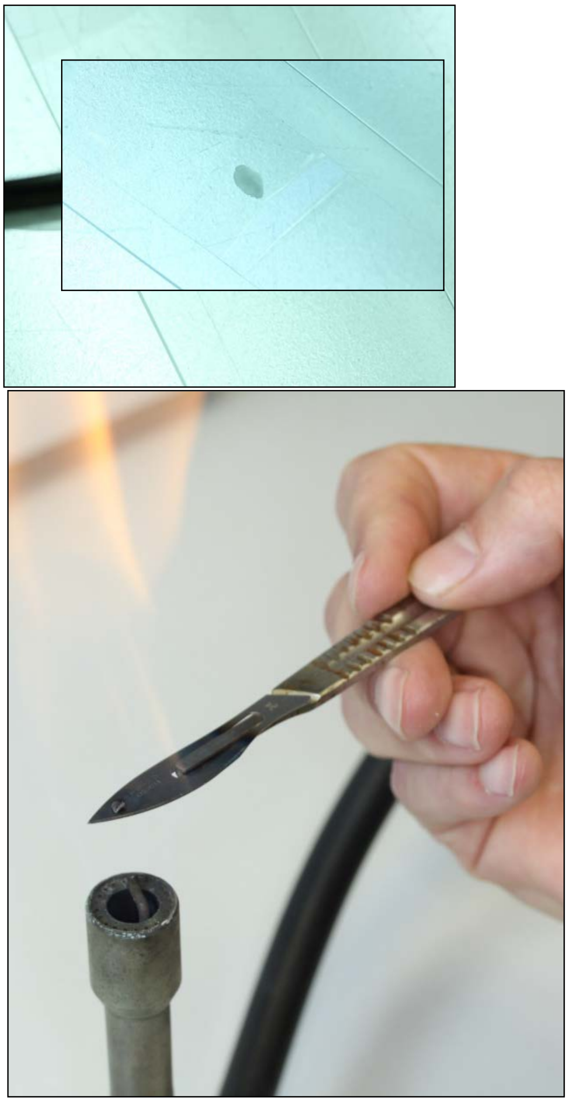
[NOTE: Wear safety glasses when burning otoliths]



The otolith is very delicate after being burnt and must be handled with care. The otolith halves are picked up using a pointer, which has been dipped in a small amount of the sealant and gently mounted in a small bead of silicone sealant that has been placed on a microscope slide, with the prepared reading surface pressed against the slide surface (Figure 5.6). The otolith should be slid sideways into the sealant in order to exclude any trapped air bubbles. By carefully turning the microscope slide it can be checked weather the otolith is placed the right way… if not some alterations to the mounting can be carefully made, but this needs to be done quickly before the silicone begins to cure.

The silicone is left to set for approx. 45-60 minutes before a second bead of silicone sealant is placed on top of the first to seal in the otolith halves and make a permanent mount.

The otoliths are read through the glass of the inverted slide (Figure 5.8) under a stereo microscope using reflected light under magnification between x20 and x50.



Photos: Peter van der Kamp

Figure 5.7: Cutting the otolith through the nucleus and burning in a Bunsen flame.

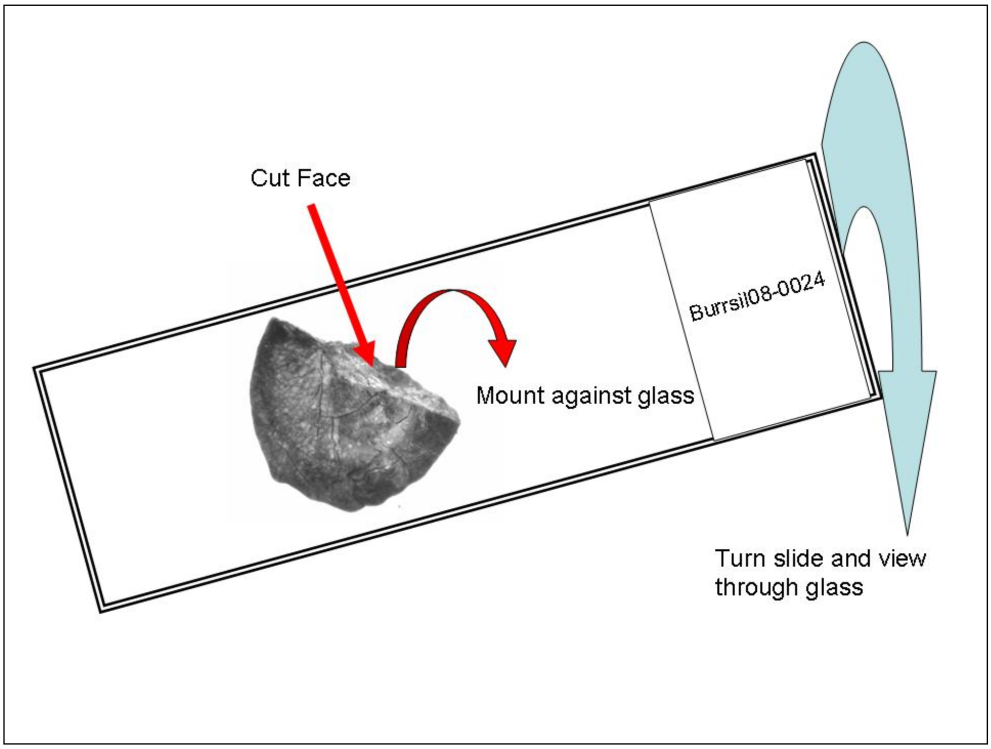


Figure 5.8: The cut face of the burnt otolith is mounted in silicone against the glass and the slide is inverted for viewing under reflected light.

5.4.6 Safety

Care should be taken with the gas supply and when using lit Bunsen burners. Care should be taken with the naked flame from the Bunsen burner, as it is not always visible, and a naked flame sign should be placed on the bench beside the Bunsen. The laboratory should be kept well ventilated and laboratory coats and eye glasses should be worn at all times.

5.4.7 Variation – traditional cracking and burning

5.4.7.1 Principle of the Method

Before cutting and burning, cracking and burning was traditionally used for the preparation of eel otoliths. The whole otolith was placed on a scalpel blade and placed into the blue part of a moderate Bunsen flame for up to 30-60 seconds, by which time they have darkened to a slate grey colour. This will follow initial darkening from light brown to black. It is important that the flame is not too hot as incomplete, or patchy, incineration can occur.

[NOTE: Wear safety glasses when burning otoliths]

The otolith is very delicate after being burnt and must be handled with care. It is placed on a glass slide convex side down and pressure is gently placed on both ends of the otolith using the fine pointers and the otolith splits in two along the transverse axis. (see Chapter 2: glossary) Alternatively, the burnt otolith can be placed concave side down and gentle pressure applied to the centre of the otolith, to split it in two parts. The split halves are placed on a microscope slide and each half mounted in silicone as described above.

5.4.7.2 Advantages of cutting and burning

Cutting the otolith before burning produces a flat surface along the correct transverse axis for mounting against the glass slide, allowing for consistently higher quality otolith preparation (Graynoth, 1999). This in turn makes the otoliths easier to age and facilitates better quality digital images. It removes the problems associated with the traditional burning and cracking method, reducing the occurrence of irregular surfaces on the reading plane. Overall it is a more precise method and can result in reduced sample damage. It is recommended that this variation should be considered as a new standard technique.

5.4.7.3 Disadvantages of cutting and burning

The burning process is somewhat subjective and variable depending on the heat of the flame, the thickness of the blade, moisture content of the otolith and the operators experience. There is a danger of the otolith breaking or shattering and also a danger of charring and smoke damage obscuring some of the annuli.

Once the otolith has been burned it can't be used for other analysis such as micro-chemistry or genetics.

5.5 Whole Otolith Clearing – in toto

Clearing whole otoliths (in toto) is still used to age young eel populations (Panfili *et al*. 1994a; Cullen & McCarthy, 2003; Melià *et al*. 2006).

The whole right and left otoliths are immersed into rosemary essential oil or 96% ethanol (in order to enhance the visualization of the annuli) and read under a binocular microscope with reflected light against a dark background.

Other media used to immerse otoliths were: propandiol, camomile essential oil or less so now, creosote oil.

5.5.1 Advantage

A quick method that is efficient for young eels.

5.5.2 Disadvantage

This method is not appropriate for slow growing eels and it underestimates age of eels older than about 5 years old (Vollestad & Næsje 1988; Panfili *et al.* 1994b). Care should be taken to observe health and safety aspects of clearing agents.

6 Digital Image Capture

Capture of images of otoliths using digital cameras mounted on microscopes is increasingly being used in both age and growth analysis of eels. It is particularly useful for back-calculated growth analysis as well as an inter-calibration tool for the quality assurance of eel ageing. A standard protocol and data format for image capture has not as yet been developed but it is seen as an important area for development in otolith studies across many species including eel (Pierra *et al*. 2005). Described here is the more recent experience of participants of the Workshop and some guidelines for current best practice.

6.1 Image Format

Saving images in .tiff file format provides lossless image compression and is therefore recommended. More precisely, 8 bit tiff format is further recommended as it is more universally compatible with general software programmes.

Jpeg is another option providing smaller file size but will result in a lower quality image and can introduce artefacts due to the compression method used. Deterioration over time and with sequential opening also occurs with jpeg files.

An image storage system should be developed linking a unique id between the image file and the associated eel data. A link to a file can be included within a database but caution should be taken to avoid image loss due to file relocation.

6.2 Colour or Grey Scale

Certain preparation or staining techniques benefit from the image being taken in colour. However, for general purpose ageing and growth measurements, clearer contrast and reduced file size grey scale imaging should be used in preference.

6.3 Resolution of picture

Images should be between 500 and not more than 2048 pixels. If the image is in black and white and 8-bit, 256 grey levels is sufficient as the human eye can only differentiate between about 60 shades of grey (Troadec & Benzinou, 2002). If a colour image is captured it should have the same number of pixels and 24-bit.

Printing standards for publishing in scientific journals or good quality reports require 300dpi images.

6.4 Orientation

Try to get the full otolith face in the frame of the image (the whole structure should appear on the picture).

For the sagittal plane section of ground and polished otoliths, keep the same position of the structure in the picture (i.e.: rostrum always on the left end side). This may be more difficult with burnt and cracked otoliths but its good practice to standardise the orientation. This facilitates identifying ring continuity and may benefit future image analysis of morphological features.

If the section is curved and requires multiple focus images for reading, use a composite image of different focal planes.

6.5 Magnification

For an older system where there is no calibration synchronisation between the microscope and the software available, use the same magnification for the whole picture data set.

6.6 Calibration

For new systems, synchronise the calibration of the picture with the magnification of the microscope (parameter: number of pixels per micron). Use click magnification on stereoscopes to facilitate calibration.

6.7 Light

Ground otoliths are generally analysed using transmitted light and burnt and cracked otoliths require reflected light. Light conditions should be adjusted on both microscope and software to achieve the greatest contrast before the image is captured.

Post processing of digitally acquired images should be undertaken with extreme caution to avoid introducing erroneous artefacts in the image.

Using both images and otolith samples to estimate age (to avoid problems with variable focal planes) provides the greatest accuracy and is useful to identify uncertain or unclear structures or images.

In some recent adaptations, a polarising filter is used with transmitted light instead of staining the otolith (D. Evans, pers. comm..) and in Sweden transmitted light is used in combination with differing levels of oblique angled reflected light (Wickström, pers. comm.).

A combination of varying the focus and different levels and types of stain and light achieves the optimum visualisation of the annuli and the best readings of age.

7 Guidelines for interpretation

7.1 Reading transect

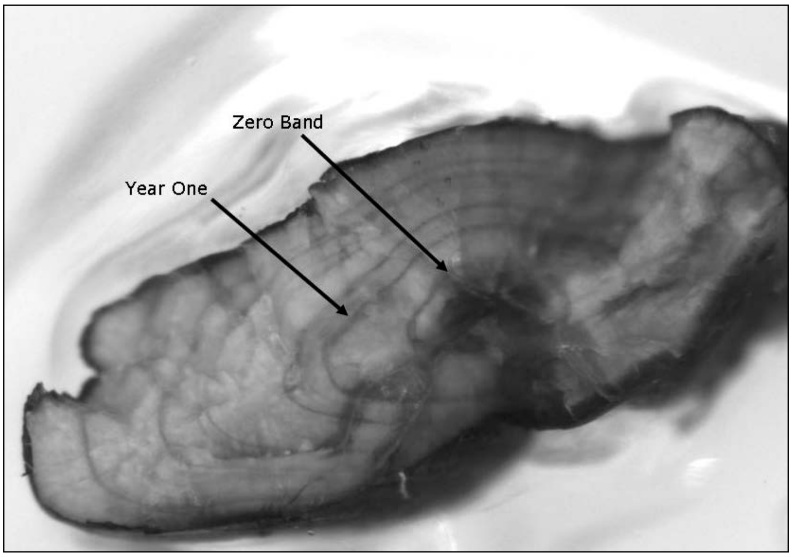
Regardless of the plane of view, the reading transect begins from the centre of nucleus to the furthest edge of the otolith. The path of the transect is dependent upon the quality and characteristics of the otolith being aged, but recommendations are made to read along the proximal plane, in the case of burning and cracking, to intersect each annulus at its widest point. In the case of ground and polished otoliths, it is possible that, in order to obtain a complete reading transect, annuli can be traced around the sagittal plane to areas that offer an increased distinction, in order to obtain a complete reading.

7.2 Zero Band

The ageing of the eel for the continental phase, has traditionally commenced from the first clearly marked band outside the nucleus. This 'zero' band is assumed to be the beginning of continental growth in the eel, equivalent to the total length for the glass eel (Moriarty, 1983; Poole *et al*., 2004). The next annulus is considered to be the end of the first year of growth (Figure 7.1).

For the determination of the continental age of the eel, counting of annuli begins after identification of the zero band, with the following annulus representing the first years winter band in the continental phase, a process considered in more detail within section 6.2 of the ICES WKAREA 2009 report, but has been summarized below:

- The 'zero band' is assumed to be the end of the oceanic/metamorphosis phase and the beginning of continental growth in the eel, equivalent to the total length for the glass eel, resulting in the next annulus to be considered as the end of the first year of growth.
- In the case of ambiguity in the identification of zero bands, the radius from the centre point of the nucleus to the assumed zero band proximity is on average 170µm for *A. anguilla*. In addition, for ground and polished otoliths, the expected diameter of the zero band has an average value of 340µm. In *A. rostrata*, the radius and circumference have average values of 120µm and 240µm respectively.
- Care must be taken to identify check marks which are often observed between the nucleus edge and the zero band, so to define the zero band with confidence.



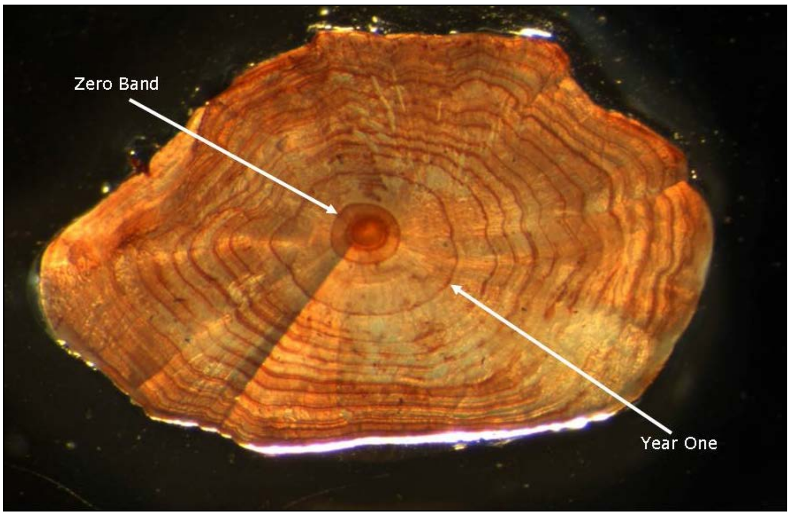


Figure 7.1: Upper image: A burnt and cracked otolith (transverse plane) from *A. anguilla* showing the location of the zero band and first winter annulus.

Lower image: A ground, polished and stained otolith (sagittal plane) from *A. anguilla* showing the location of the zero band and first winter annulus.

7.3 Annulus identification

A year's growth consists of both translucent (winter growth) and opaque (summer growth) zones. Accurate analysis requires a confident distinction to be made between;

- clearly visible as a bold growth check to be considered an annulus,
- in the case of sagittal plane ground otoliths the growth checks should, in theory, be visibly continuous around the otolith to be annuli
- in the case of burnt and cracked otoliths, the growth checks should be clear and continuous, at least down the inside edge of the otolith

- false annuli are usually of lesser strength to the annuli, are not continuous and/or merge with other checks.
- eel growth is highly variable and therefore it is difficult to predict 'normal' patterns of annual growth to facilitate the identification of false annuli. Checks that appear too close to the neighbouring checks may be false annuli and should be treated with caution. For additional information see Graynoth (1999).

The counting of consecutive annuli will provide an estimation of age, provided that check marks are successfully identified and excluded from the final count. The distinction between annulus and check marks are defined as such in the glossary and discussed further in section 6.3 of ICES WKAREA 2009 report.

It is important, but difficult, to identify and discount the inclusion of false annular growth checks, often caused by stress associated with quarantine/marking and tagging of stocked glass eel, periods in aquaculture, or due to natural stresses and growth variability in the wild.

7.4 Otolith Reading Calendar

For both *A. anguilla & A. rostrata* the following recommendations are made:

- The age reference date is set as 1st of January.
- Age is estimated based on the counts of winter rings.
- The term '+' growth can be used as an indication of additional growth but this should not confuse the calendar age of the eel.

For yellow eels, it is recommended that attention be drawn to the timing of capture and local conditions. Depending on the growing season of the capture location, the appearance of winter annuli on the otolith will vary. In the early part of the year the outer most winter annulus is generally not apparent until the summer growth begins in the new year. This will vary between years and between locations. Therefore, an additional year should be added to these eels sampled early in the year after January 1st in order to account for this undifferentiated annulus from the outer margin of the otolith.

For silver eels, flexible interpretation in some local situations may be required. Silver eel traditionally migrate as an annual migration 'cohort'. This 'cohort' receives its age from the next January as the eels have completed their annual growth period and we assume a putative annulus on the outer margin of the otolith (e.g. silver eels migrating in October 2008 take their age from the January 2009).

In the Northern European region (e.g. Scandinavia) the silver eel run may cease prematurely due to cold temperatures in the winter months and recommence in the following spring, then both portions of the migration should be referenced to the January in the middle of the run. Similar delayed runs can occur due to low water and/or hydropower barriers that prevent migration.

8 Inter-calibration

The ICES Workshop 2009 carried out a preliminary inter-calibration exercise using 21 Swedish otoliths of eels of known age where the 25 readers were of varying experience and had varying levels of knowledge on the supporting eel data and information. This indicated considerable variation and the results were only used to support the workshop discussions on setting up reading protocols and a formal inter-calibration.

The ICES Workshop 2011 carried out further intercalibration and identified a number of issues that influenced the results, including the non-availability of meta-data for each otolith. Recommendations are made in the workshop report for future reader training and intercalibration and for reference collections of otoliths of both eels of known age and those where the age is not verified by independent means (eg. marking or date of stocking).

Svedang *et al*. (1998) using otoliths of known and unknown age from fresh, brackish and marine waters found that the accuracy of age estimation was uncertain and deviations from the correct age were dependent on reader, locality and fish age. Also while consistency within readers was high, a drift over time was noted.

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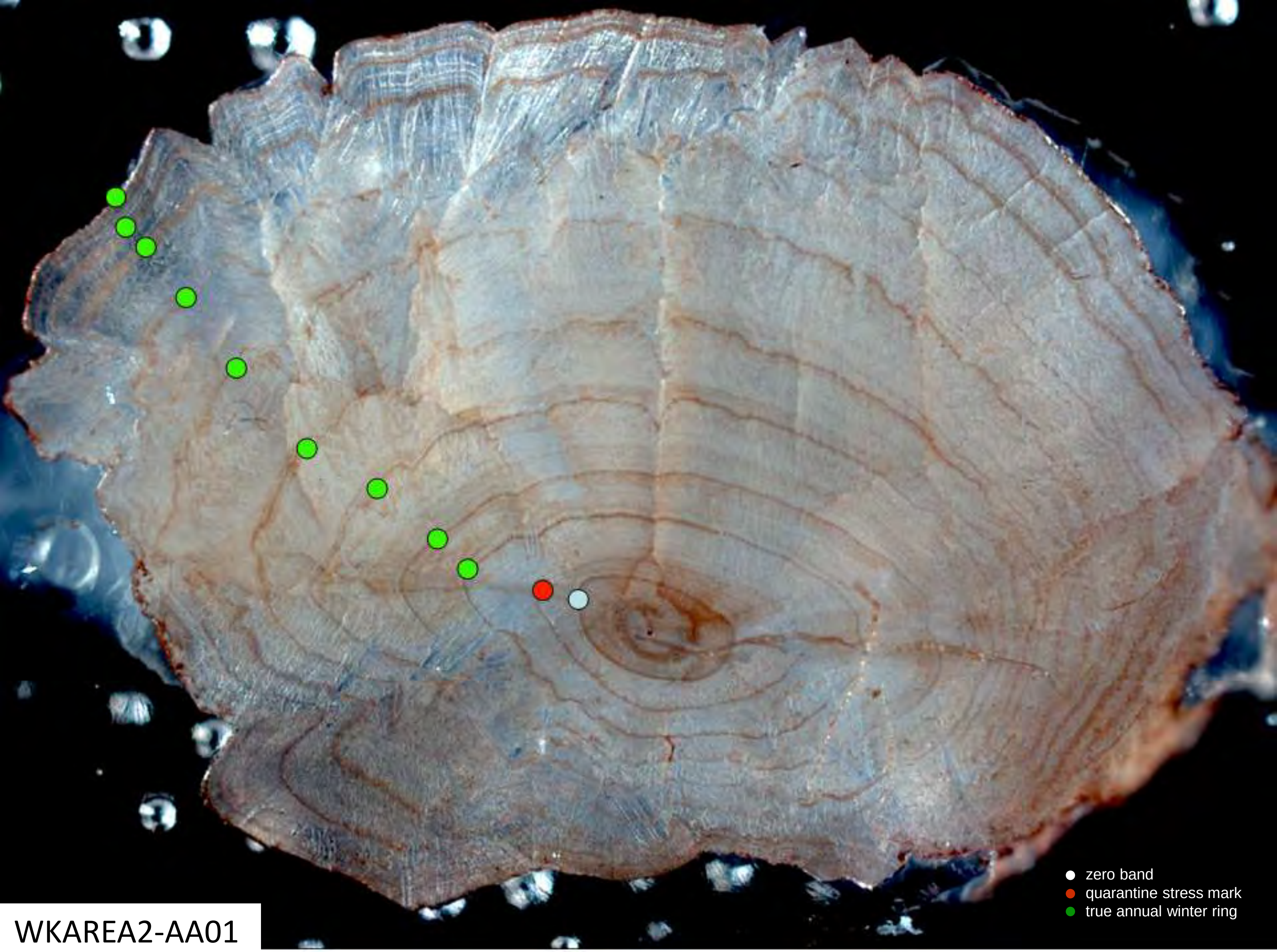
10 Image Library

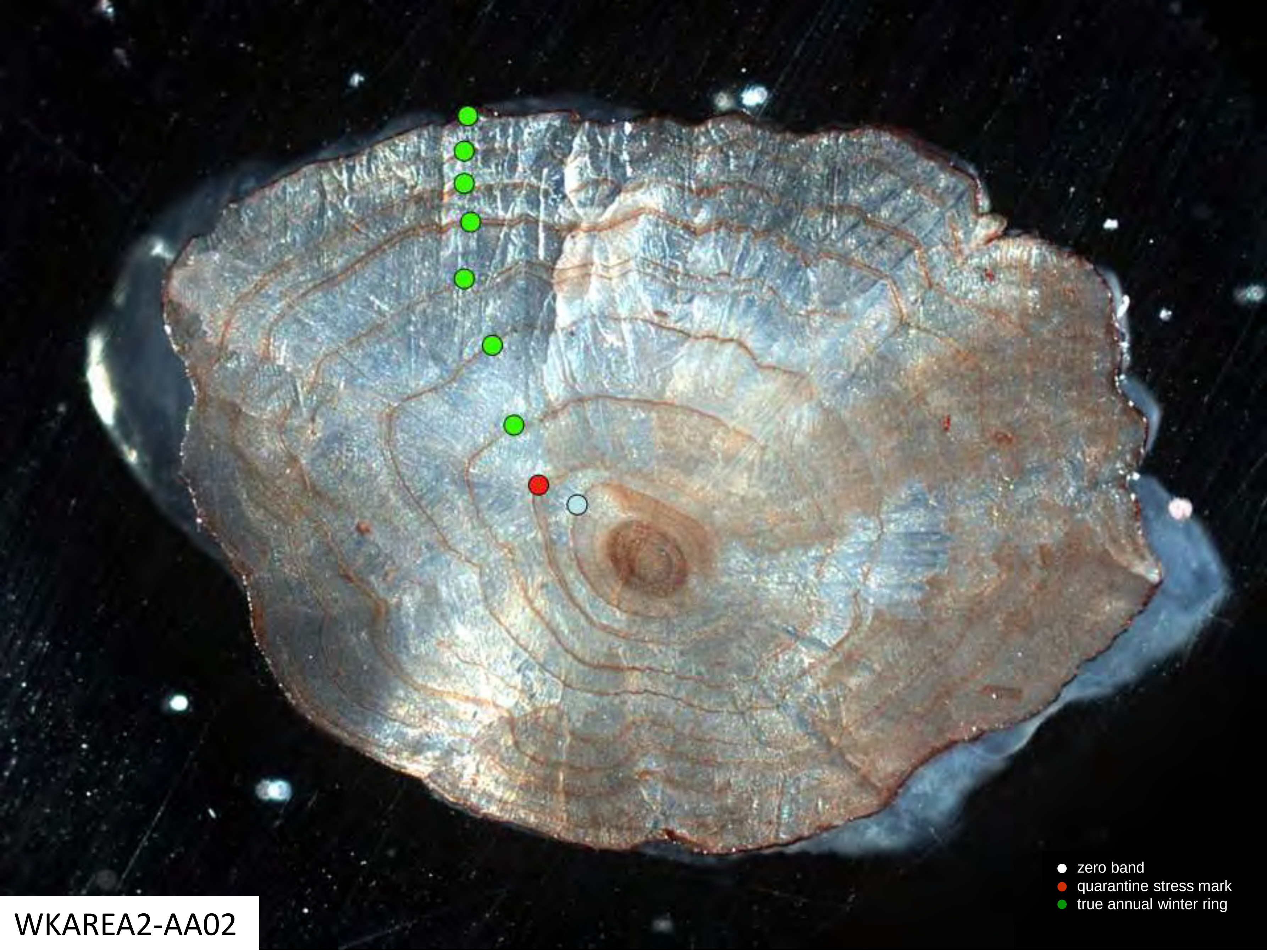
The ICES Workshop 2009 collated a demonstration set of images so as to give examples of the main otolith preparation methods and the main structures visible on the otoliths and these are available in the 2009 Version 1 of the manual.

In 2011, the Workshop collated a new set of reference images of otoliths from eels of known age, and eels where there was good agreement of the age. Version 2 of the manual refers the reader to the reference collection of images and their accompanying meta-dataset.



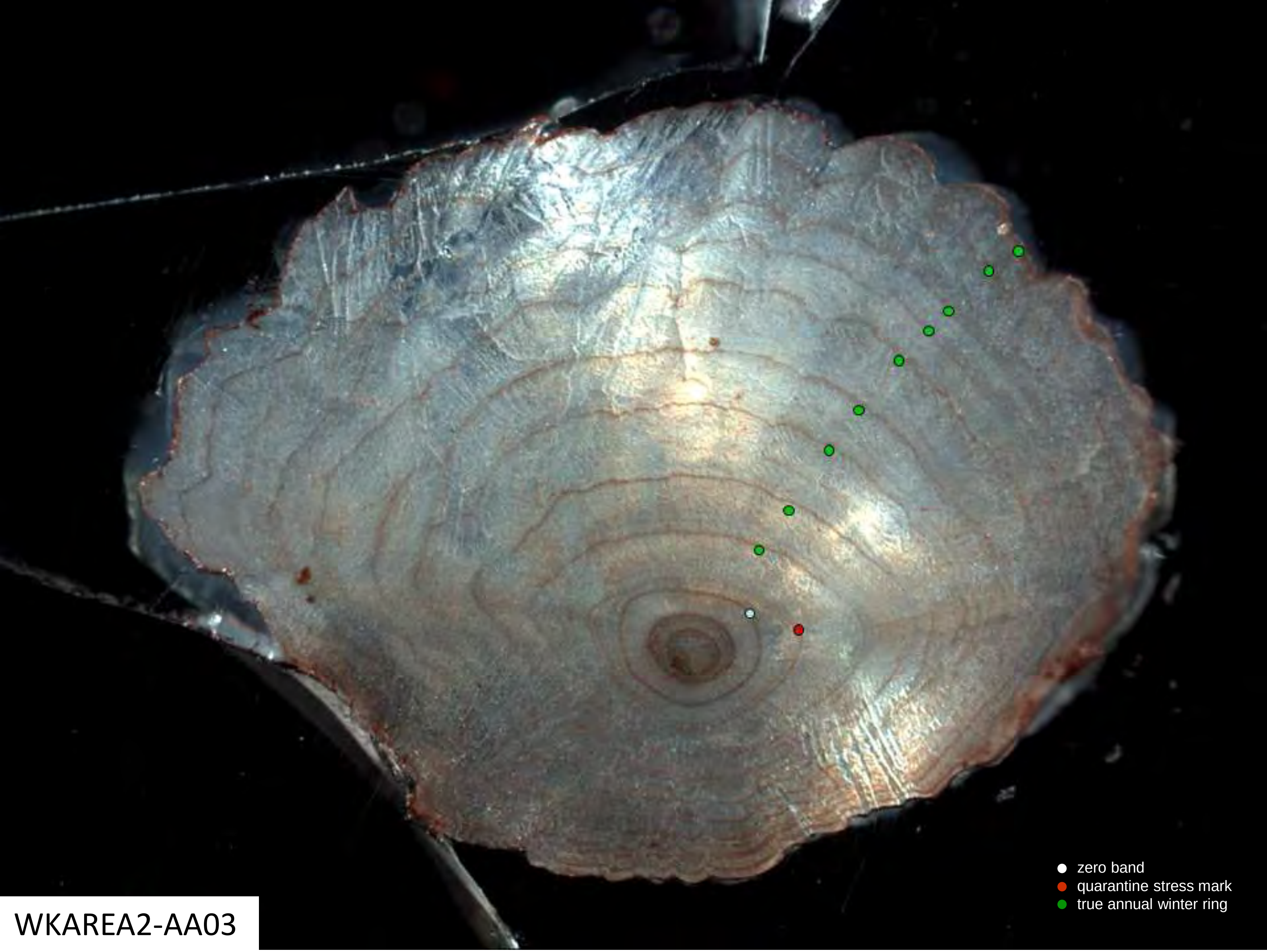
WKAREA2-AA01

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2006	5	yellow eel	503	124	female	ALC-marked and stocked as elver in 1997	9	



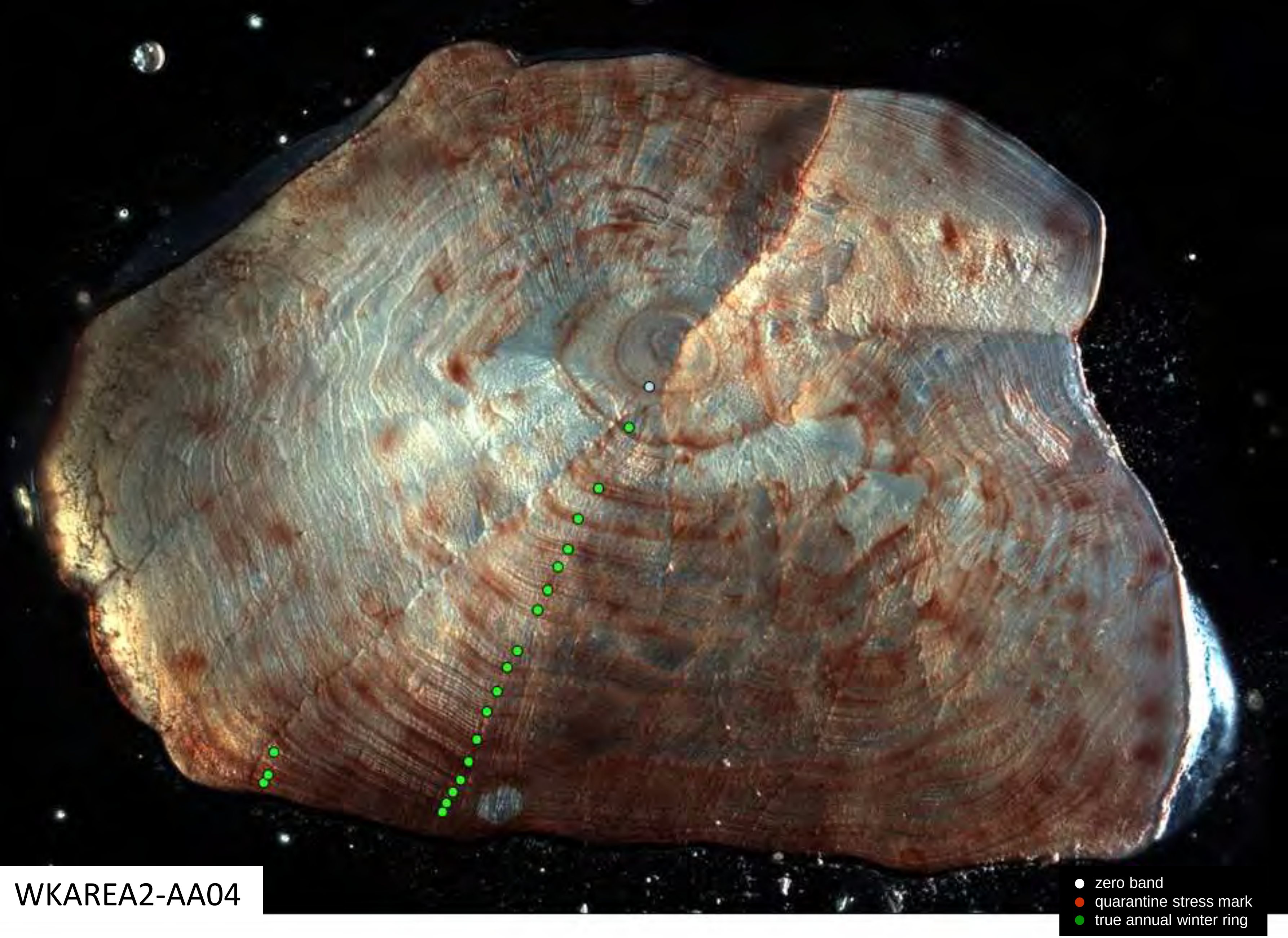
WKAREA2-AA02

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2004	6	yellow eel	70	358	female	ALC-marked and stocked as elver in 1997	7	

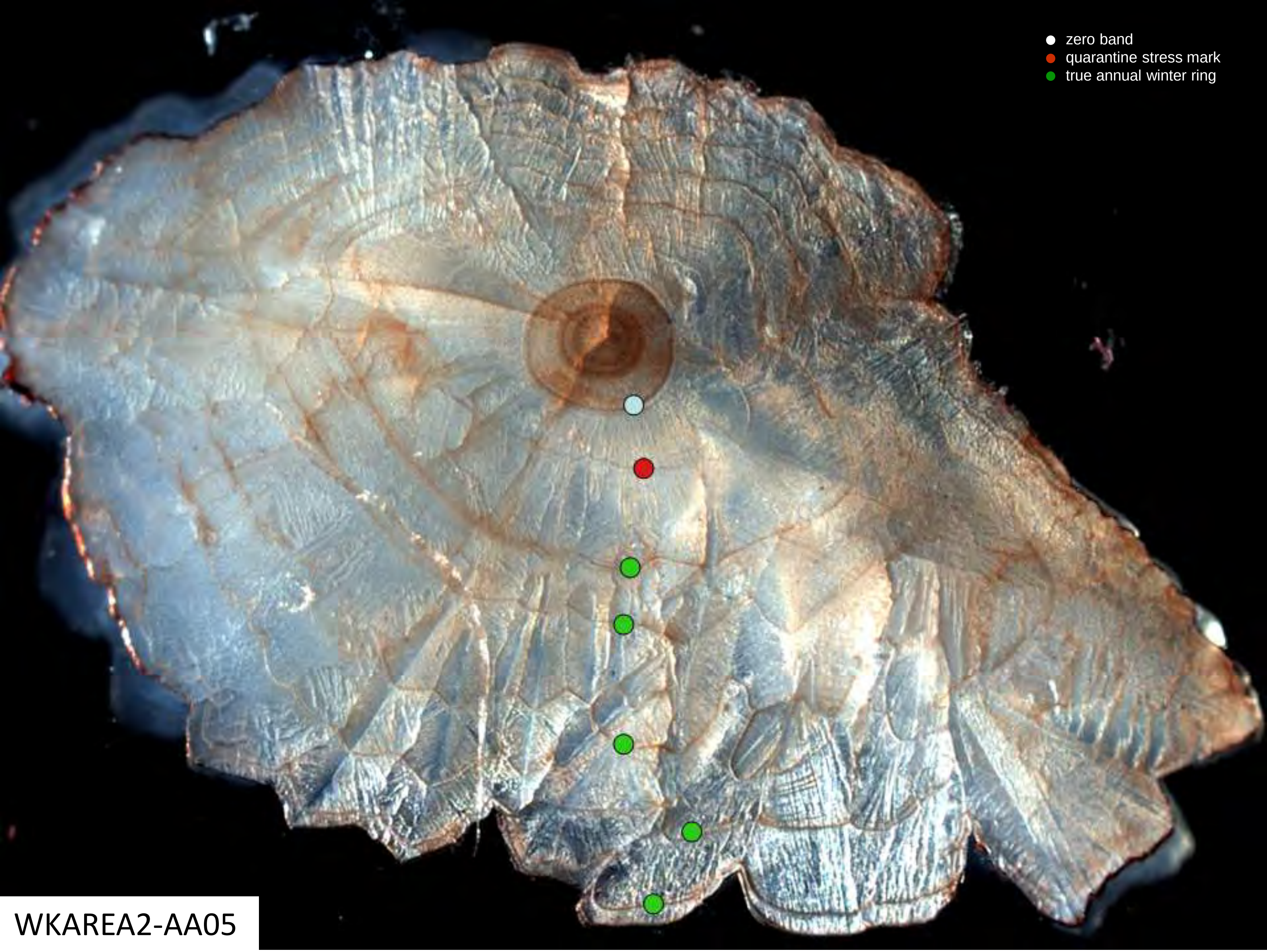


WKAREA2-AA03

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2006	5	yellow eel	415	99	female	ALC-marked and stocked as elver in 1997	9	

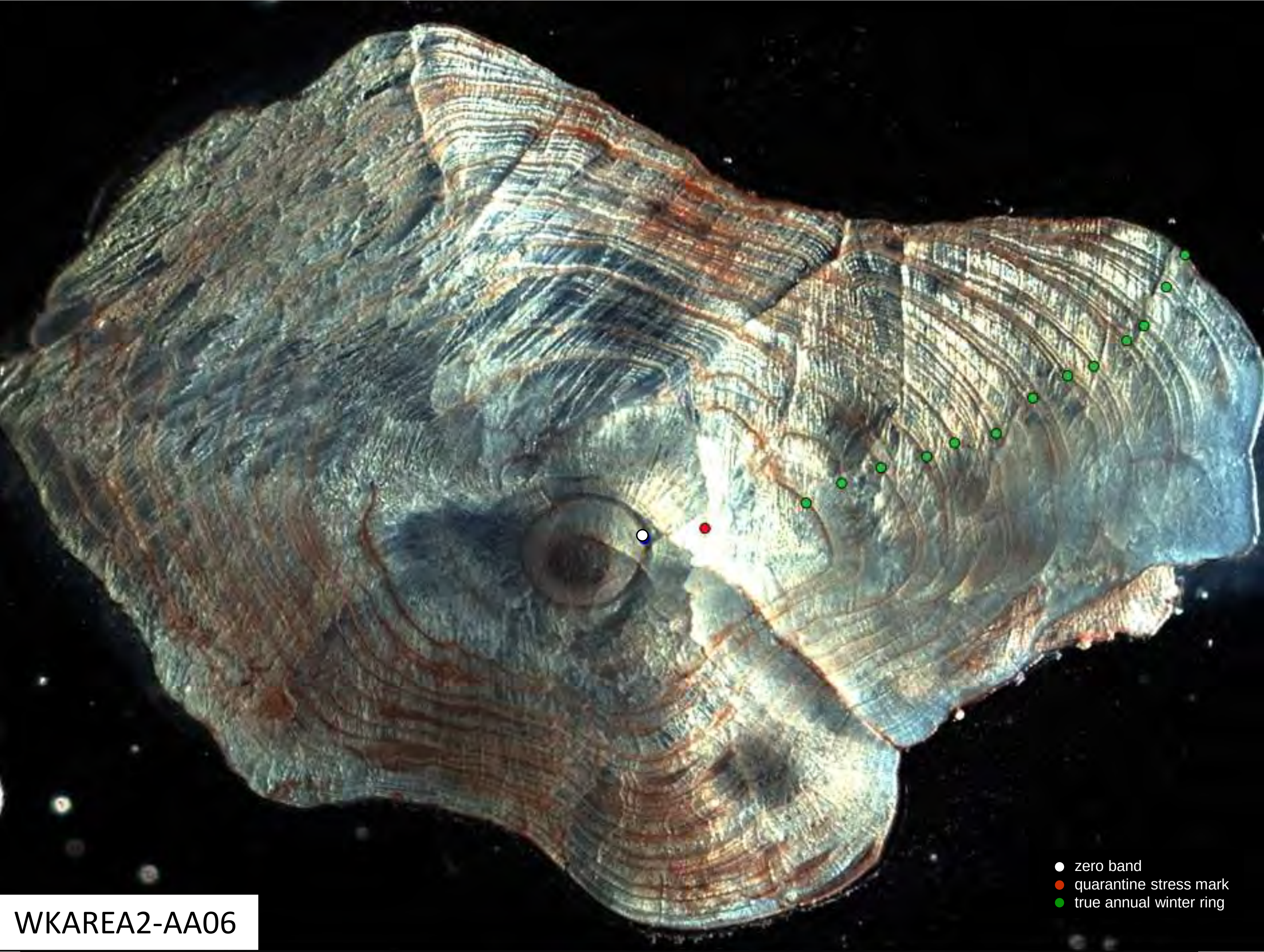


country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Fardumeträsk	2009		silver eel	834	909	female	stocked as elver in eel free lake in 1989	20	position of winter rings is uncertain



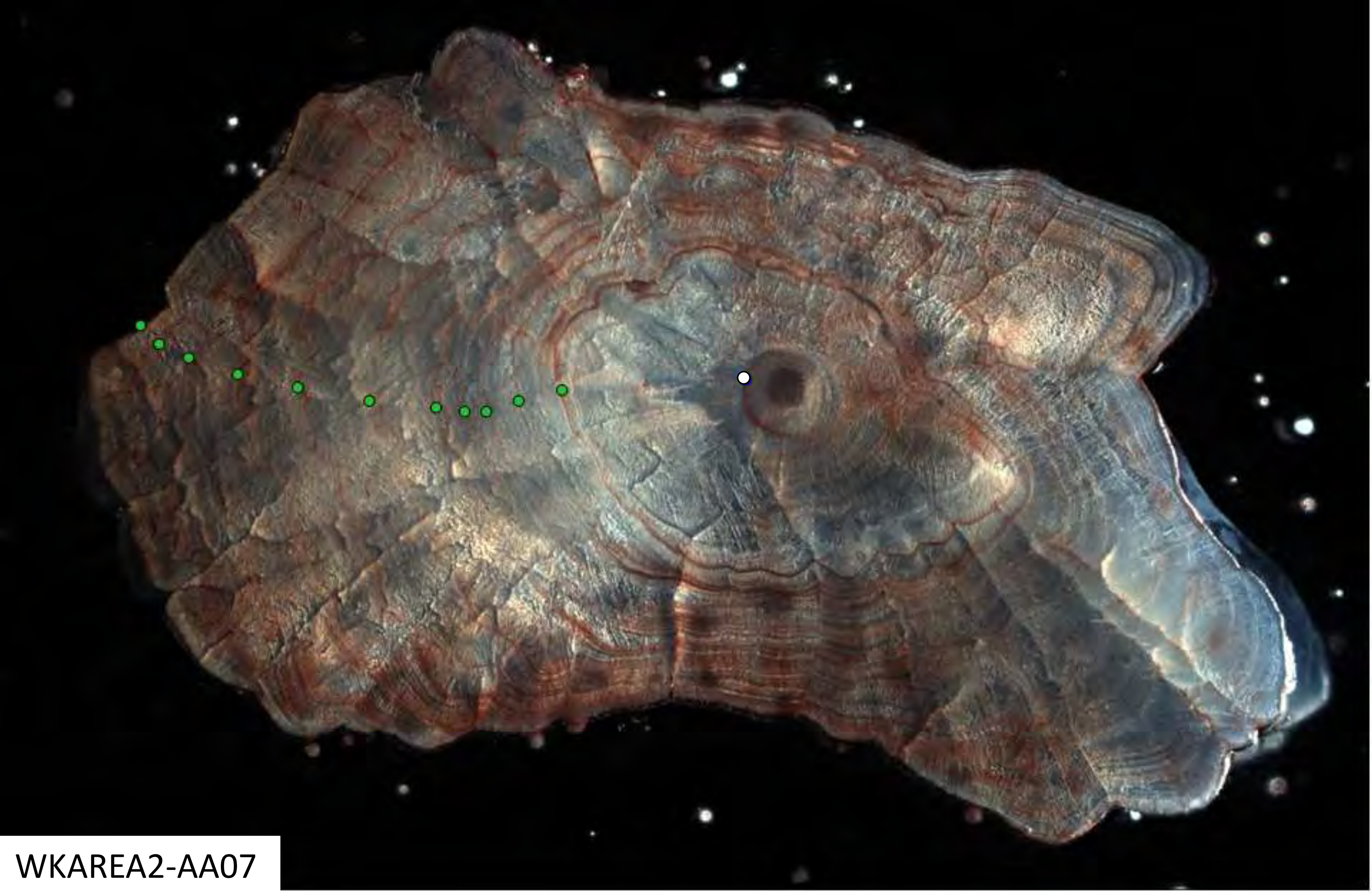
WKAREA2-AA05

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2002	6	yellow eel	478	161	female	ALC-marked and stocked as elver in 1997	5	



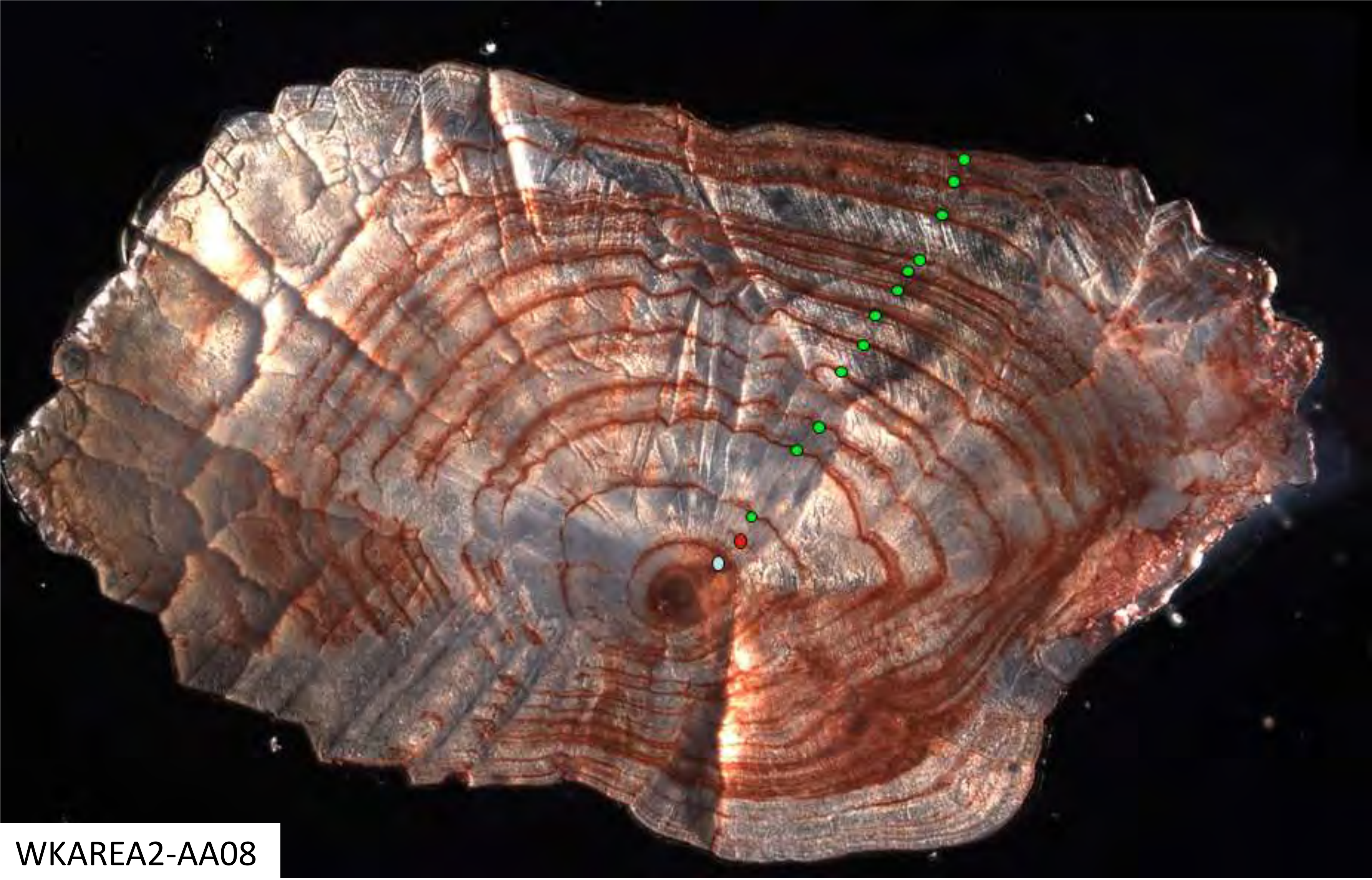
WKAREA2-AA06

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2010	5	yellow eel	488	182	female	ALC-marked and stocked as elver in 1997	13	

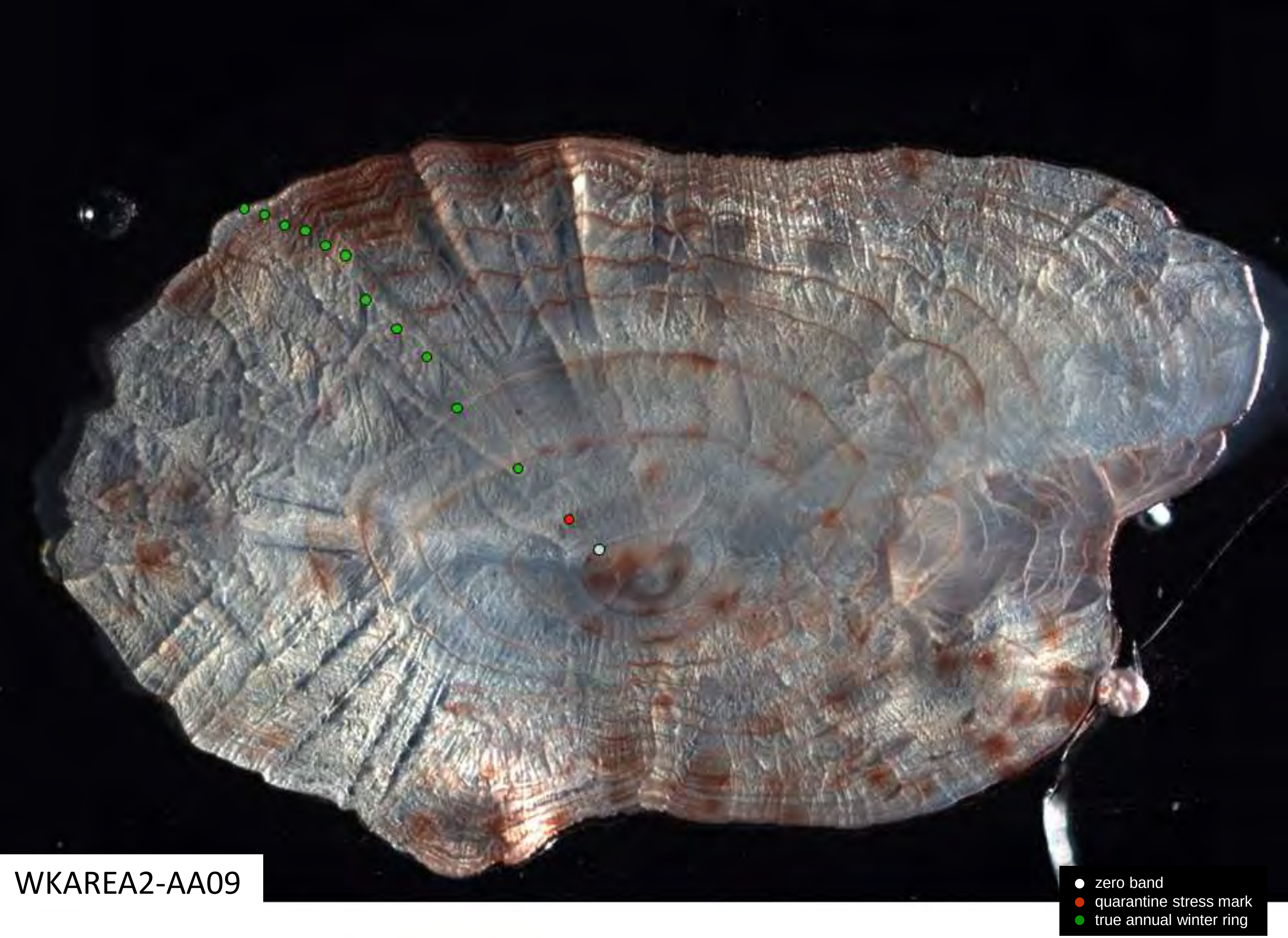


- zero band
- quarantine stress mark
- true annual winter ring

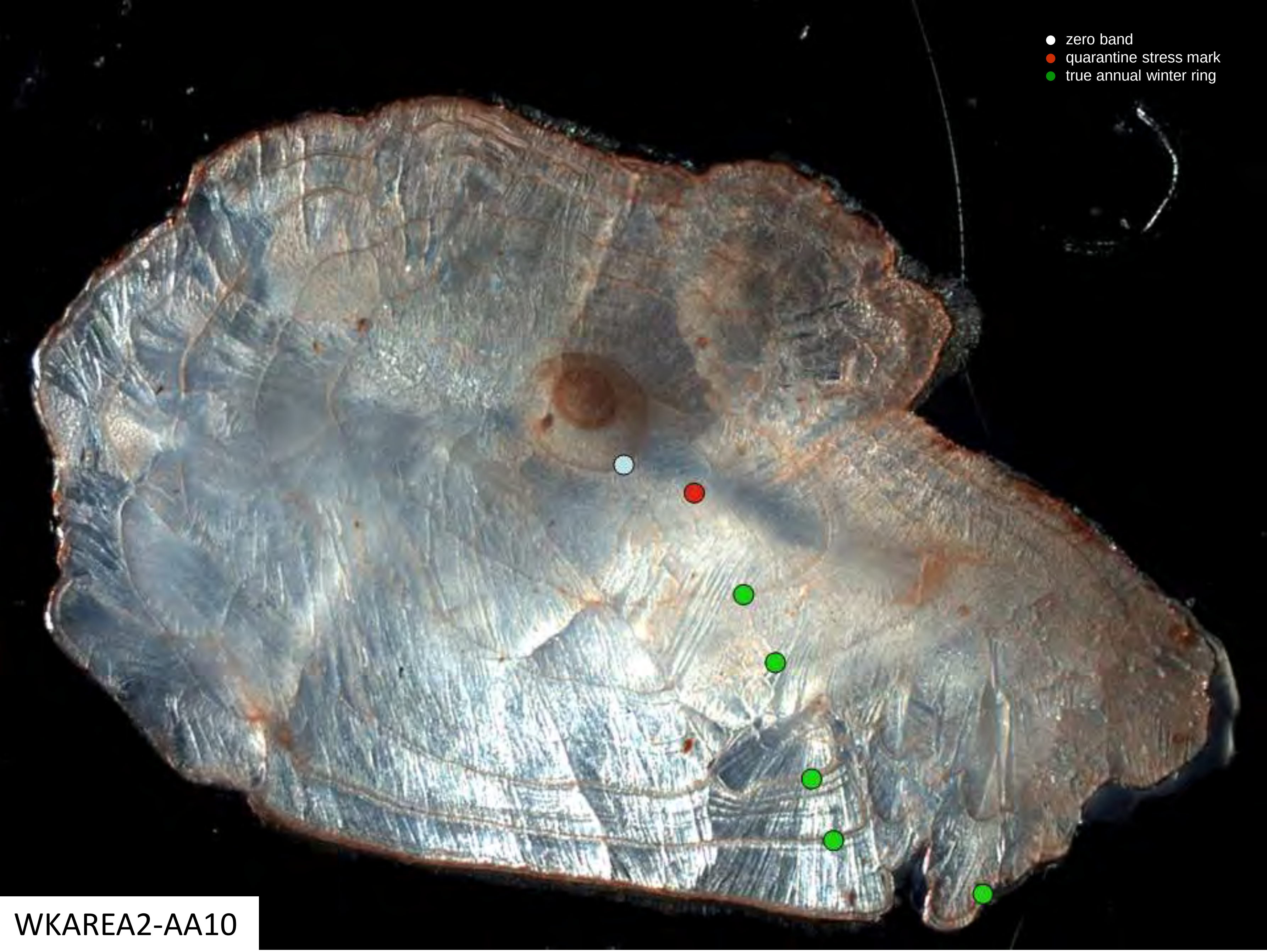
country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Ymsen	2010	5	yellow eel	743	930	female	individually pit tagged and stocked in 1999 or 2000	unknown	11 years (uncertain)



country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2009	6	yellow eel	553	186	female	ALC-marked and stocked as elver in 1997	12	

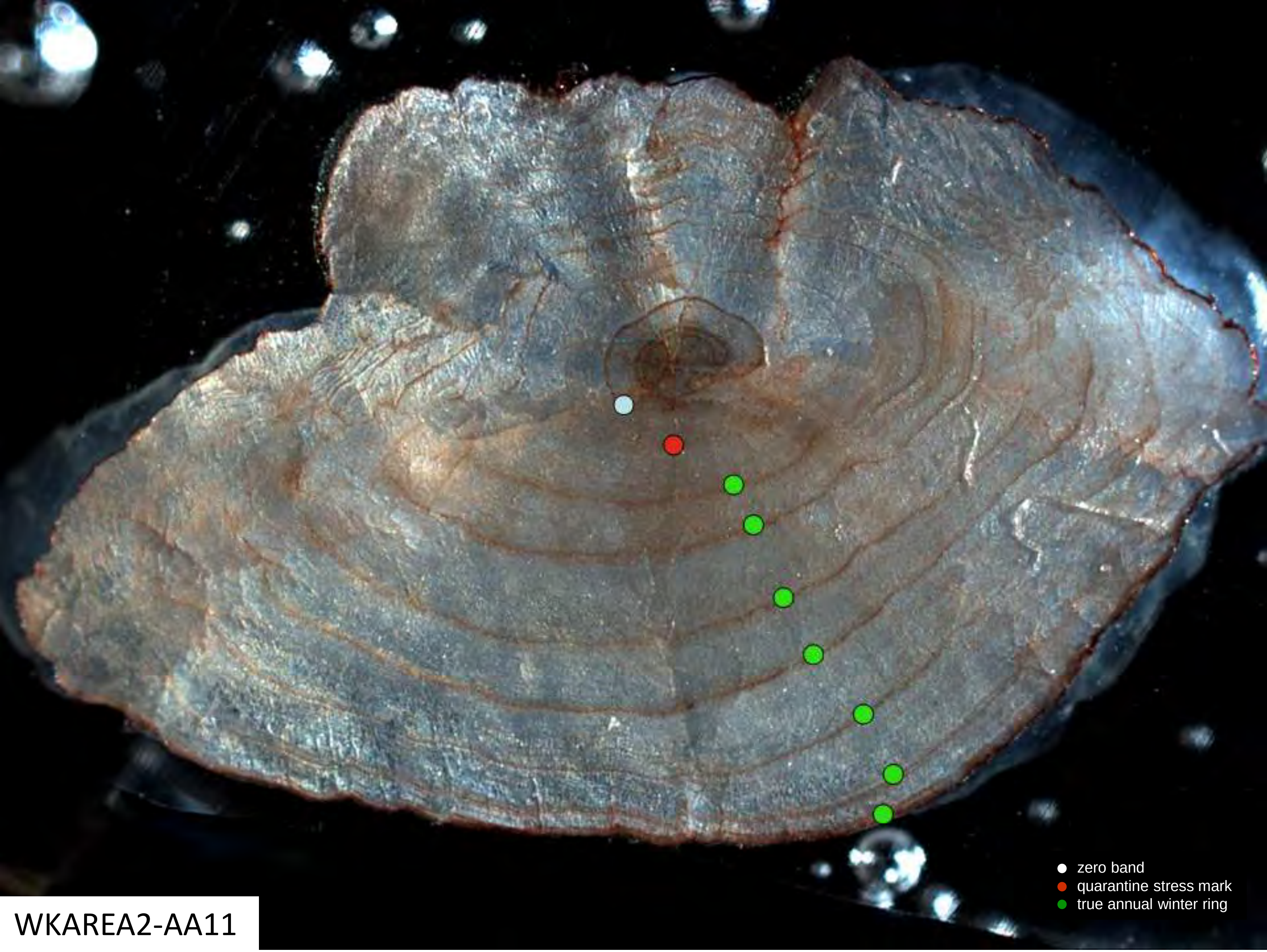


country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	484	221	female	ALC-marked and stocked as elver in 1997	11	



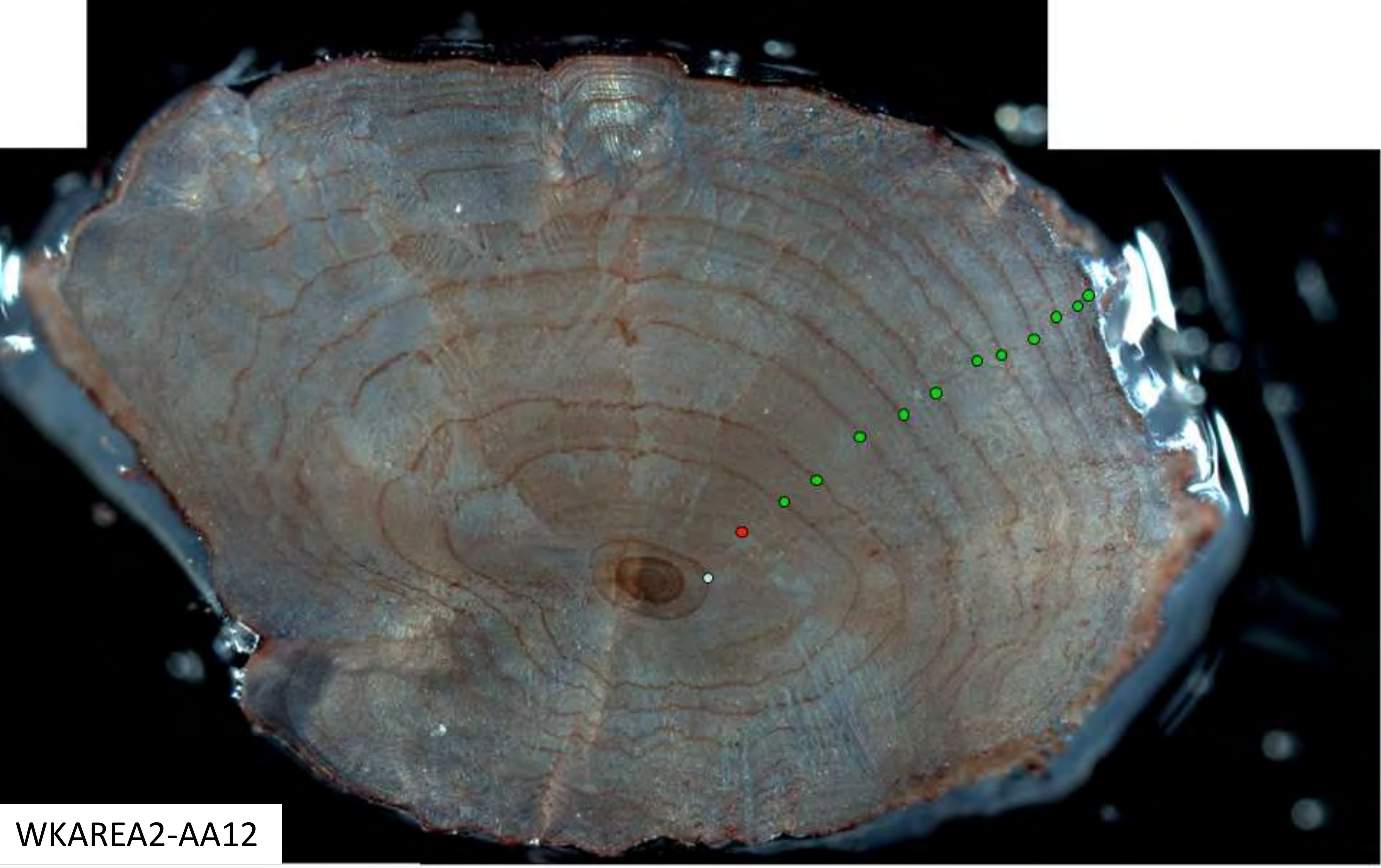
WKAREA2-AA10

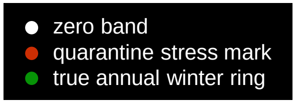
country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2002	6	yellow eel	454	149	female	ALC-marked and stocked as elver in 1997	5	



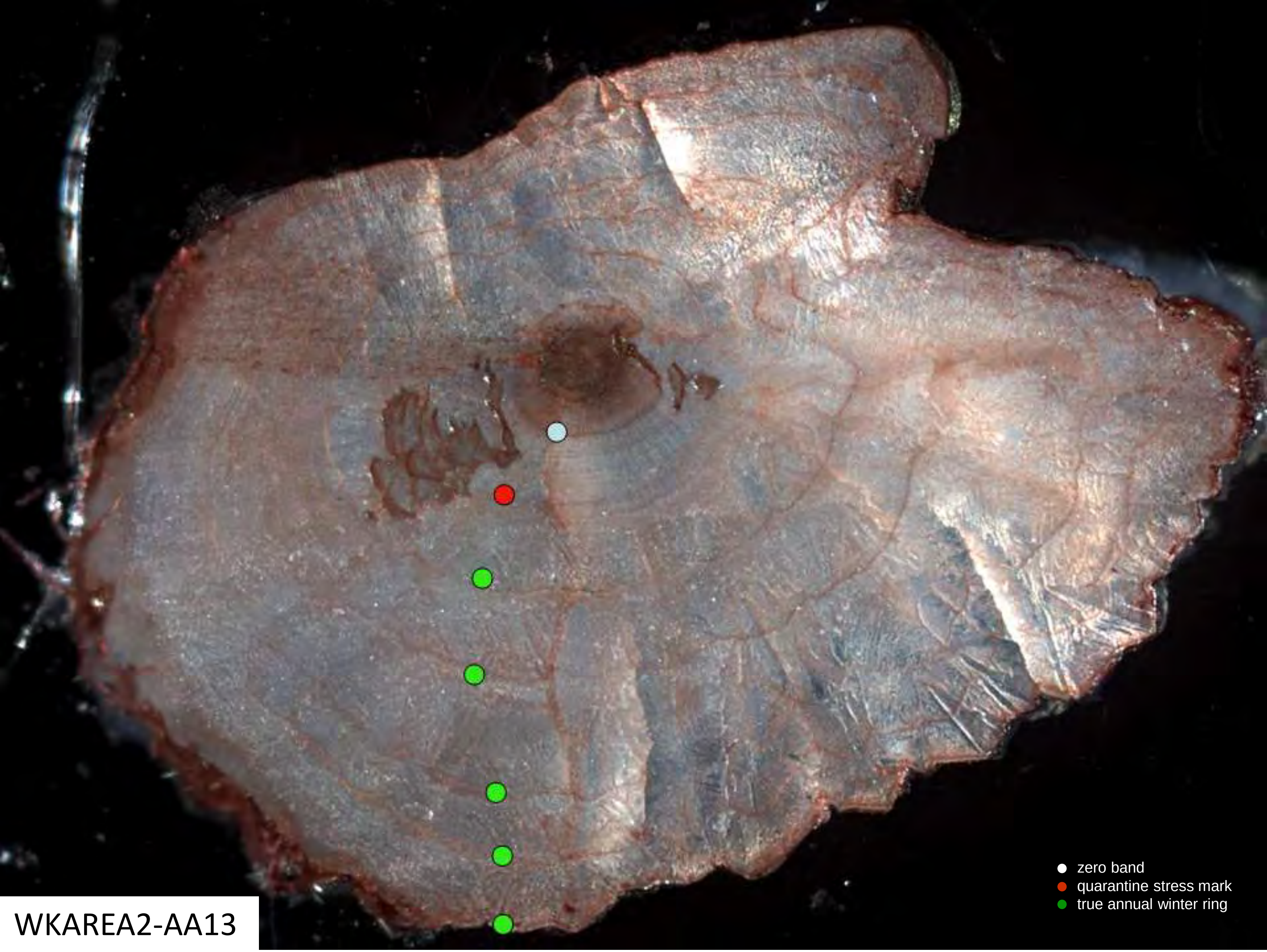
WKAREA2-AA11

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2004	5	yellow eel	440	140	female	ALC-marked and stocked as elver in 1997	7	





country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	614	360	female	ALC-marked and stocked as elver in 1997	11	



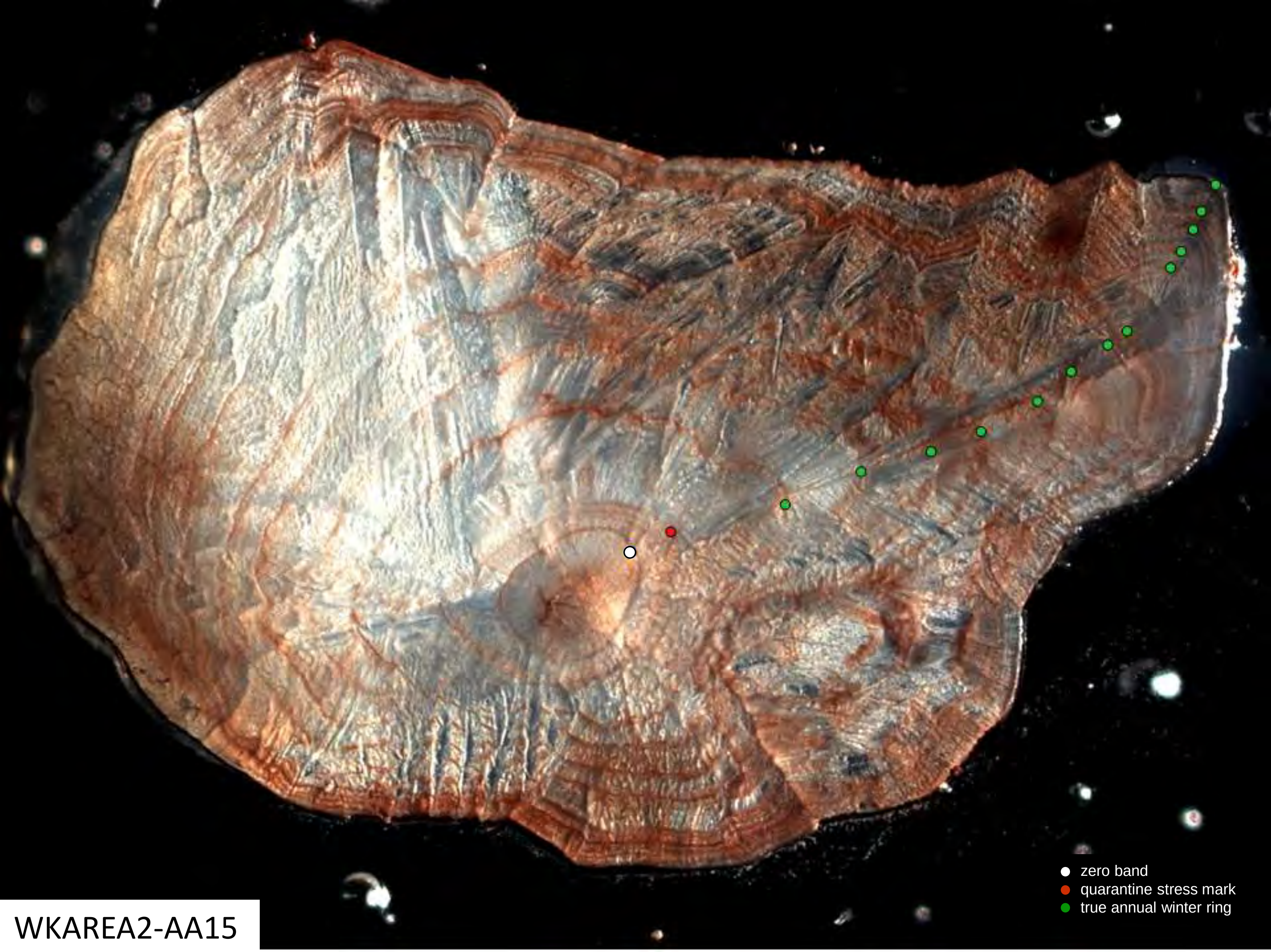
WKAREA2-AA13

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2002	6	yellow eel	466	152	female	ALC-marked and stocked as elver in 1997	5	

WKAREA2-AA14

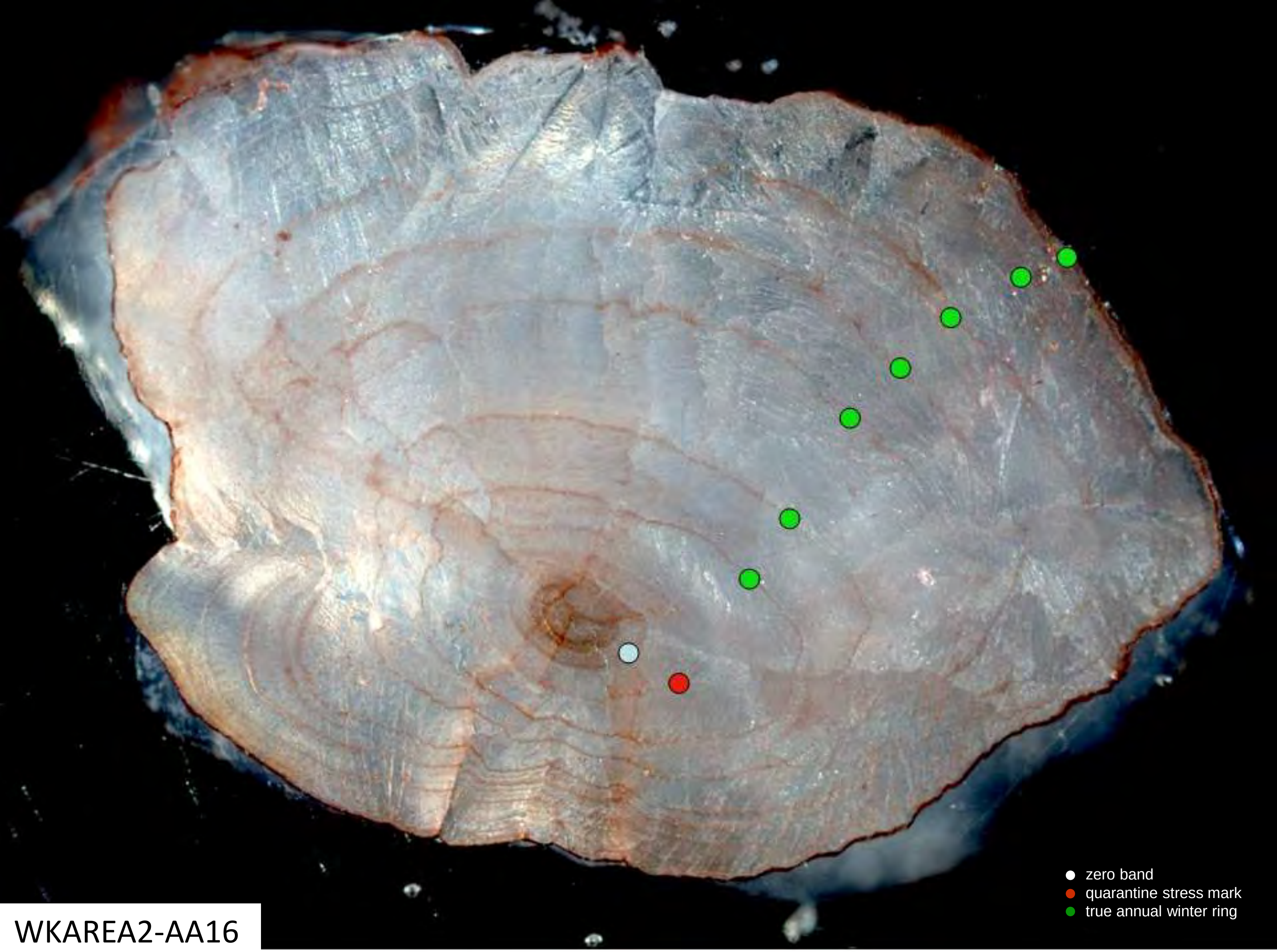
- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2006	5	yellow eel	532	226	female	ALC-marked and stocked as elver in 1997	9	



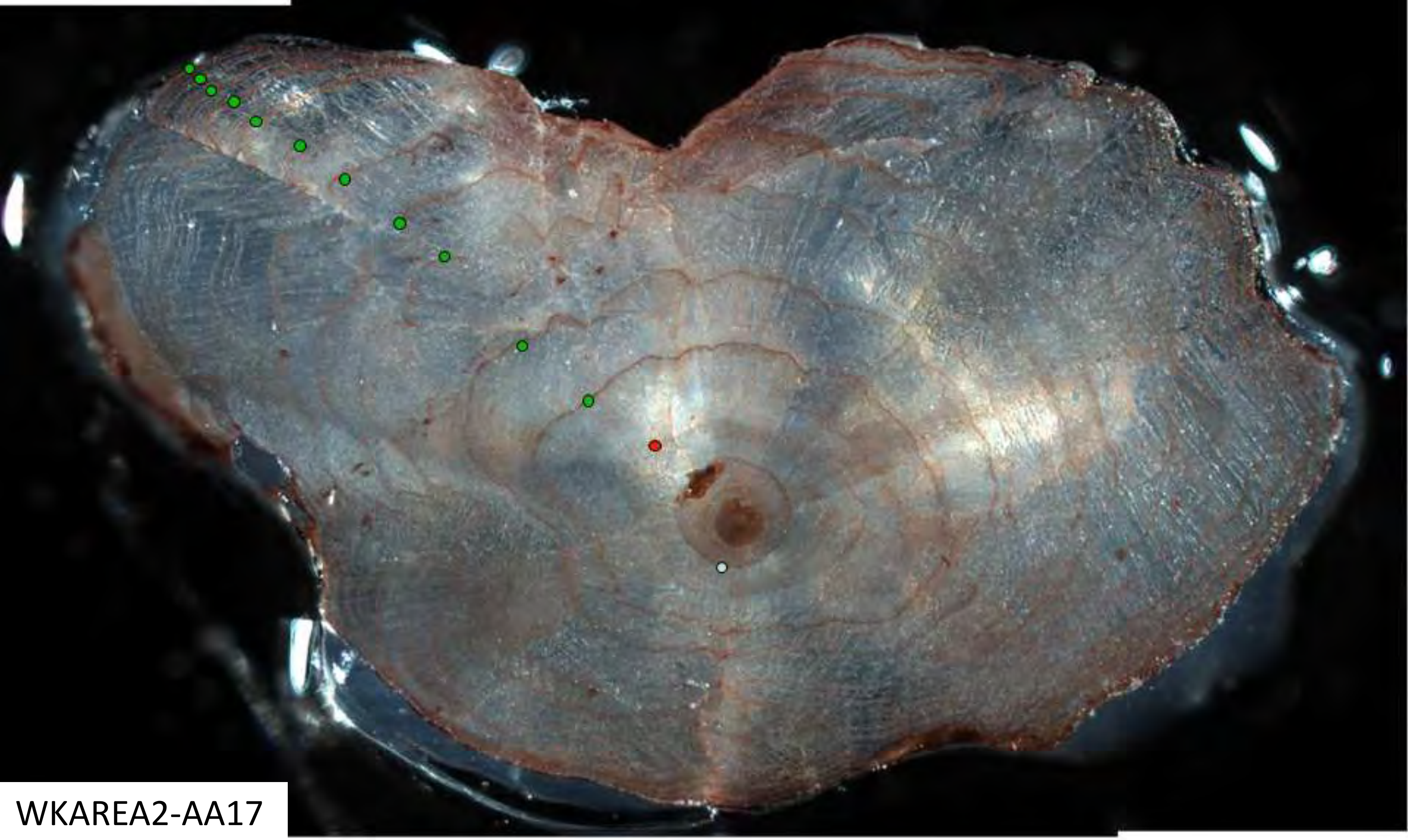
WKAREA2-AA15

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2010	6	silver eel	471	197	male	ALC-marked and stocked as elver in 1997	13	

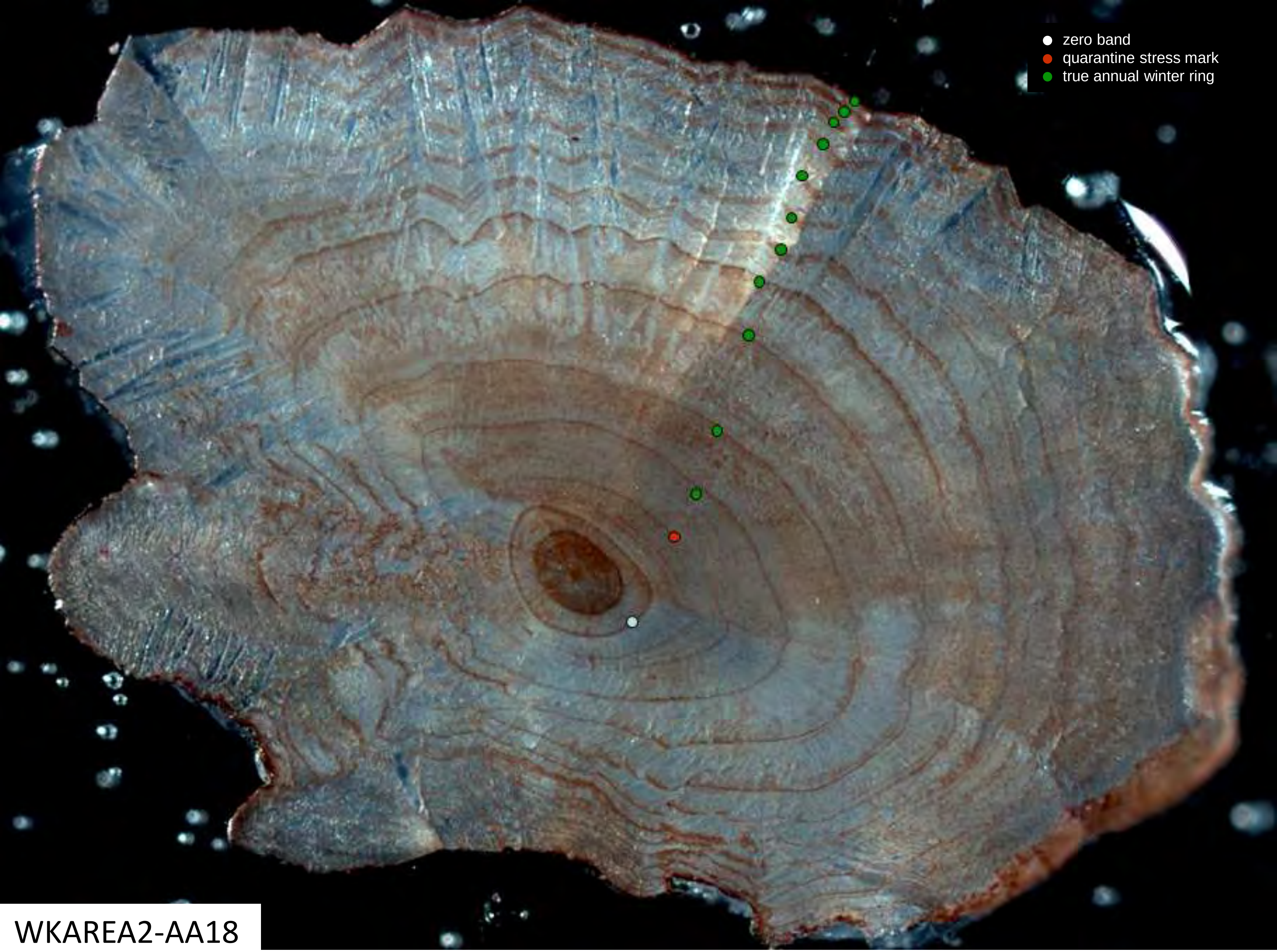


WKAREA2-AA16

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2004	7	yellow eel	444	124	female	ALC-marked and stocked as elver in 1997	7	

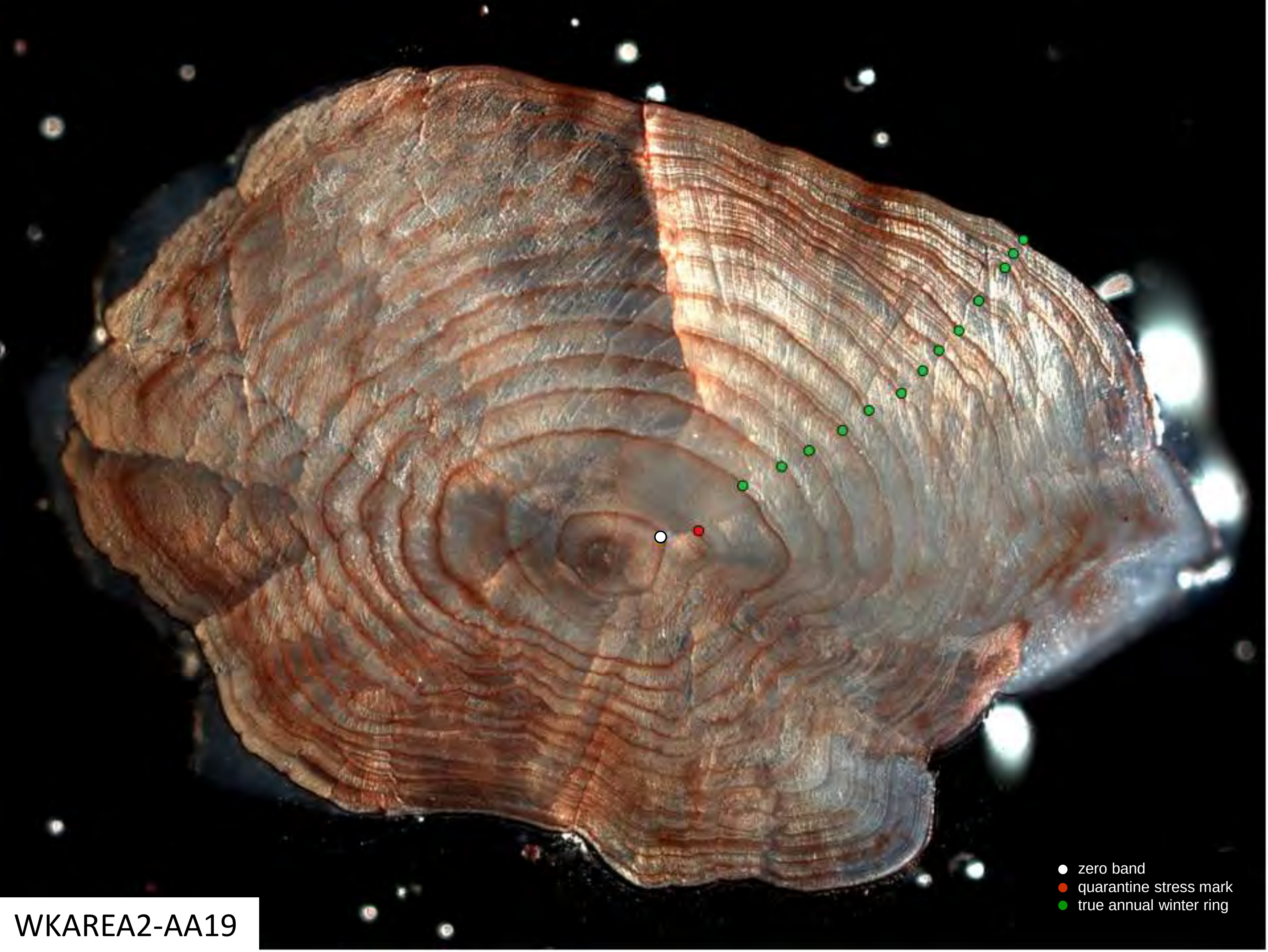


country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	589	304	female	ALC-marked and stocked as elver in 1997	11	



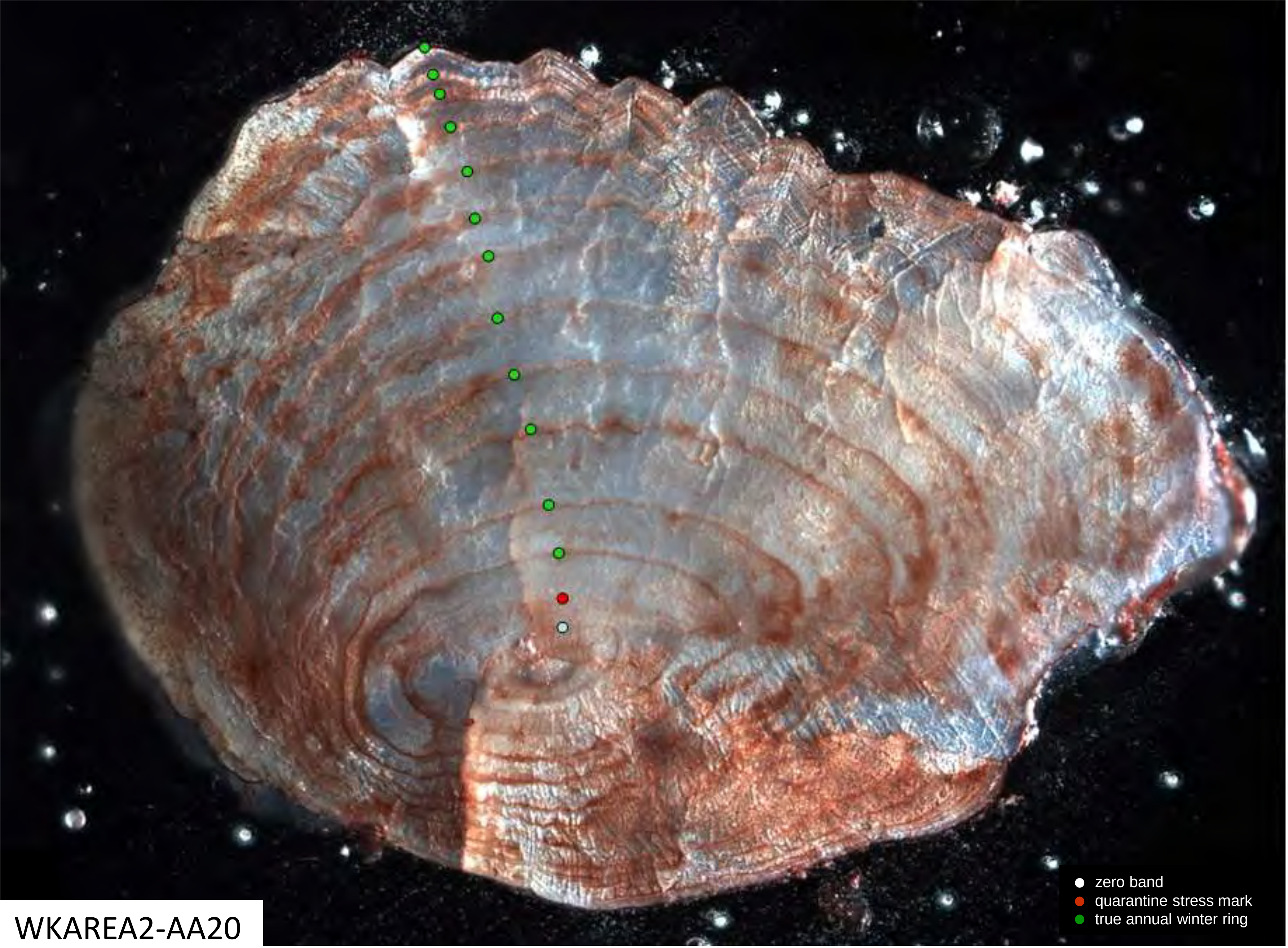
WKAREA2-AA18

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	518	233	female	ALC-marked and stocked as elver in 1997	11	same as WKAREA2-AA35



WKAREA2-AA19

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2010	6	yellow eel	546	217	female	ALC-marked and stocked as elver in 1997	13	



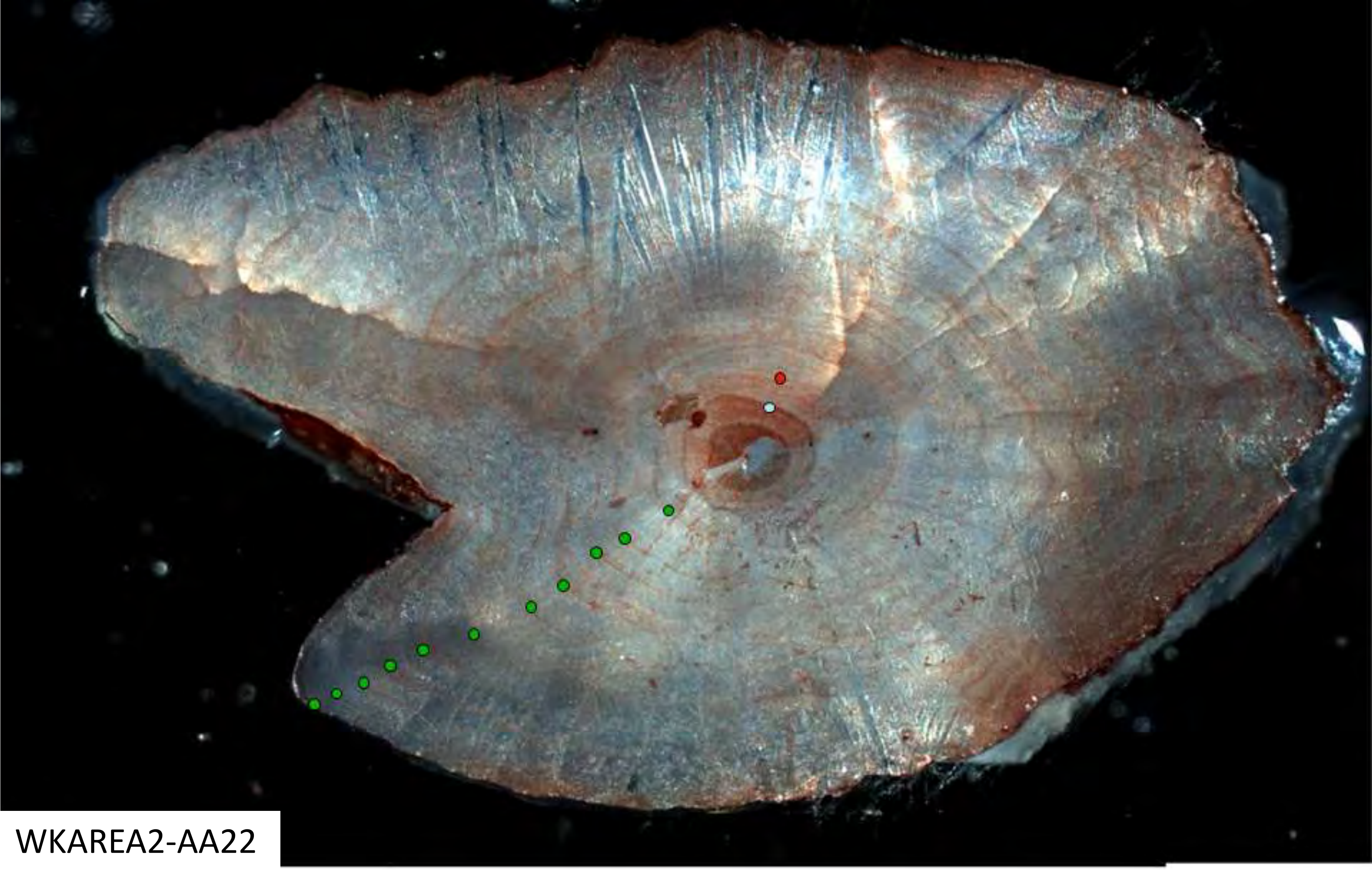
WKAREA2-AA20

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2009	6	yellow eel	529	257	female	ALC-marked and stocked as elver in 1997	12	

WKAREA2-AA21

- zero band
- quarantine stress mark
- true annual winter ring

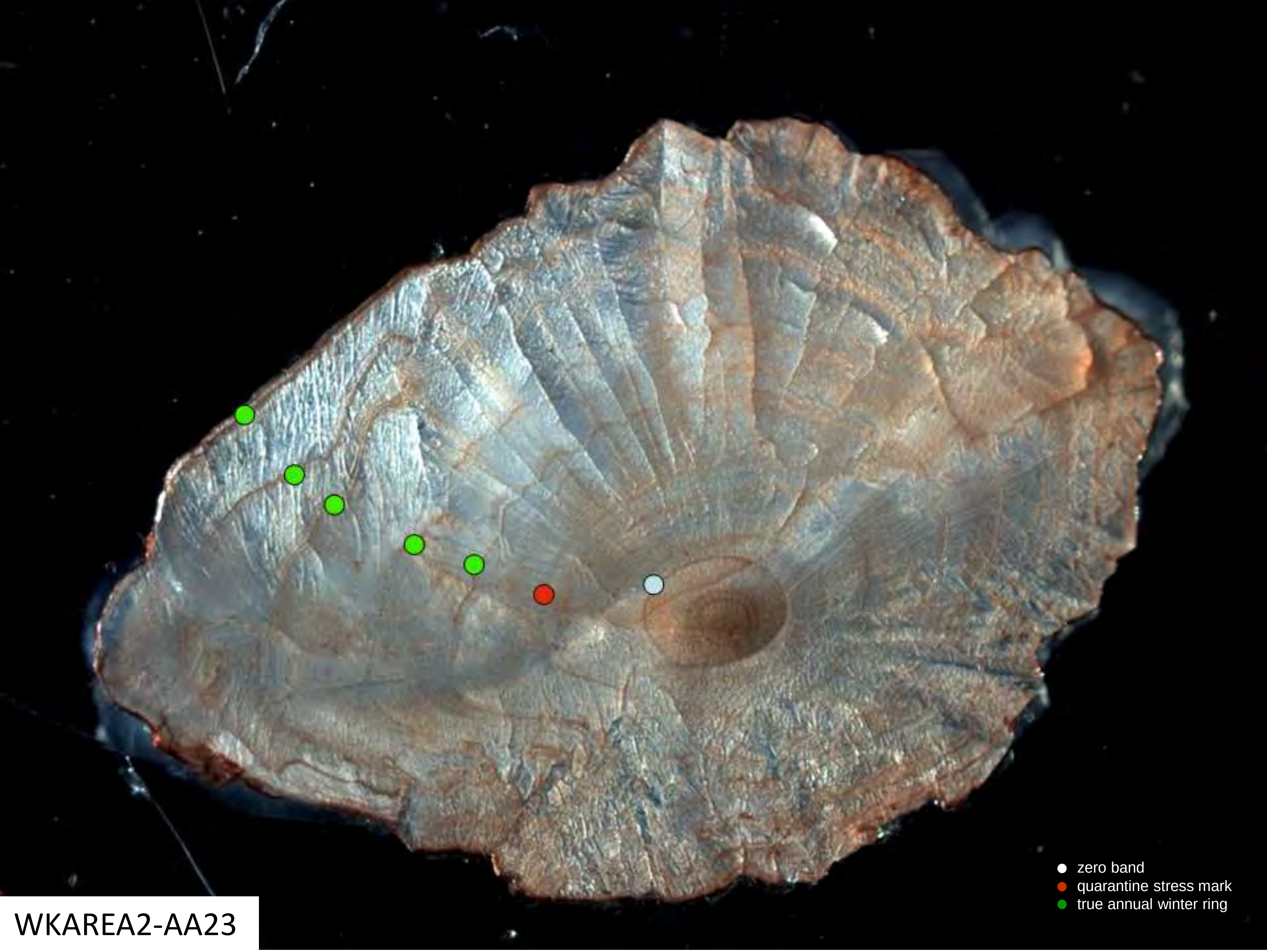
country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2009	5	yellow eel	572	358	female	ALC-marked and stocked as elver in 1997	12	



WKAREA2-AA22

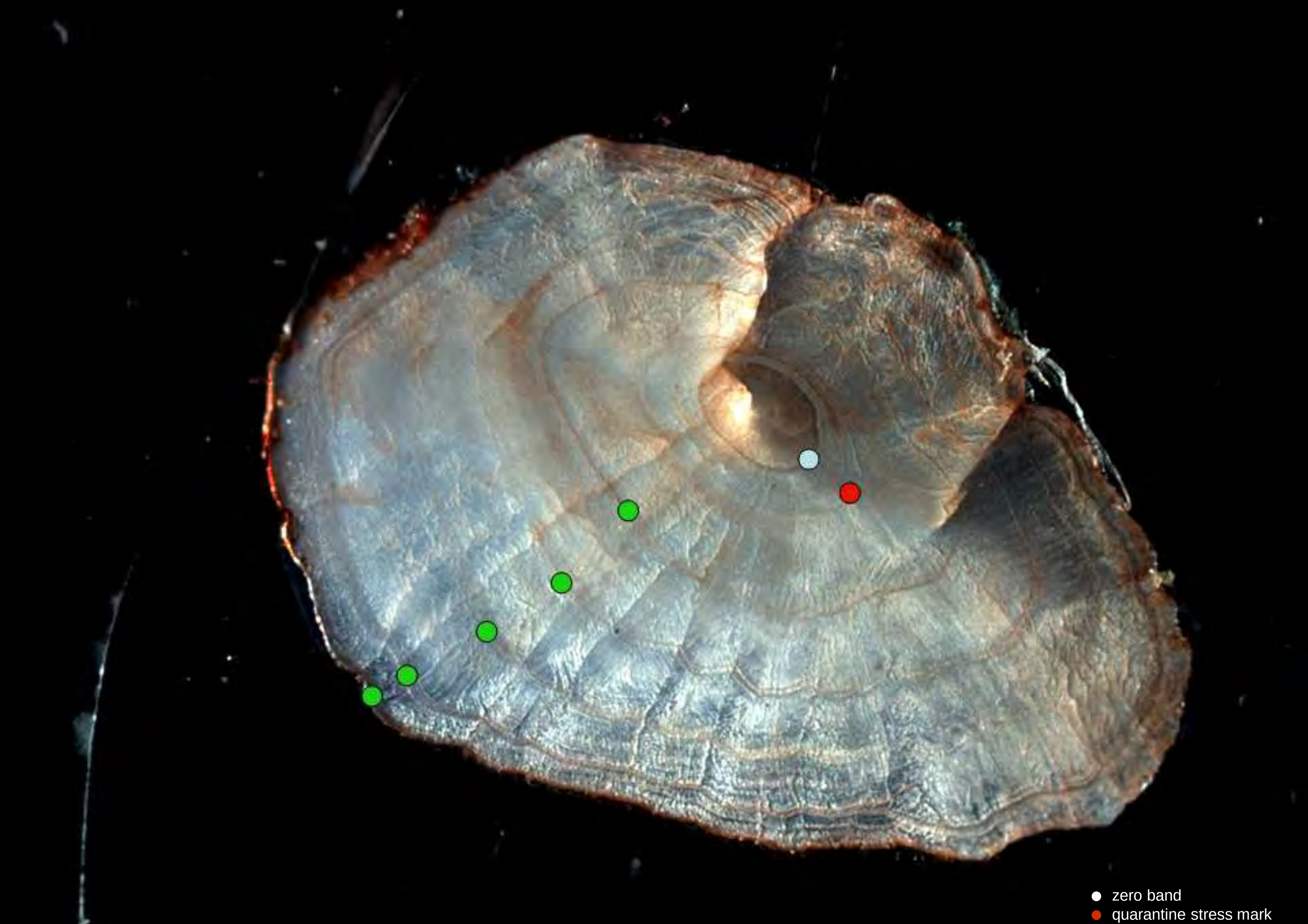
- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	544	289	female	ALC-marked and stocked as elver in 1997	11	



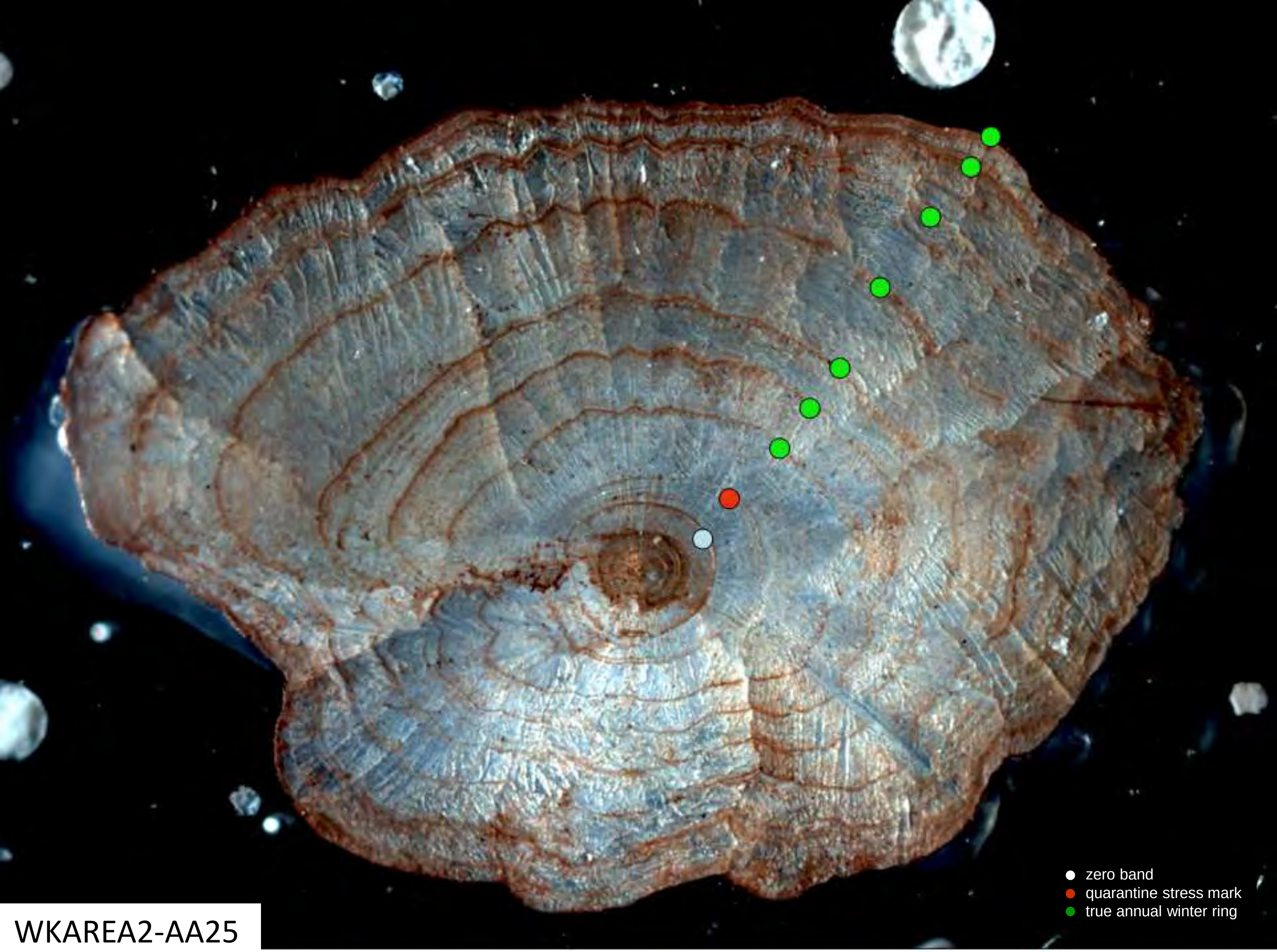
WKAREA2-AA23

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2002	6	yellow eel	328	51	female	ALC-marked and stocked as elver in 1997	5	



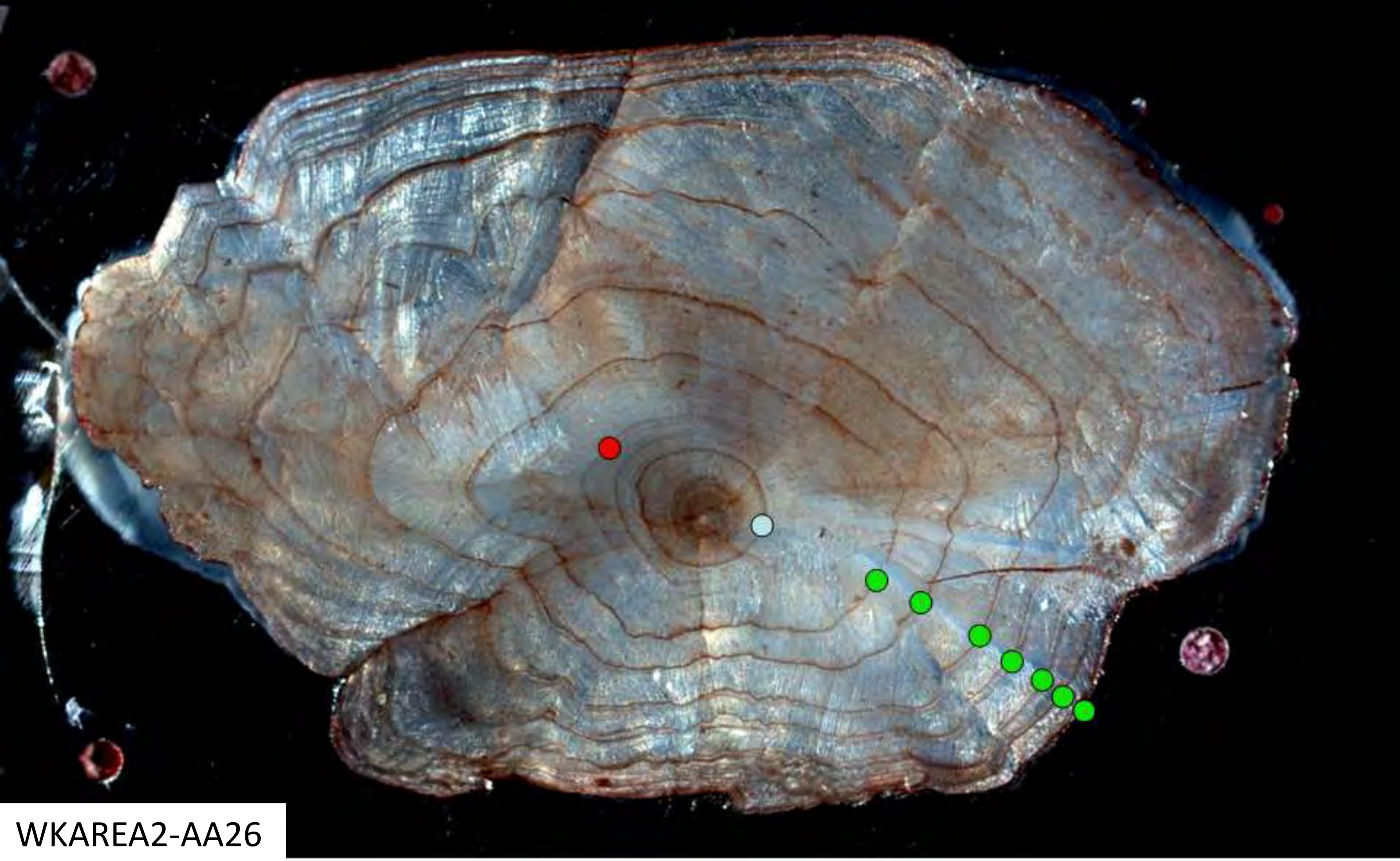
WKAREA2-AA24

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2002	6	yellow eel	410	103	female	ALC-marked and stocked as elver in 1997	5	same as WKAREA2-AA36



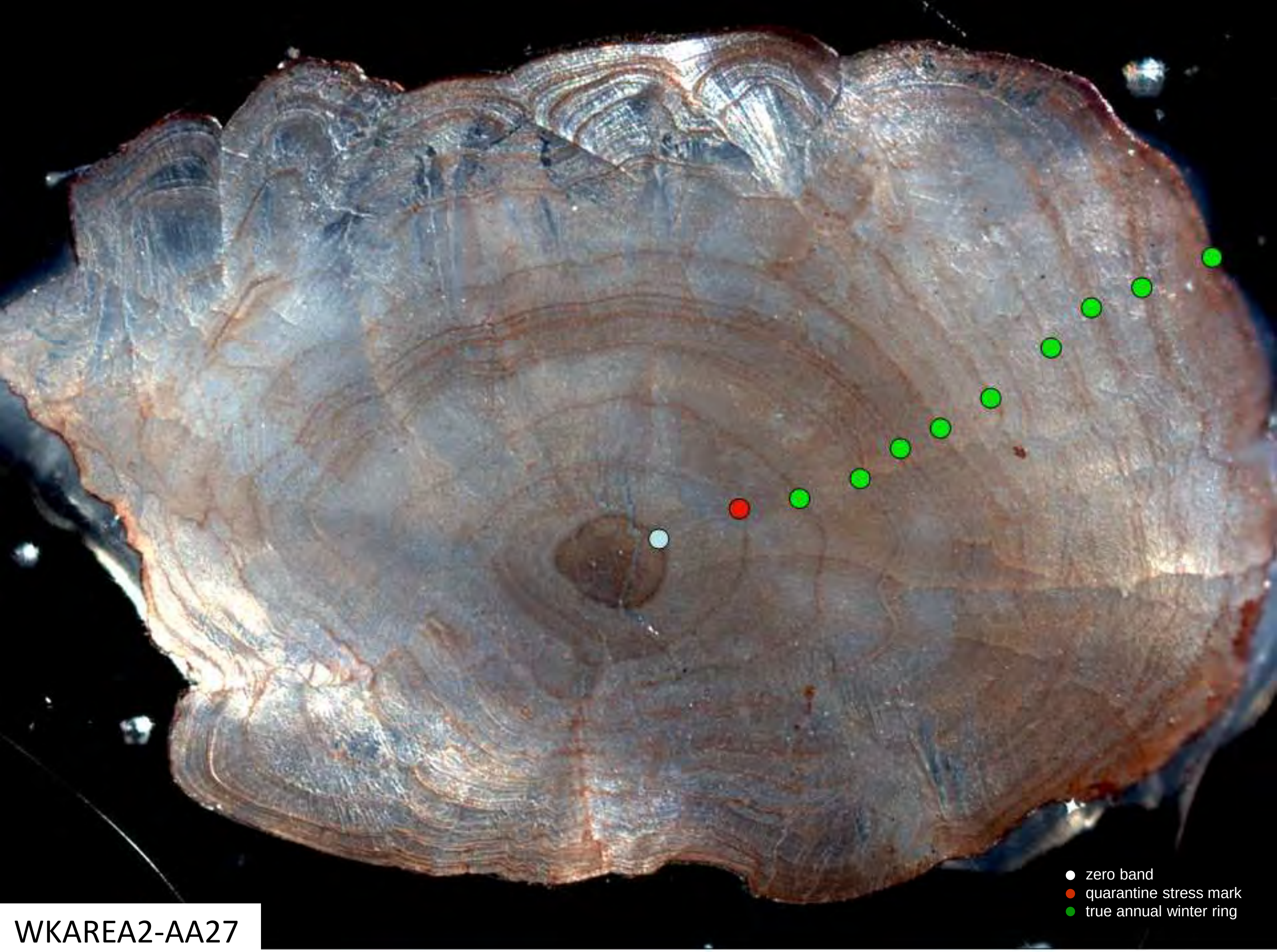
WKAREA2-AA25

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2004	7	yellow eel	485	153	female	ALC-marked and stocked as elver in 1997	7	



- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2004	6	yellow eel	558	260	female	ALC-marked and stocked as elver in 1997	7	



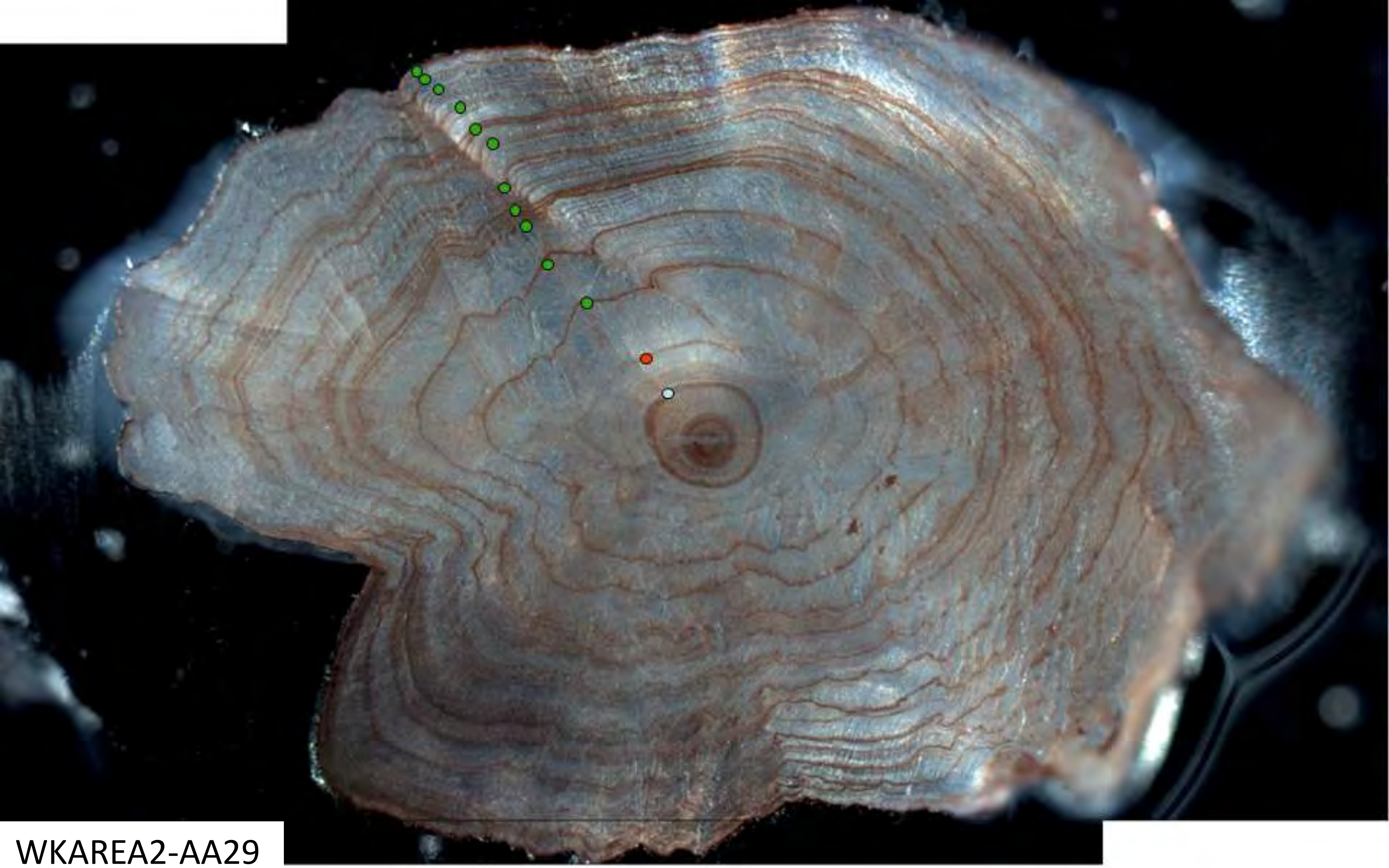
WKAREA2-AA27

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2006	5	yellow eel	526	222	female	ALC-marked and stocked as elver in 1997	9	

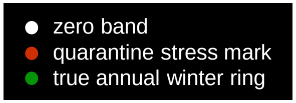
WKAREA2-AA28

- zero band
- quarantine stress mark
- true annual winter ring

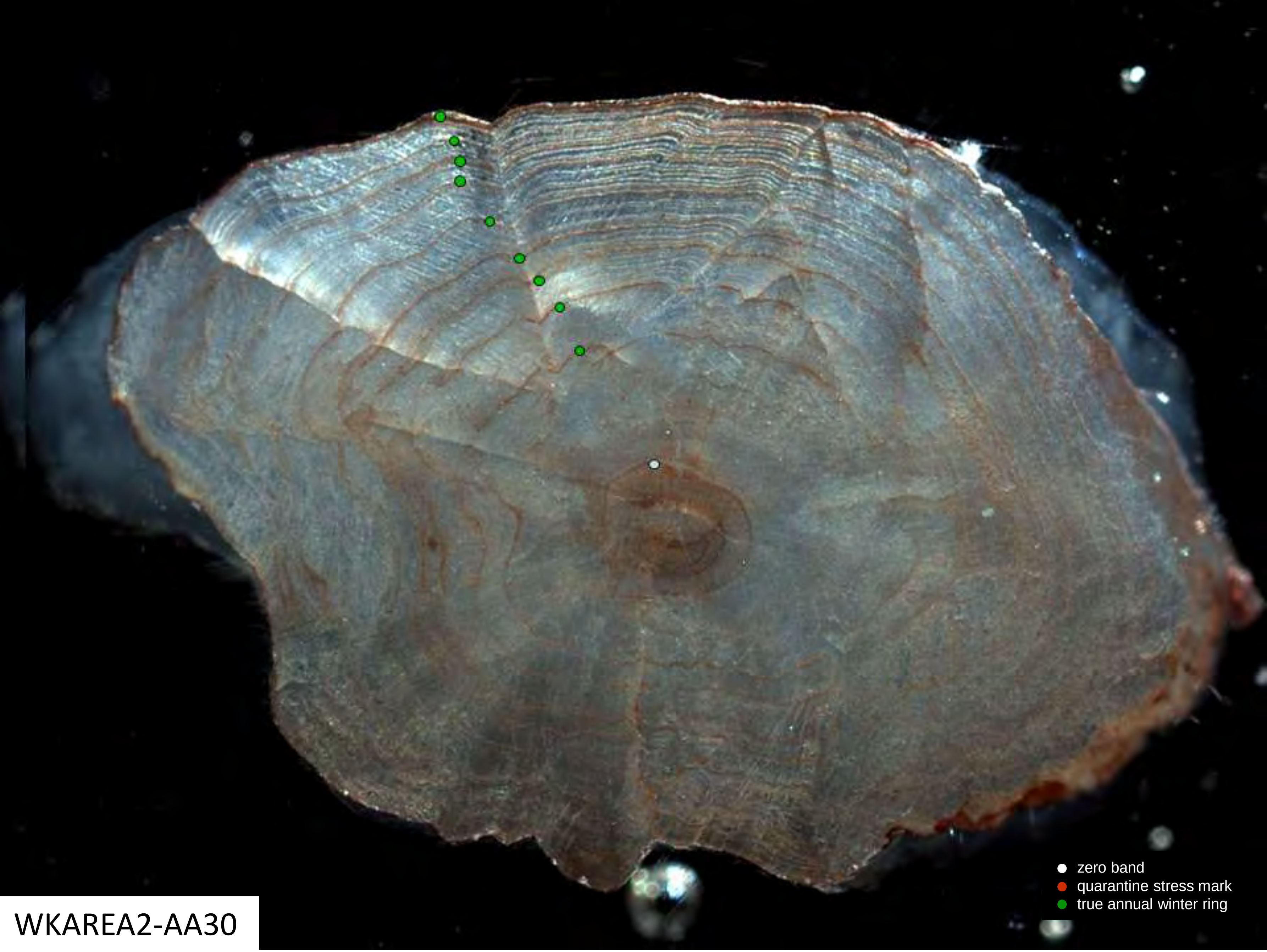
country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	6	yellow eel	545	247	female	ALC-marked and stocked as elver in 1997	11	



WKAREA2-AA29

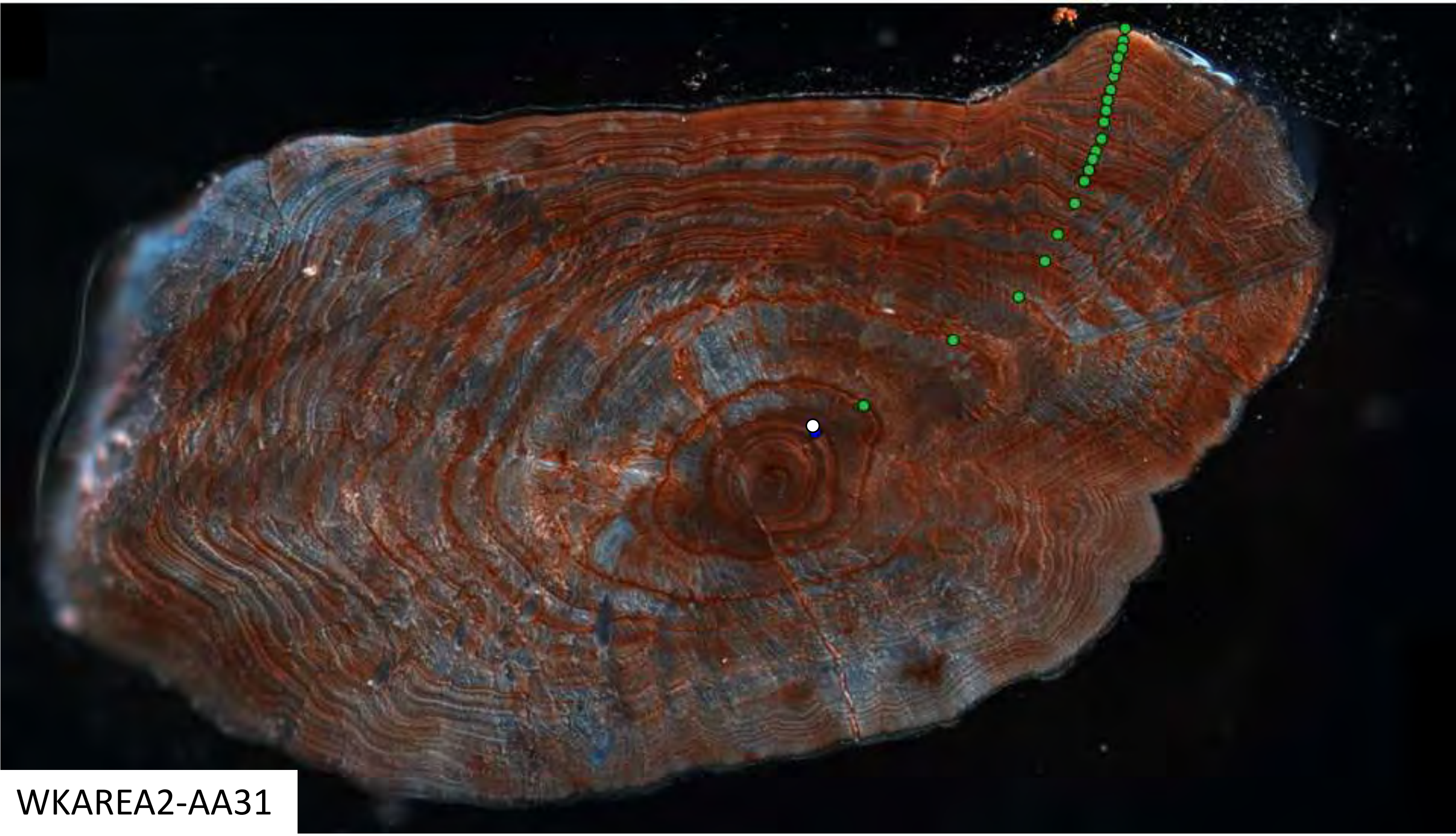


country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	549	252	female	ALC-marked and stocked as elver in 1997	11	



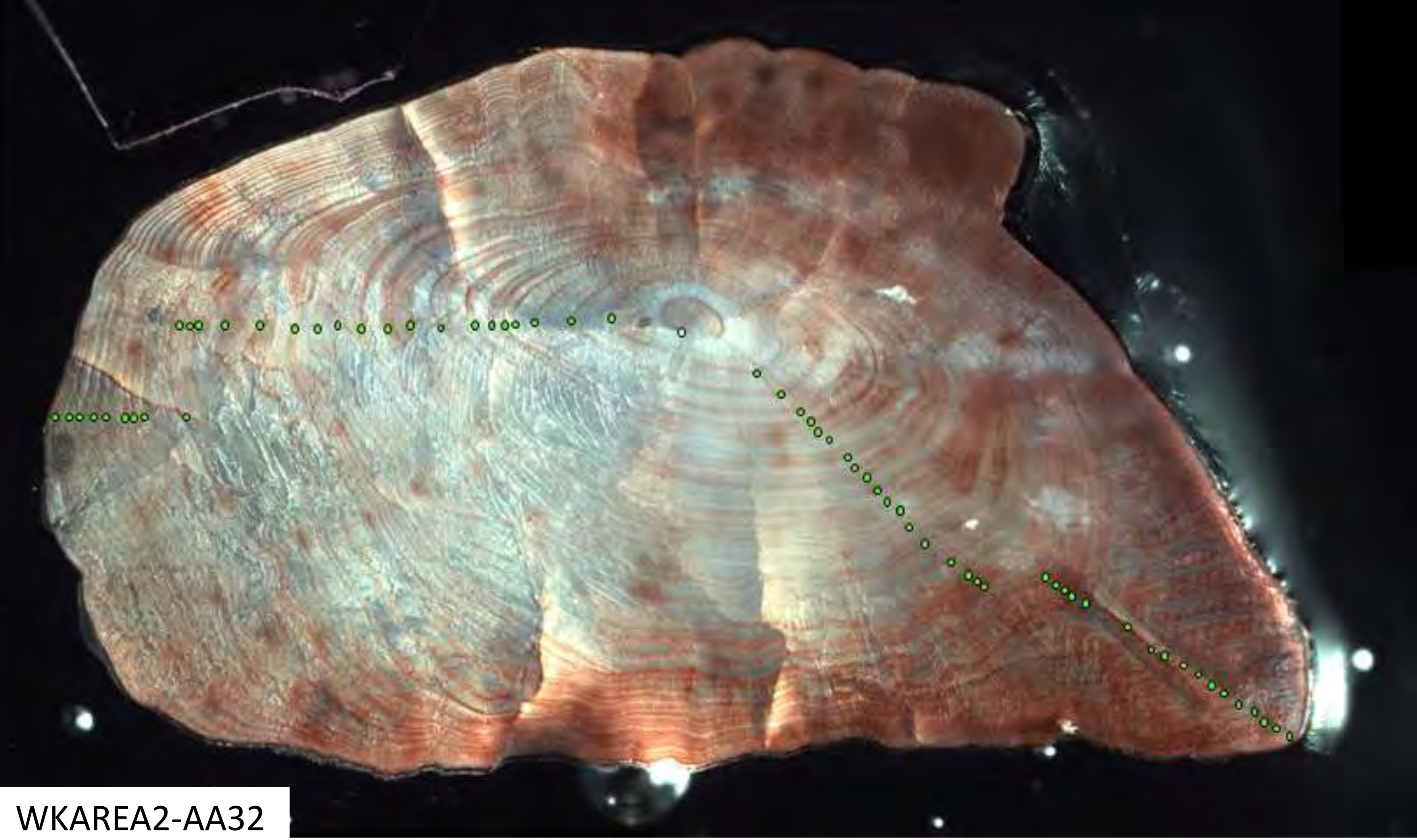
WKAREA2-AA30

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2006	5	yellow eel	404	92	female	ALC-marked and stocked as elver in 1997	9	



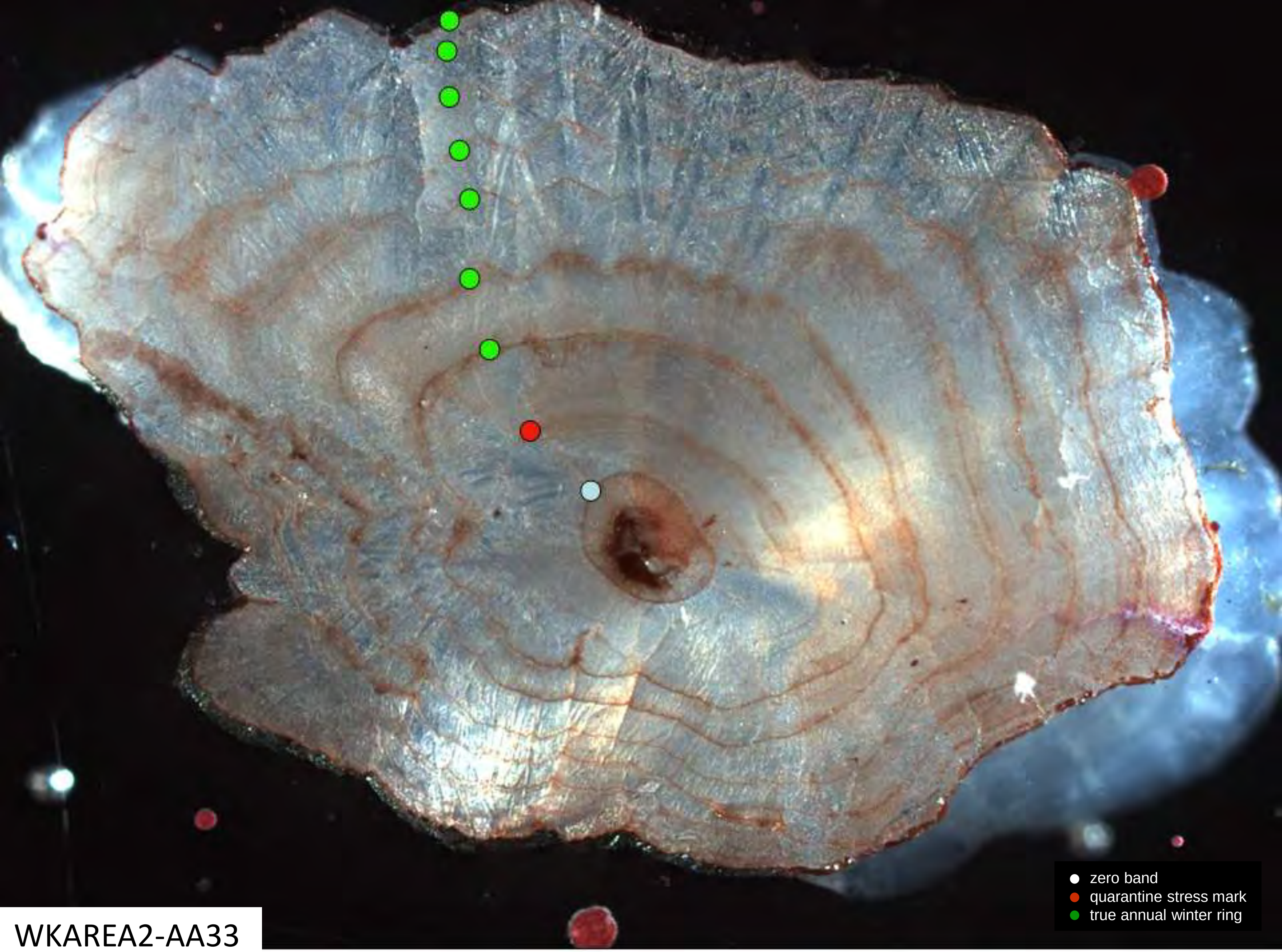
- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Fardumeträsk	2010	6-11	silver eel	762	793	female	stocked as elver in eel free lake in 1989	21	



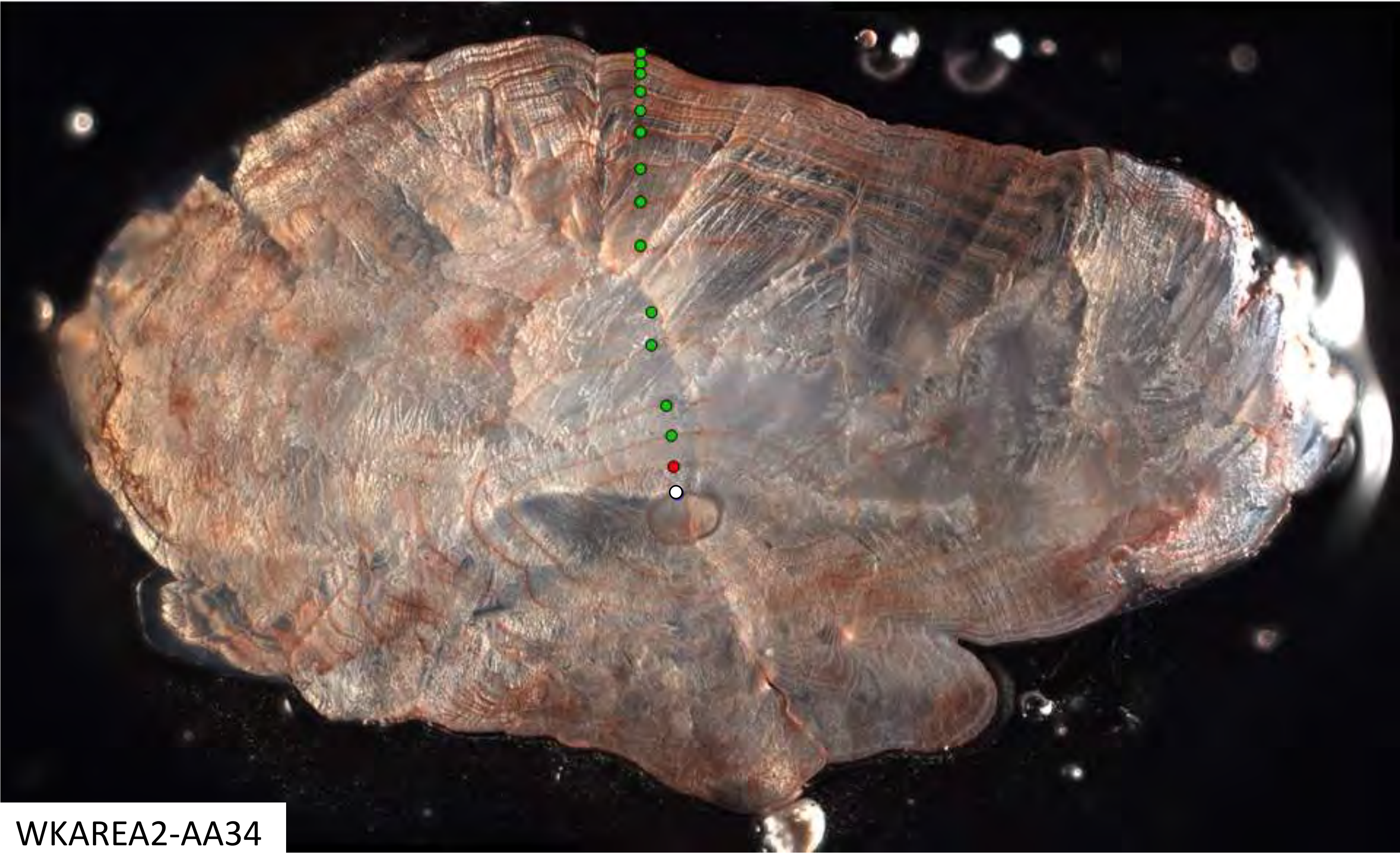
- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Ången	2008	4	silver eel	1031	3003	female	stocked as yellow eel (appr. 7 years) in 1979	unknown	2 alternatives



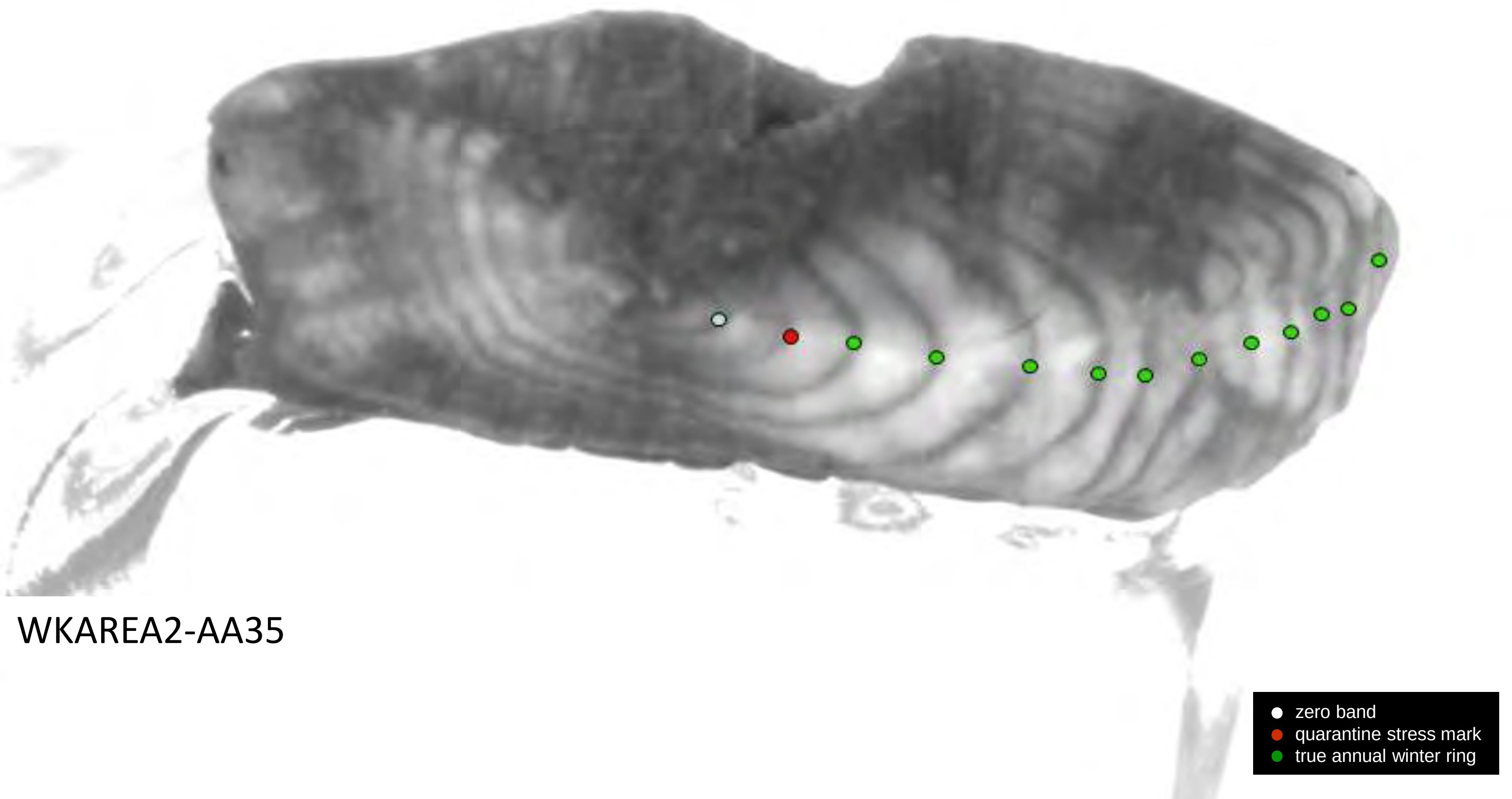
WKAREA2-AA33

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2004	5	yellow eel	469	158	female	ALC-marked and stocked as elver in 1997	7	



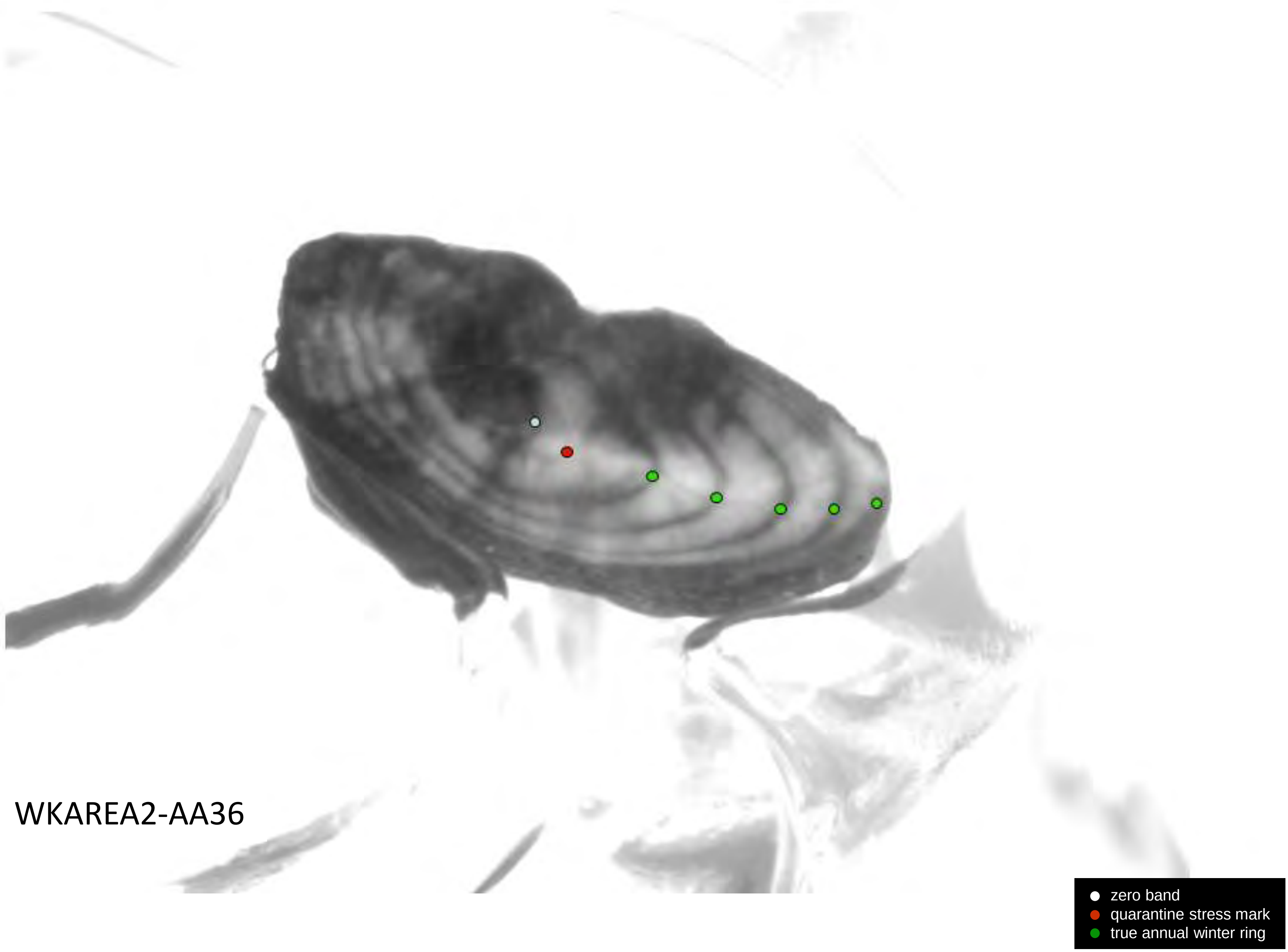
- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2010	5	yellow eel	701	548	female	ALC-marked and stocked as elver in 1997	13	

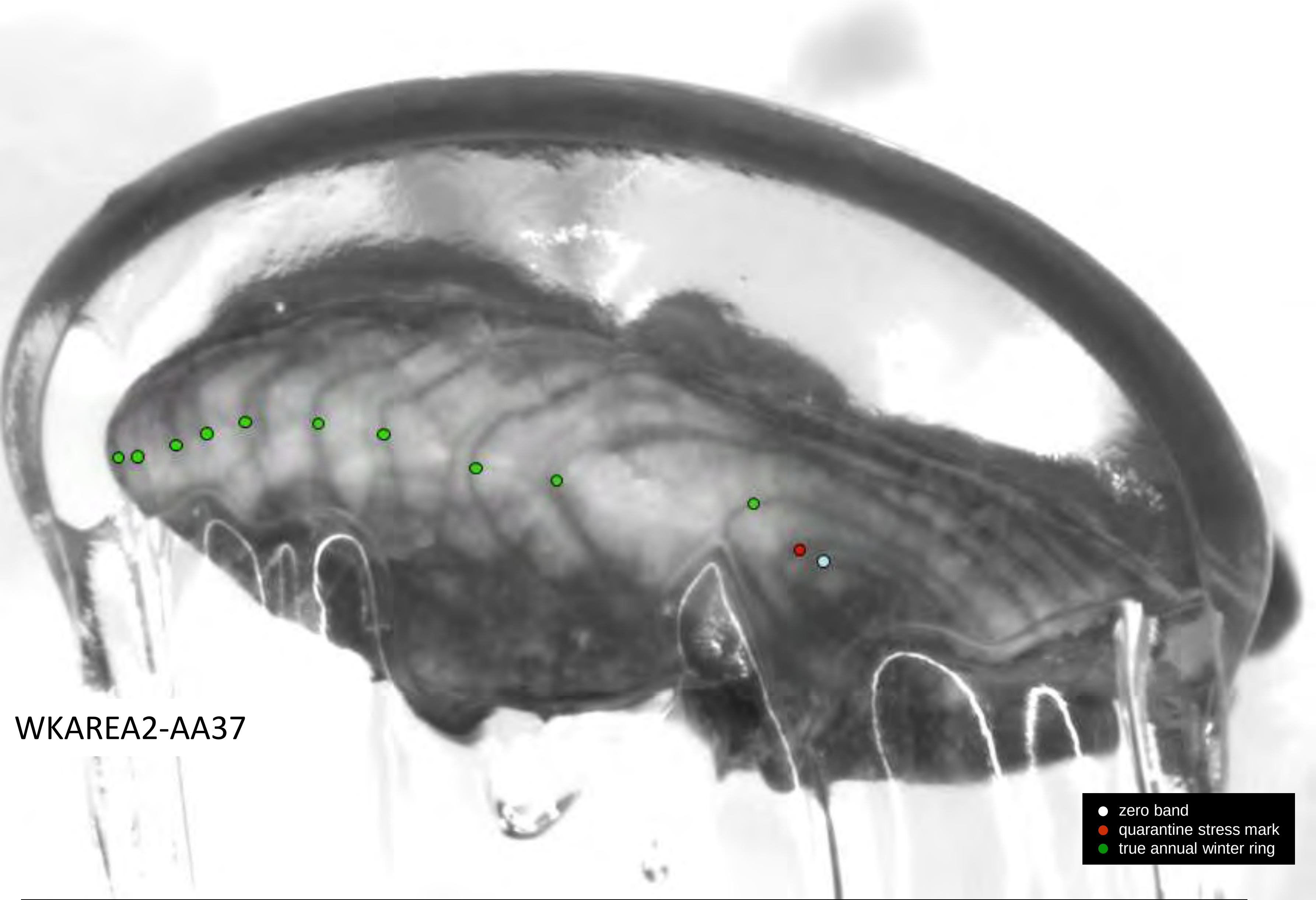


WKAREA2-AA35

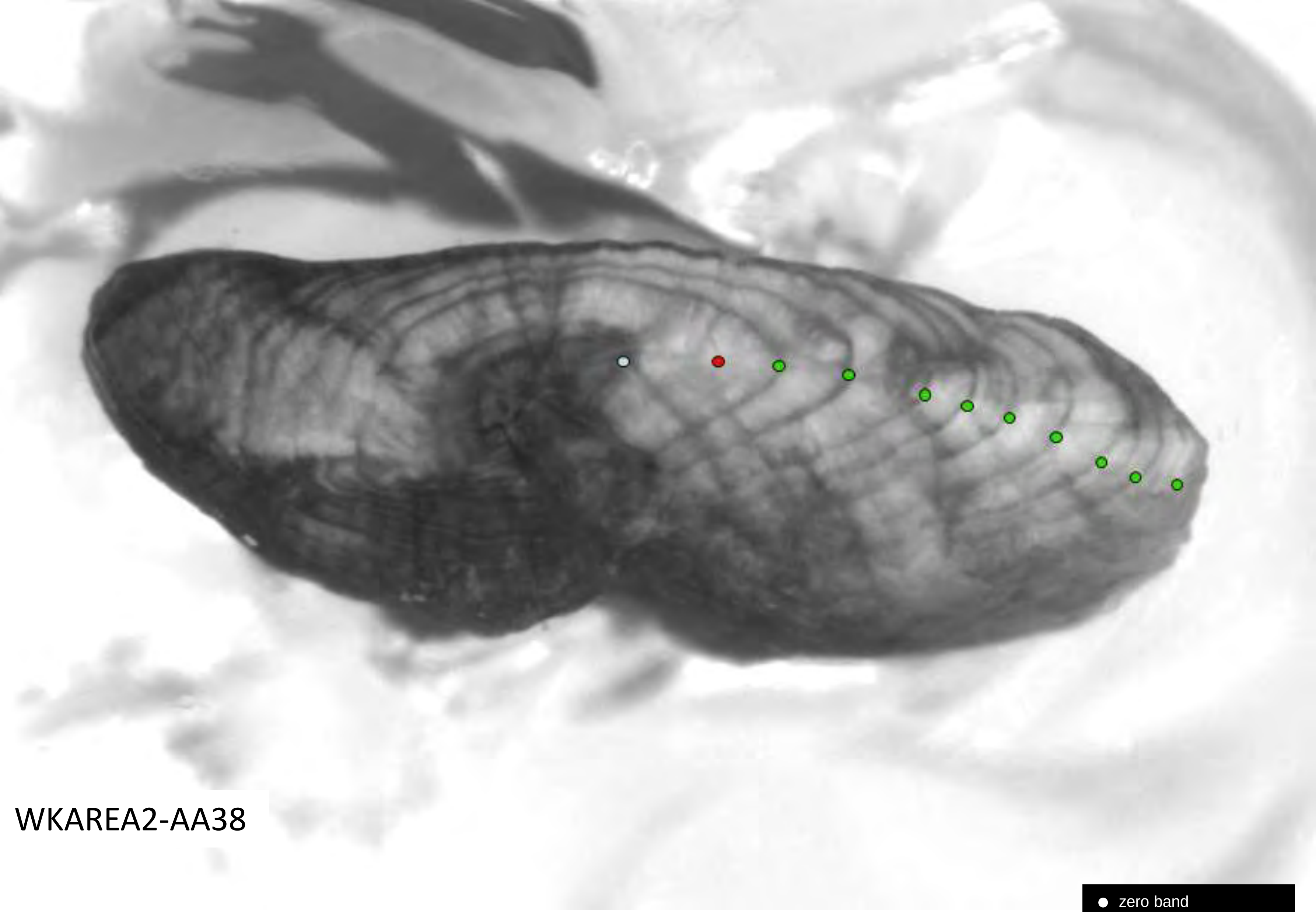
country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2008	5	yellow eel	518	233	female	ALC-marked and stocked as elver in 1997	11	same as WKAREA2-AA18



country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2002	6	yellow eel	410	103	female	ALC-marked and stocked as elver in 1997	5	same as WKAREA2-AA24



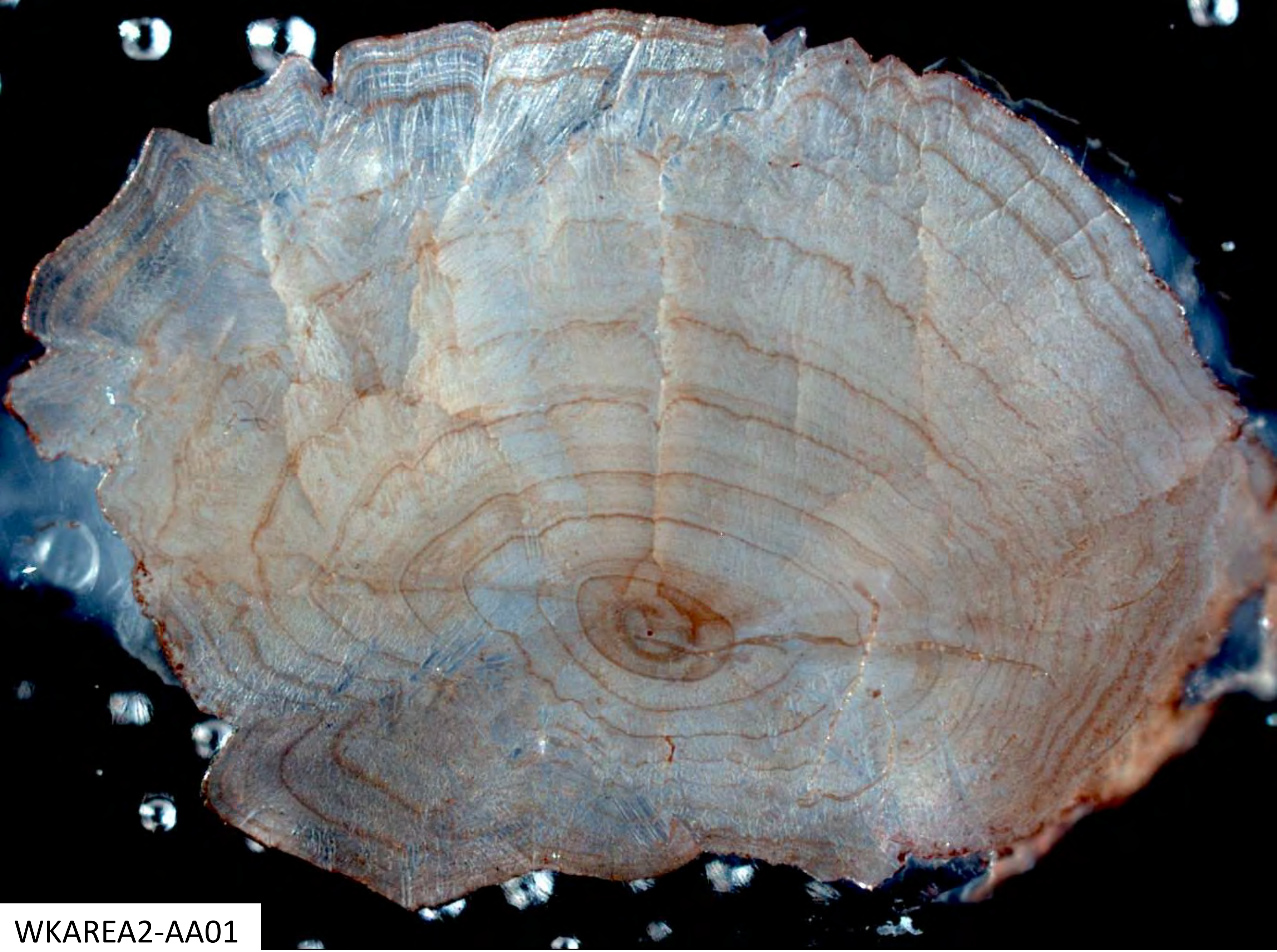
country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Fardume	1999	3	silver eel	754	848	female	stocked in 1989	10	



WKAREA2-AA38

- zero band
- quarantine stress mark
- true annual winter ring

country	location	year	month	life stage	length (mm)	weight (g)	sex	history + timing	actual age	comments
Sweden	Lake Mälaren	2006	5	yellow eel	446	145	female	ALC-marked and stocked as elver in 1997	9	

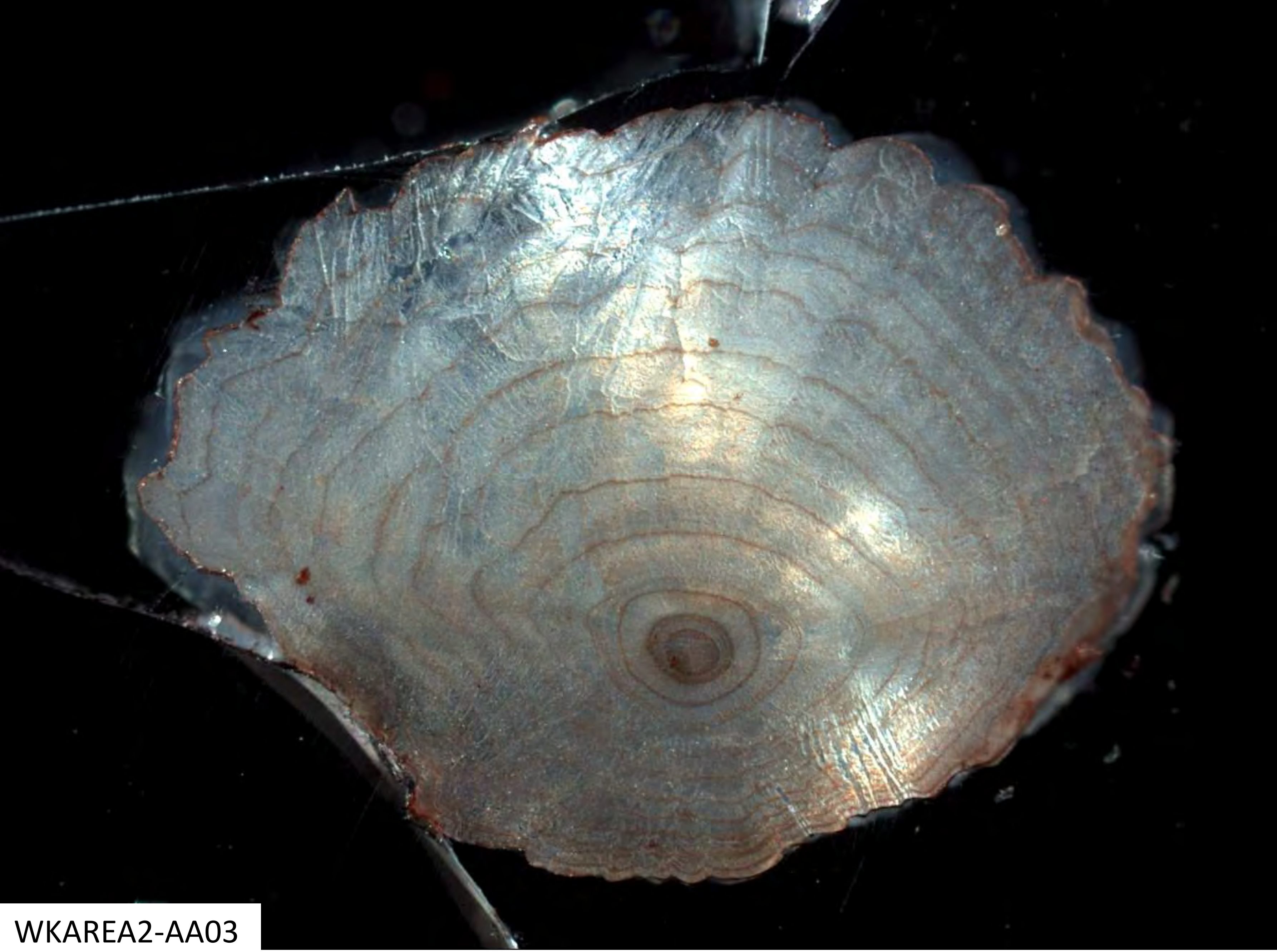


WKAREA2-AA01

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	503	124	female	ALC-marked and stocked as elver

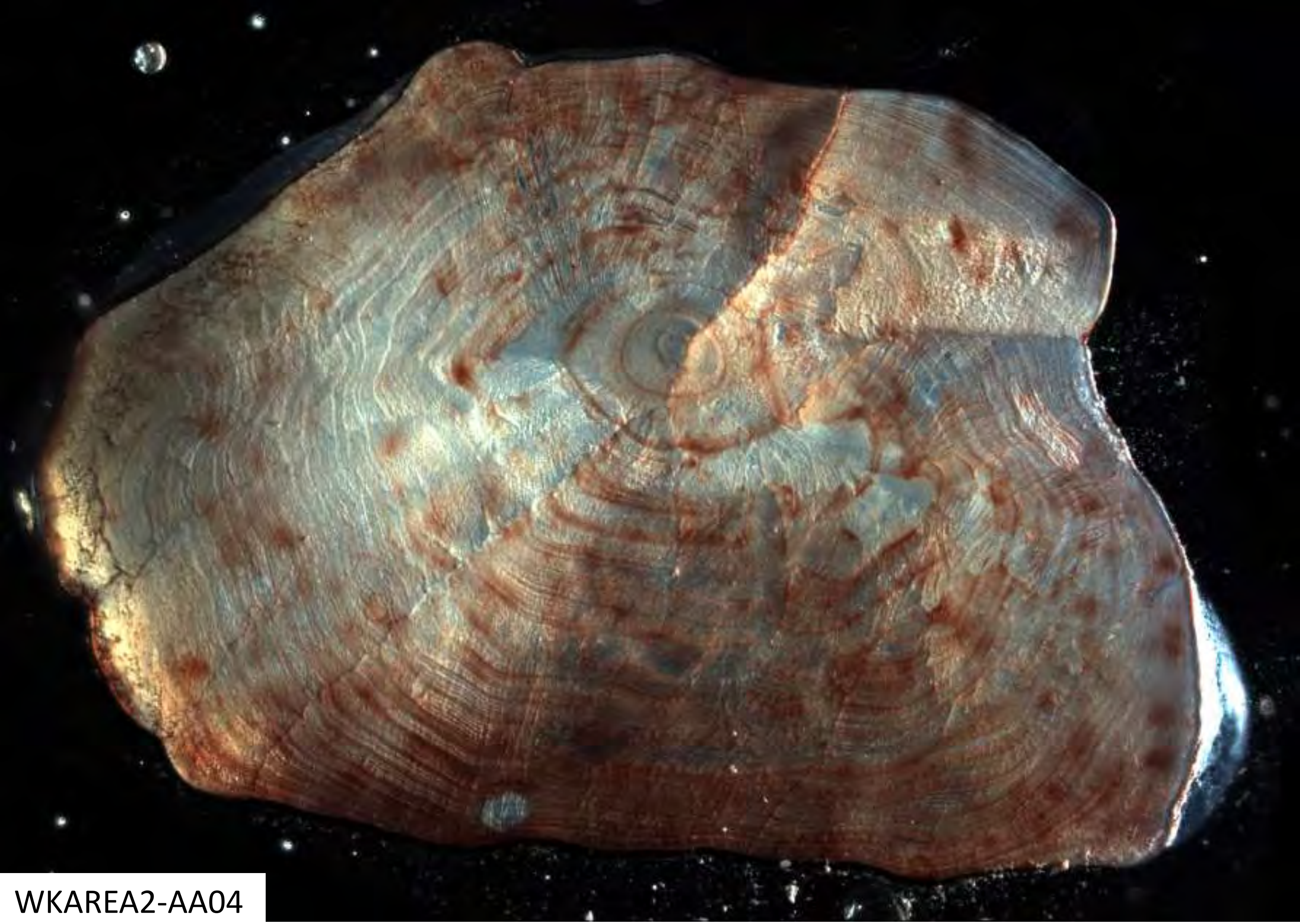
WKAREA2-AA02

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	70	358	female	ALC-marked and stocked as elver



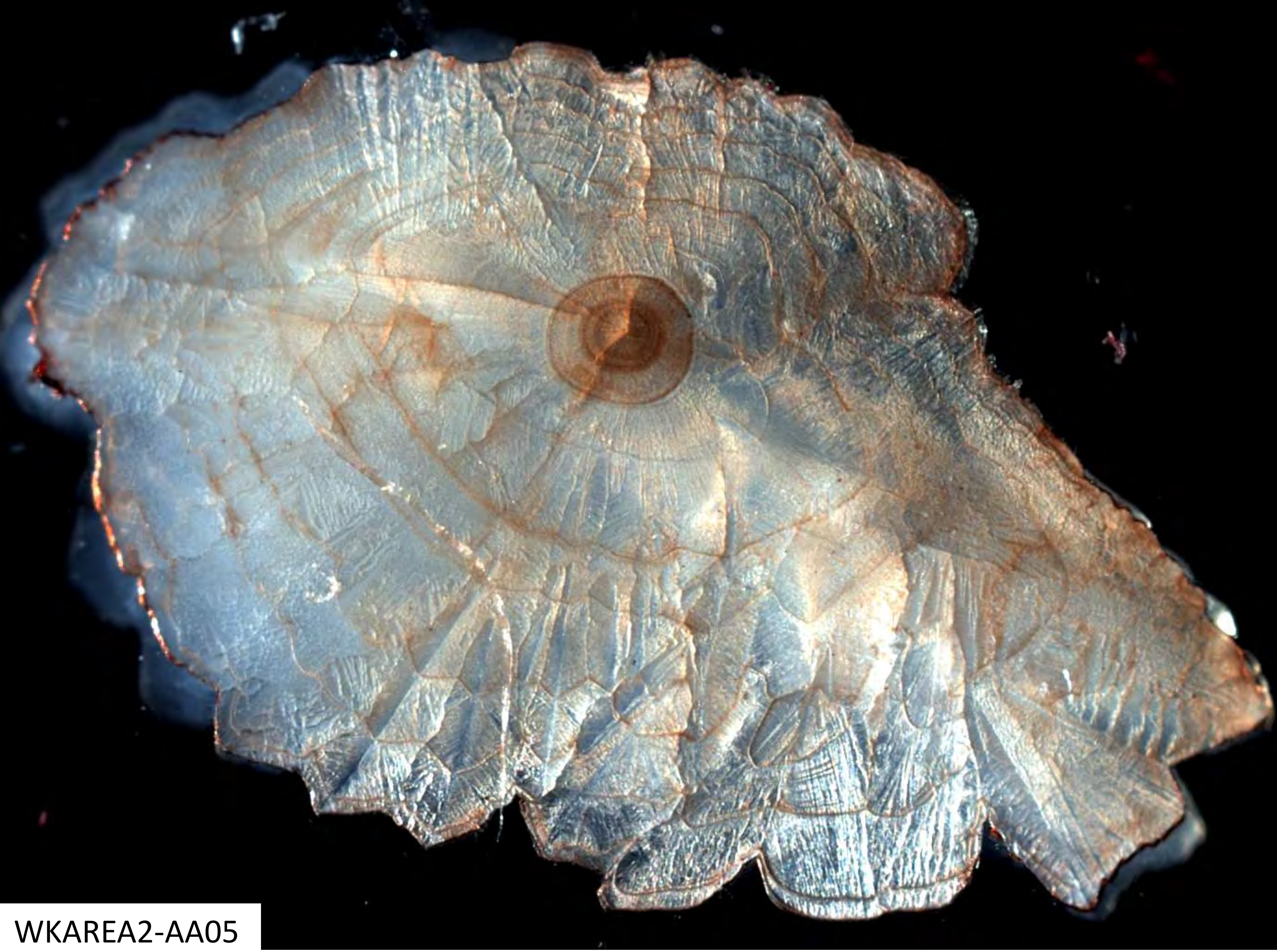
WKAREA2-AA03

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	415	99	female	ALC-marked and stocked as elver



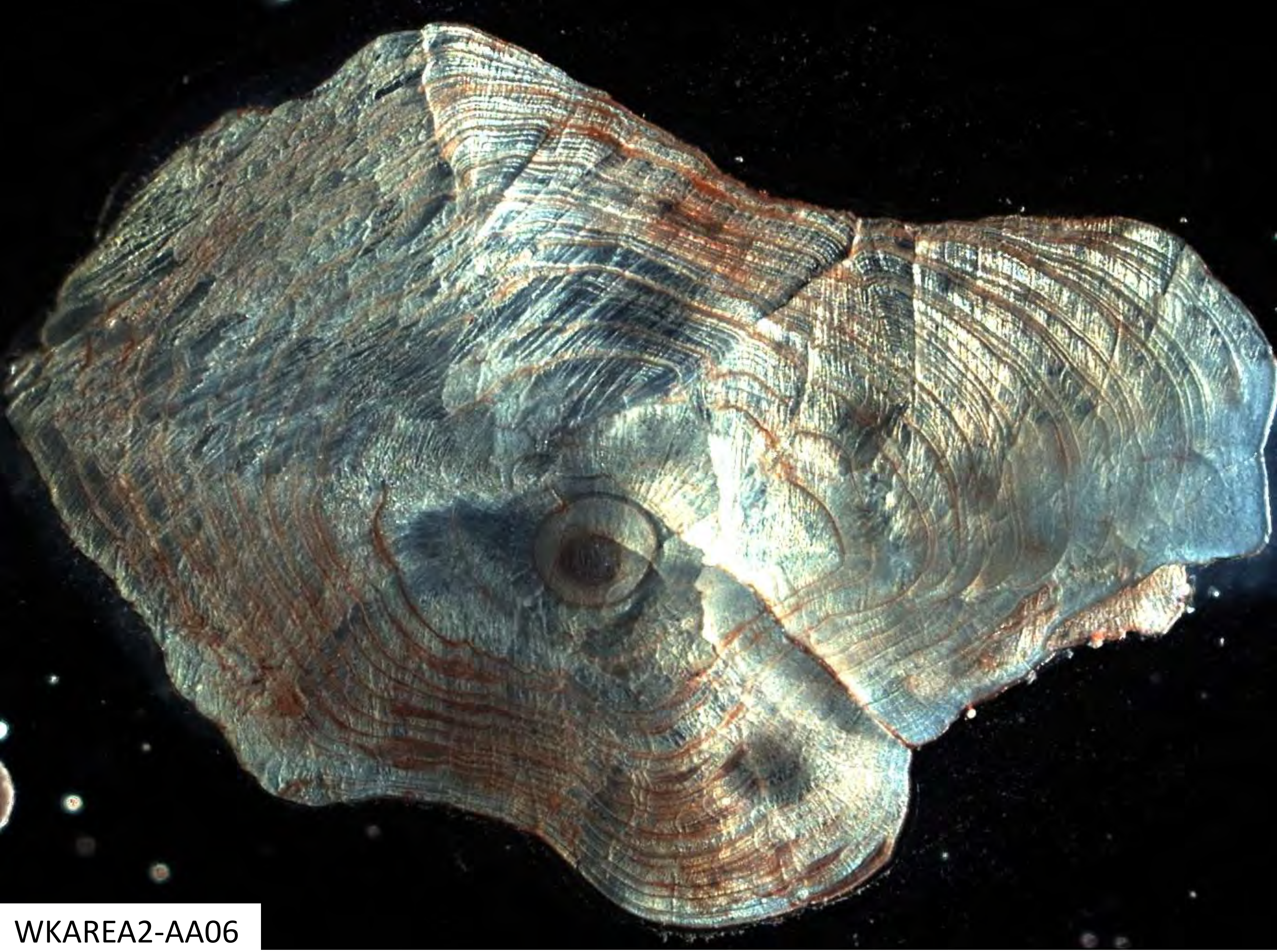
WKAREA2-AA04

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Fardumeträsk		silver eel	834	909	female	stocked as elver in eel free lake



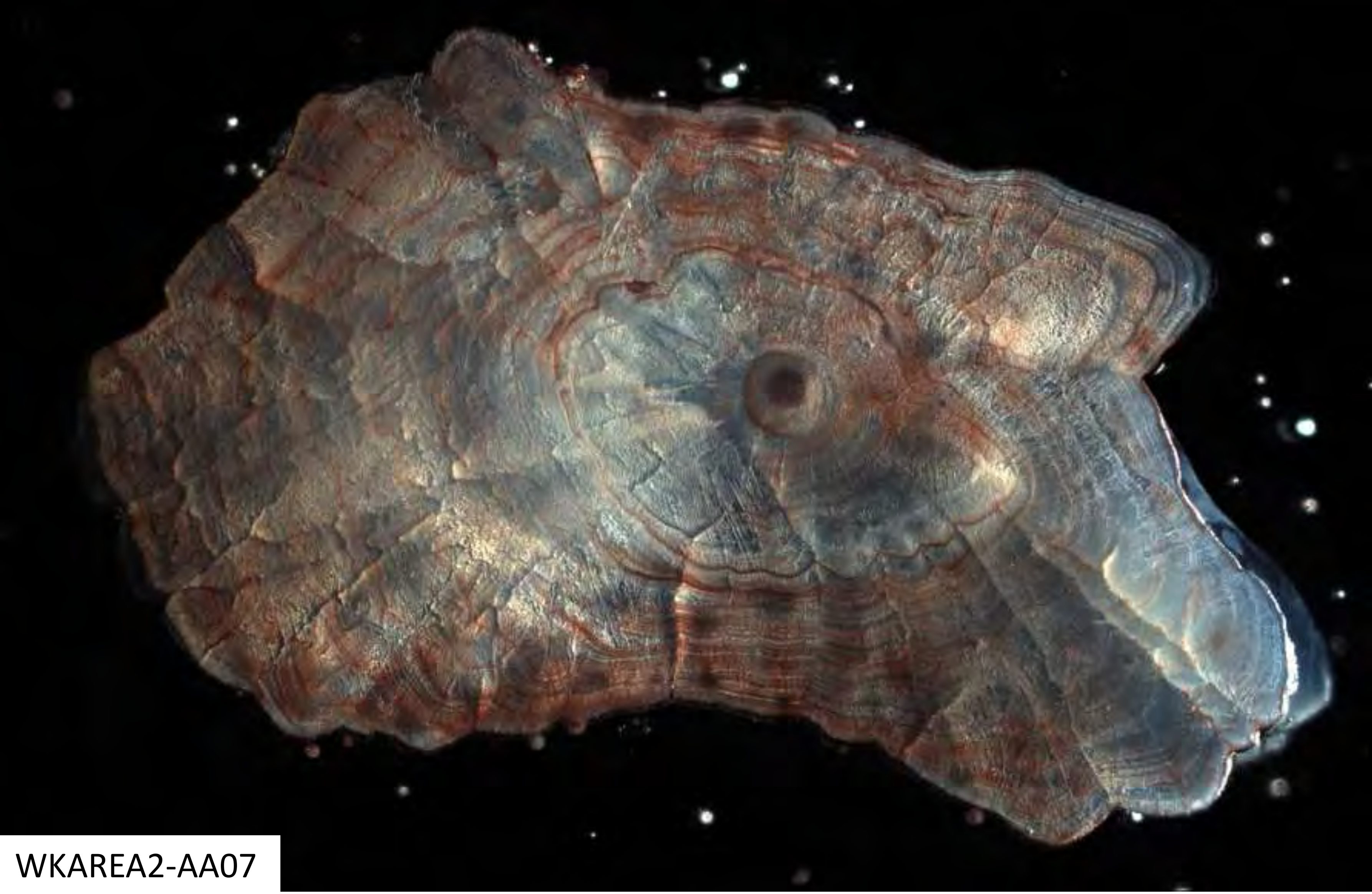
WKAREA2-AA05

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	478	161	female	ALC-marked and stocked as elver



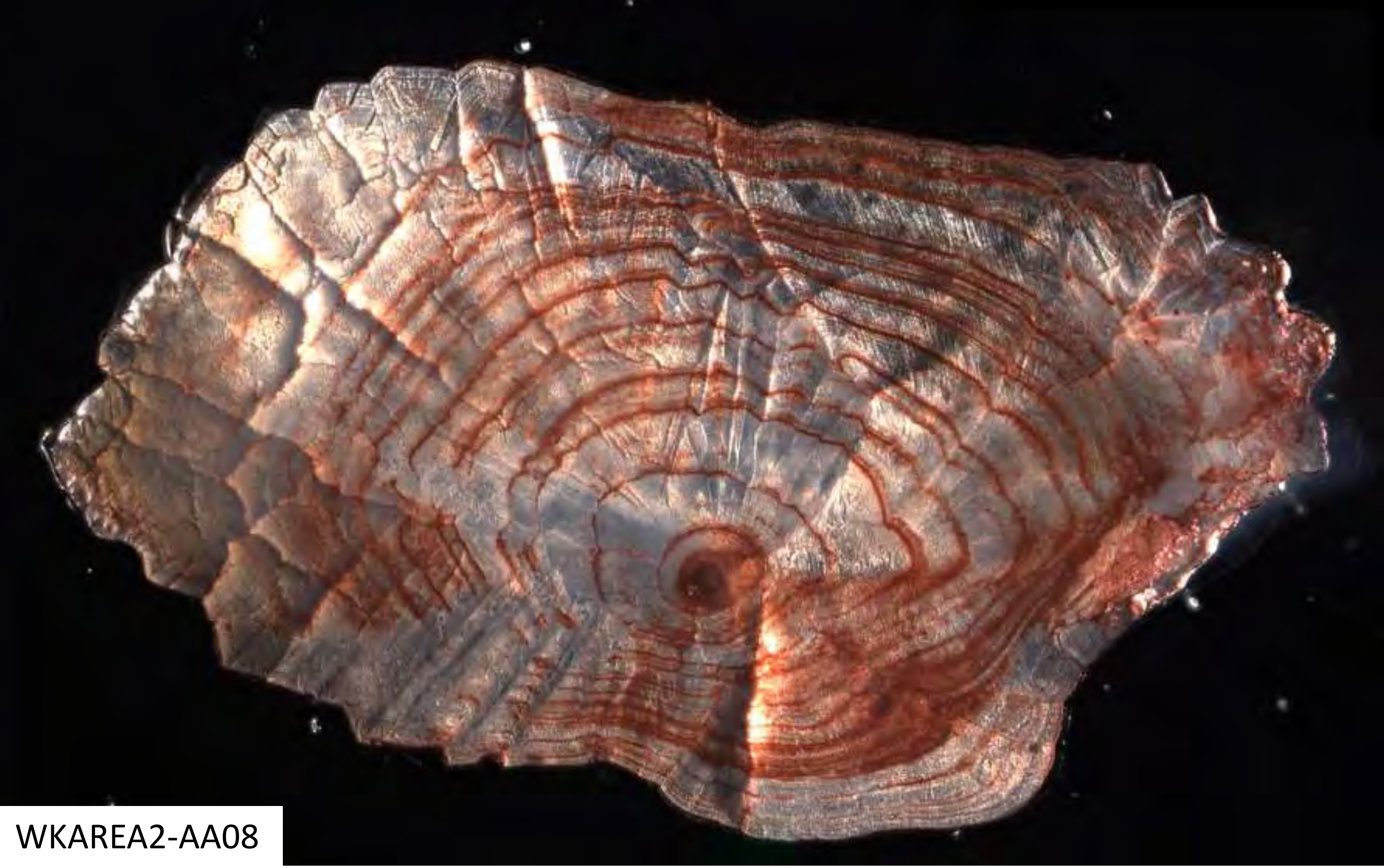
WKAREA2-AA06

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	488	182	female	ALC-marked and stocked as elver



WKAREA2-AA07

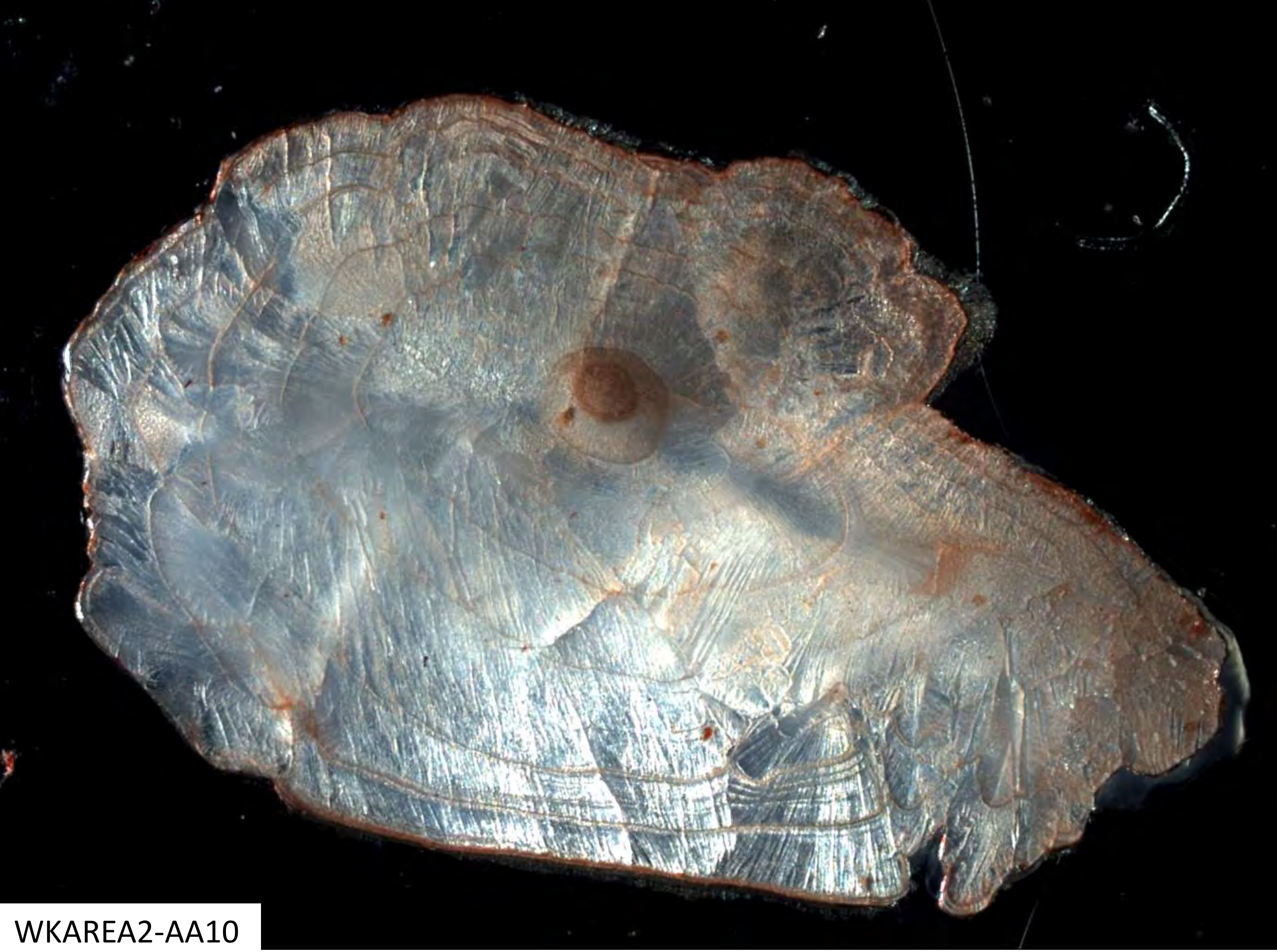
country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Ymsen	5	yellow eel	743	930	female	individually pit tagged and stocked



WKAREA2-AA08

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	553	186	female	ALC-marked and stocked as elver

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	484	221	female	ALC-marked and stocked as elver



WKAREA2-AA10

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	454	149	female	ALC-marked and stocked as elver

WKAREA2-AA11

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	440	140	female	ALC-marked and stocked as elver

WKAREA2-AA12

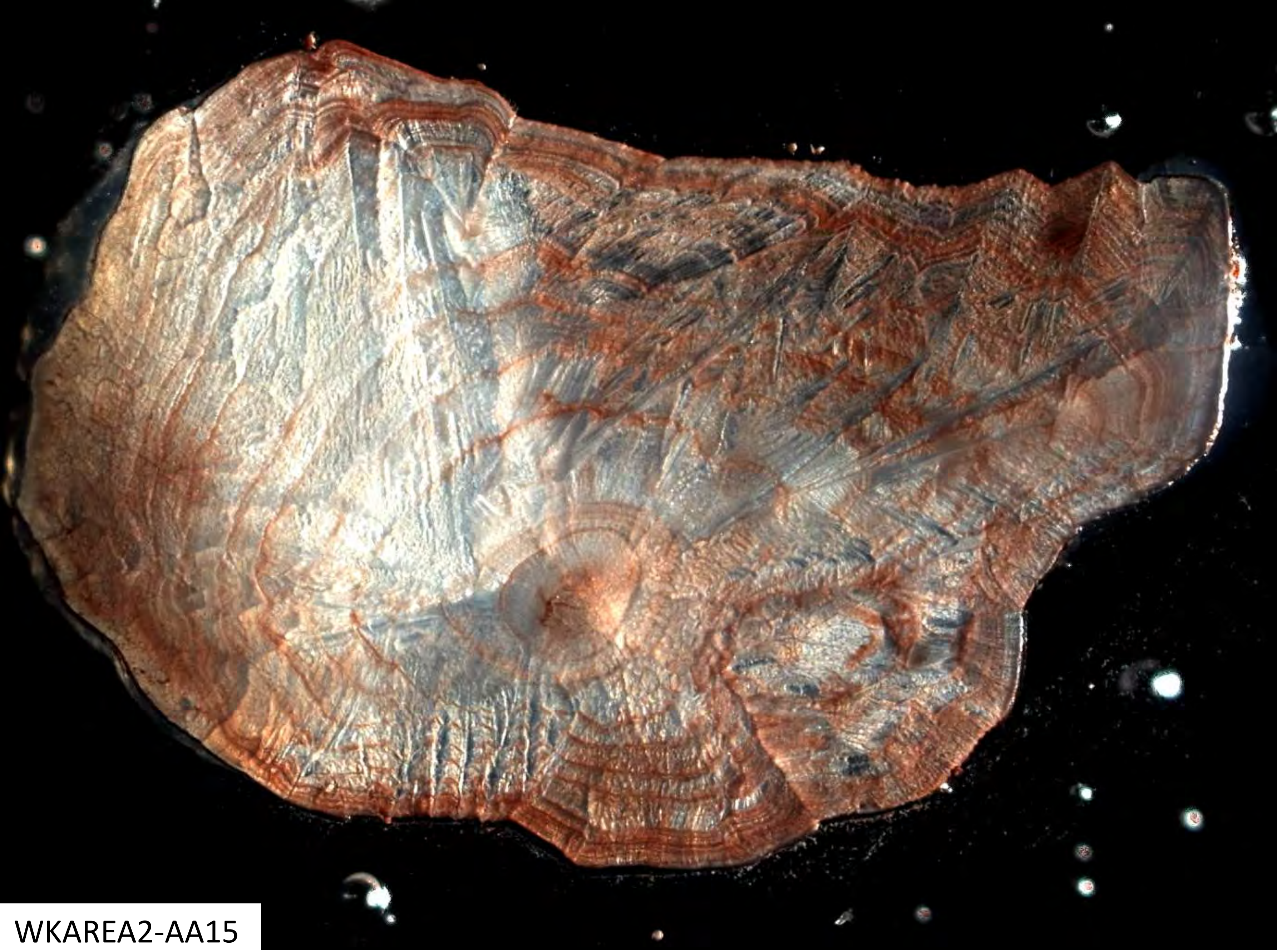
country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	614	360	female	ALC-marked and stocked as elver

WKAREA2-AA13

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	466	152	female	ALC-marked and stocked as elver

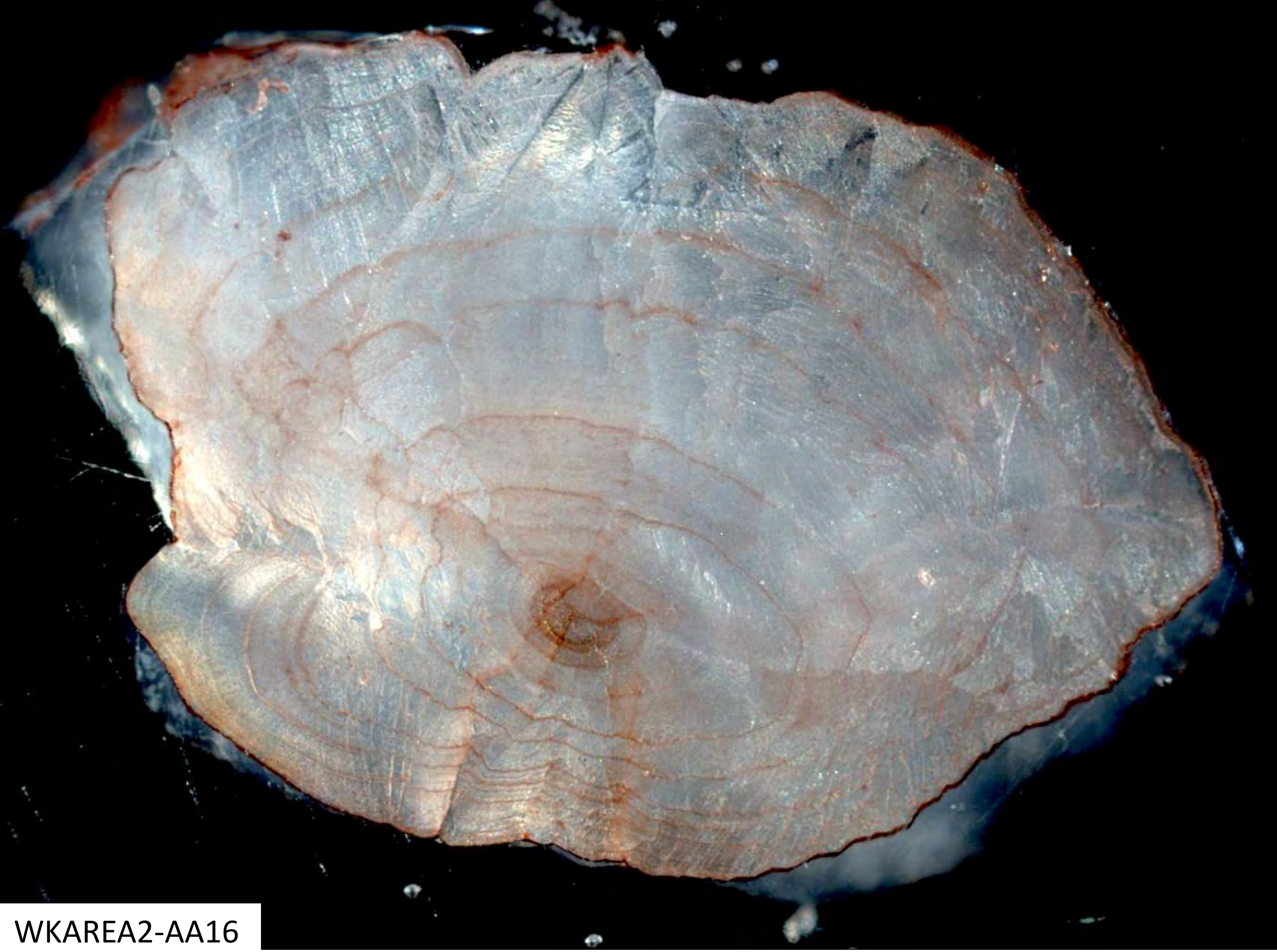
WKAREA2-AA14

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	532	226	female	ALC-marked and stocked as elver



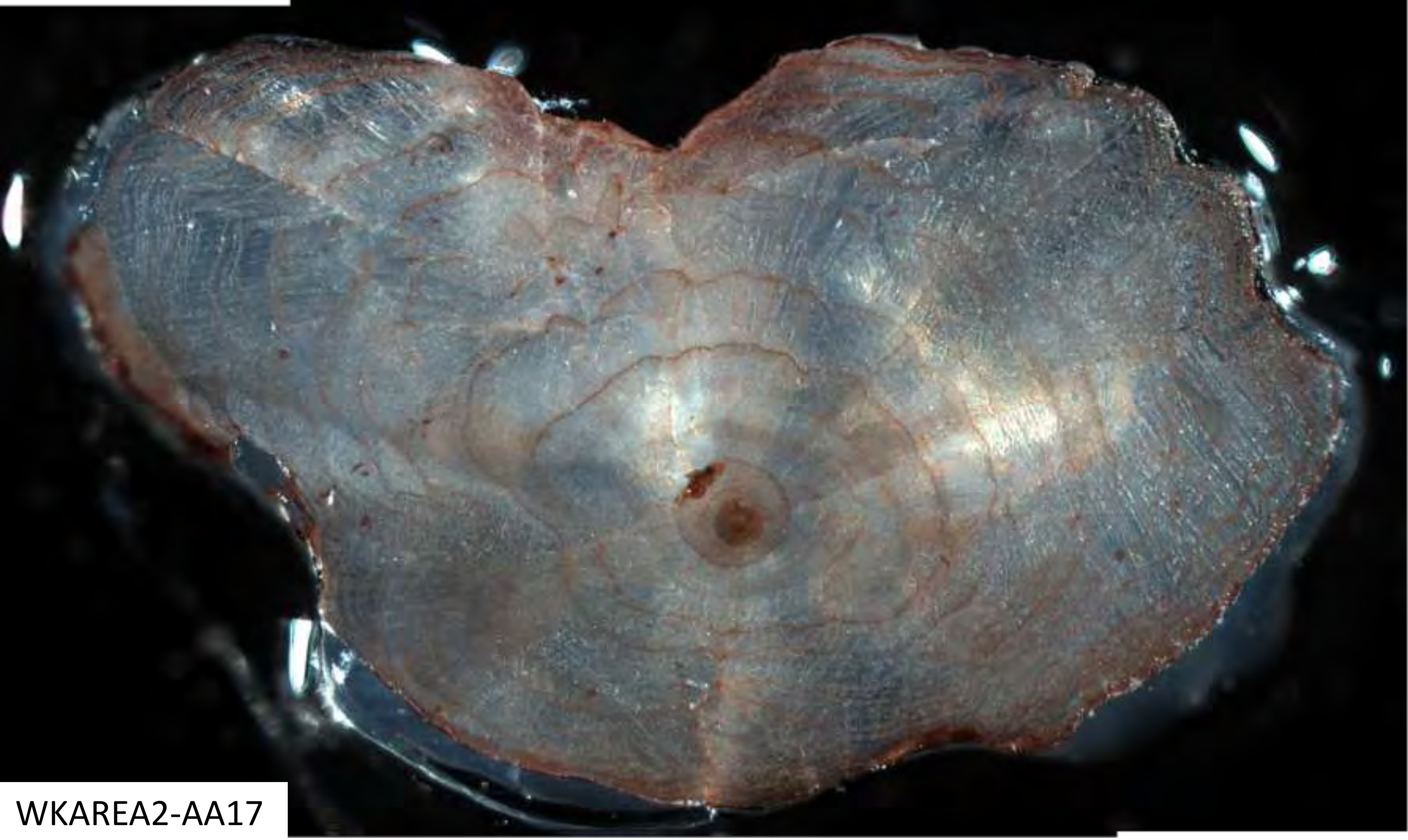
WKAREA2-AA15

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	silver eel	471	197	male	ALC-marked and stocked as elver



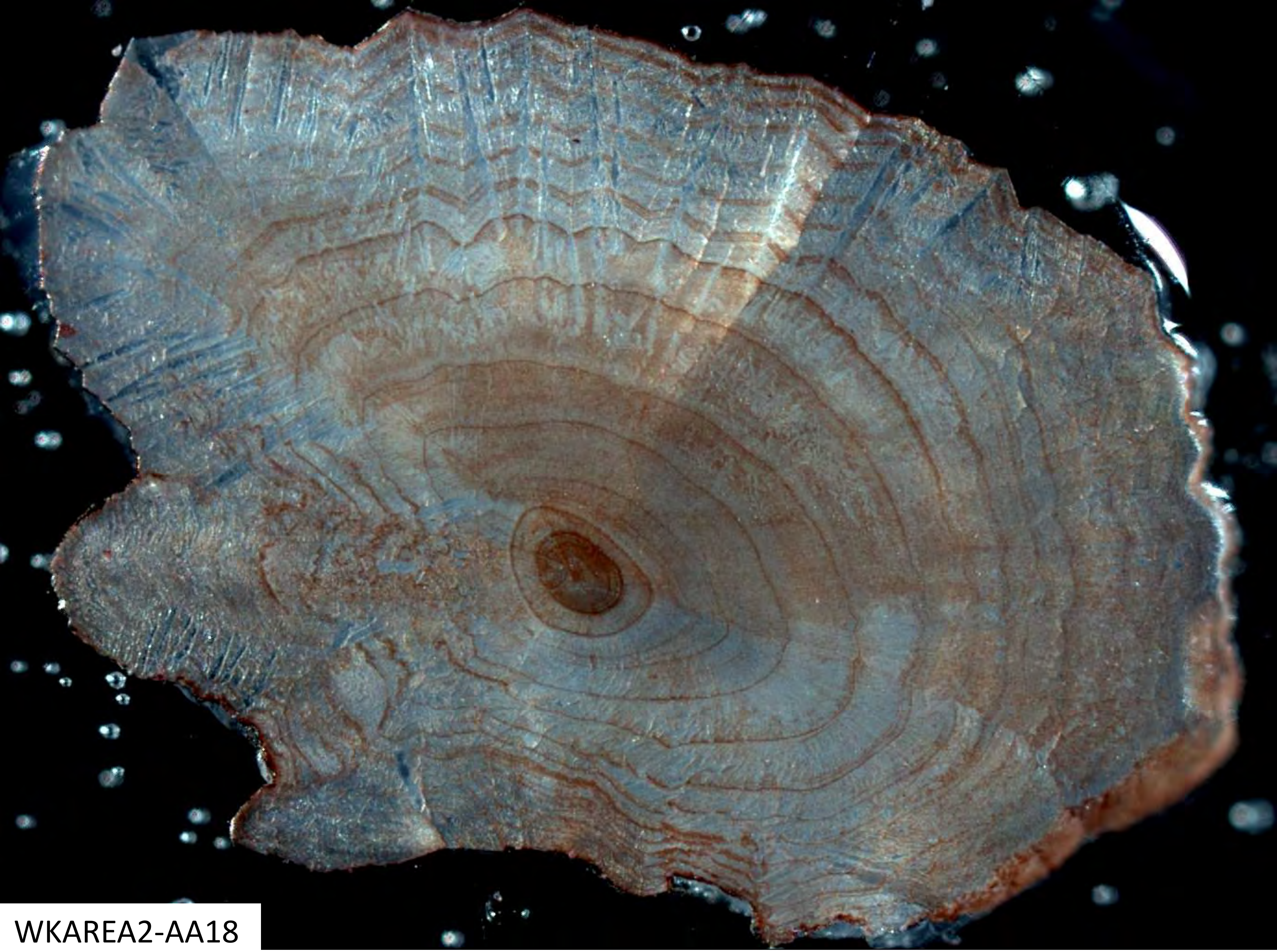
WKAREA2-AA16

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	7	yellow eel	444	124	female	ALC-marked and stocked as elver



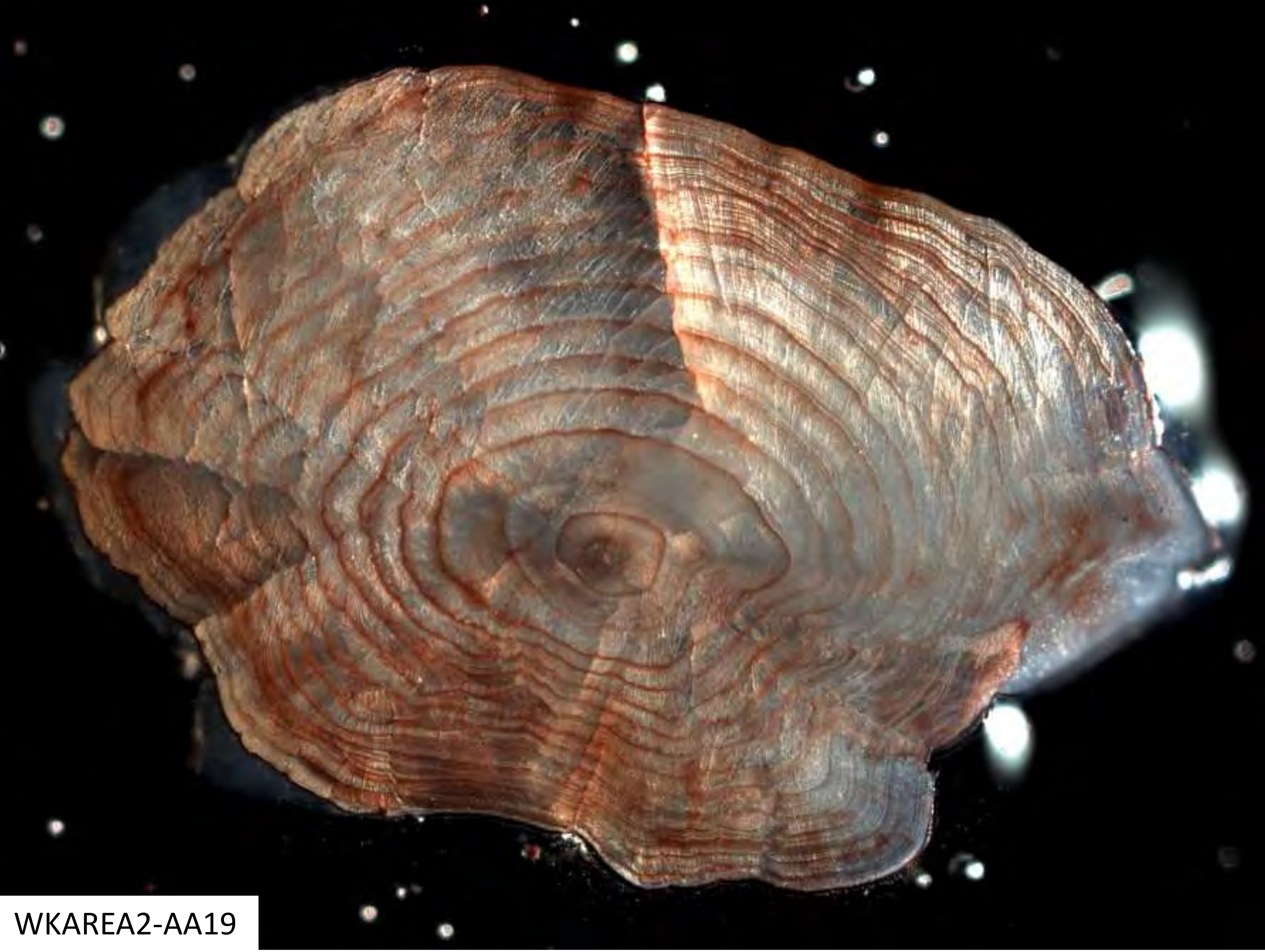
WKAREA2-AA17

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	589	304	female	ALC-marked and stocked as elver



WKAREA2-AA18

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	518	233	female	ALC-marked and stocked as elver



WKAREA2-AA19

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	546	217	female	ALC-marked and stocked as elver

WKAREA2-AA20

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	529	257	female	ALC-marked and stocked as elver

WKAREA2-AA21

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	572	358	female	ALC-marked and stocked as elver

WKAREA2-AA22

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	544	289	female	ALC-marked and stocked as elver

WKAREA2-AA23

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	328	51	female	ALC-marked and stocked as elver

WKAREA2-AA24

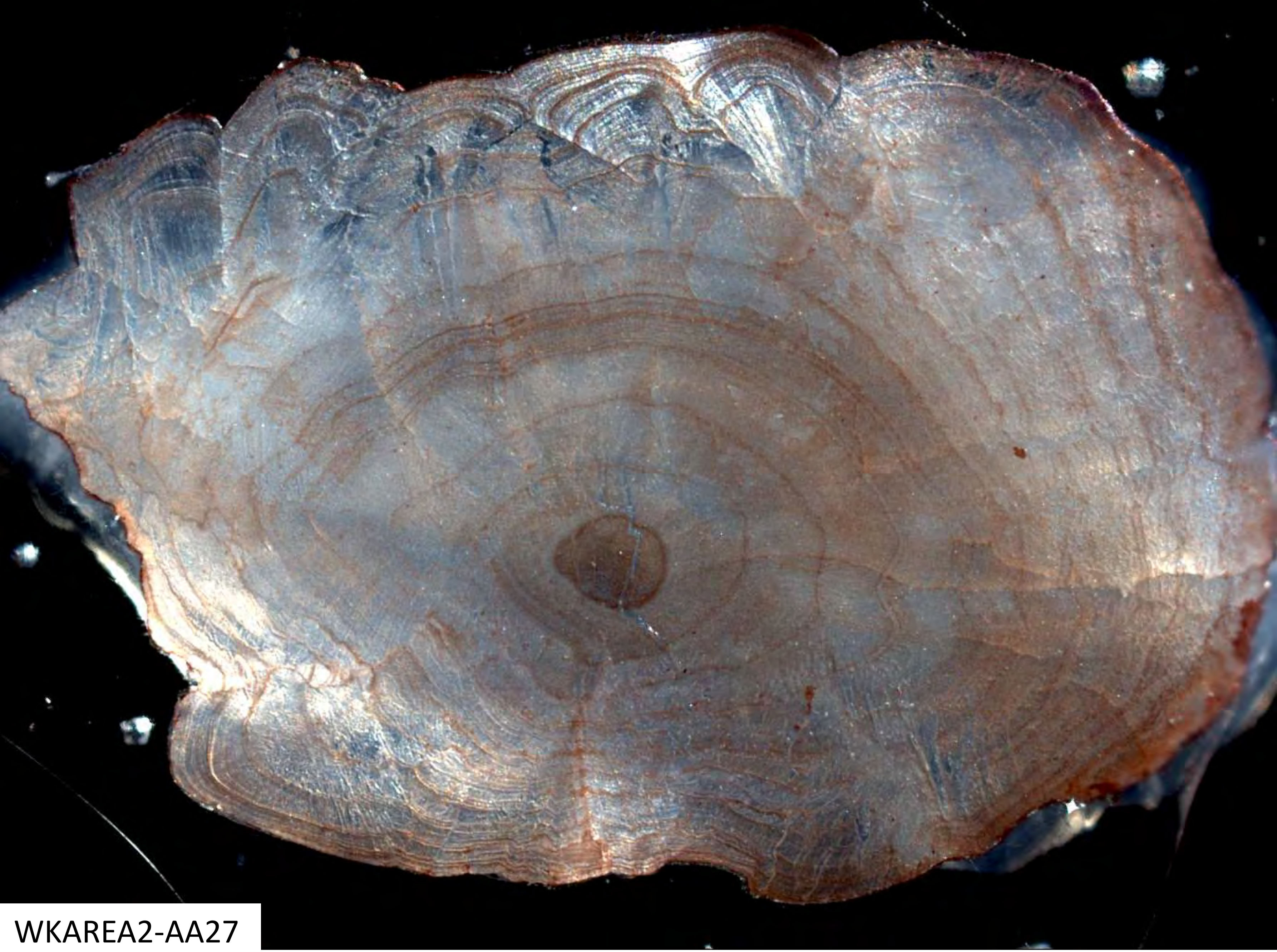
country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	410	103	female	ALC-marked and stocked as elver

WKAREA2-AA25

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	7	yellow eel	485	153	female	ALC-marked and stocked as elver

WKAREA2-AA26

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	558	260	female	ALC-marked and stocked as elver



WKAREA2-AA27

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	526	222	female	ALC-marked and stocked as elver

WKAREA2-AA28

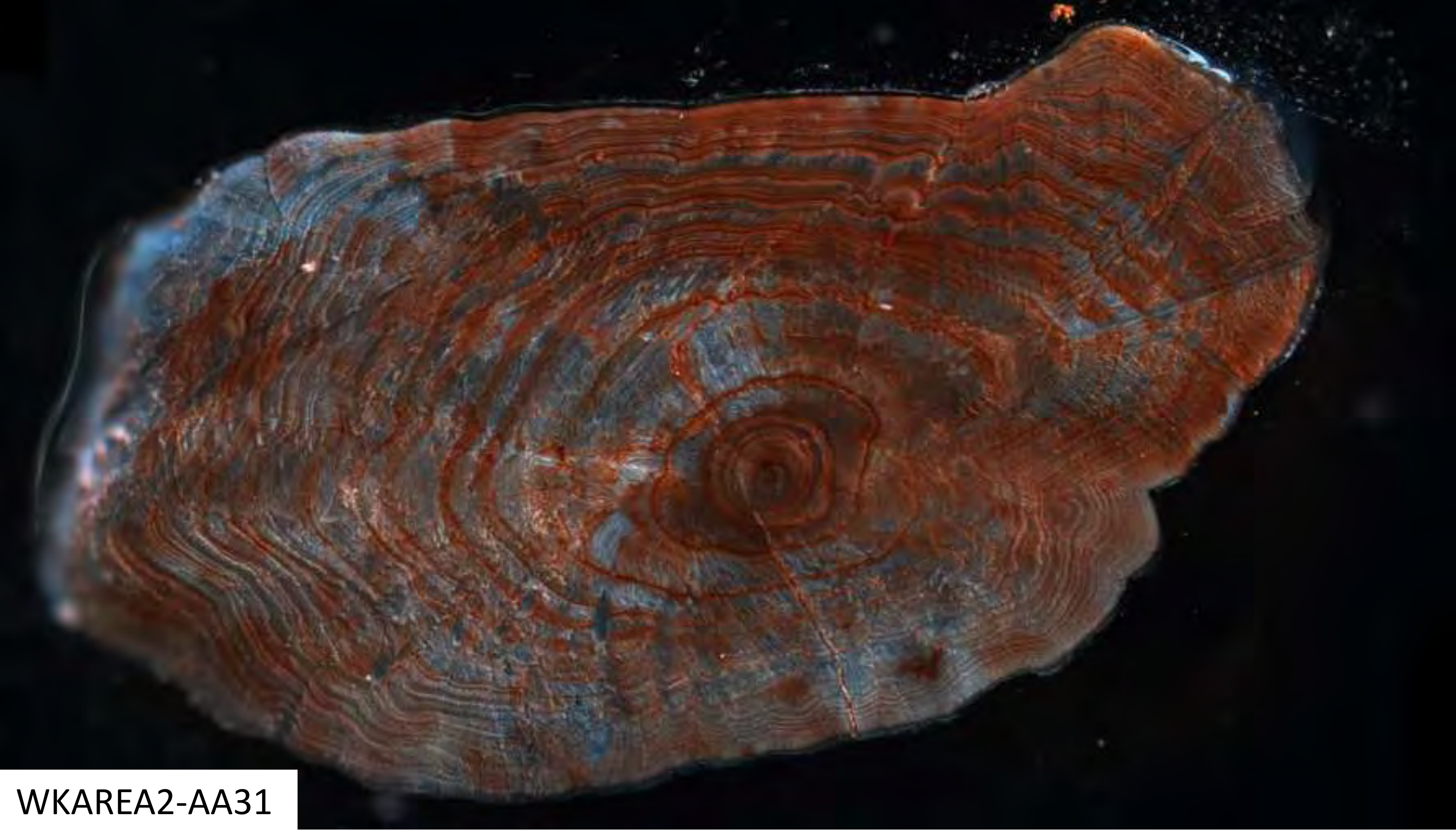
country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	545	247	female	ALC-marked and stocked as elver

WKAREA2-AA29

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	549	252	female	ALC-marked and stocked as elver

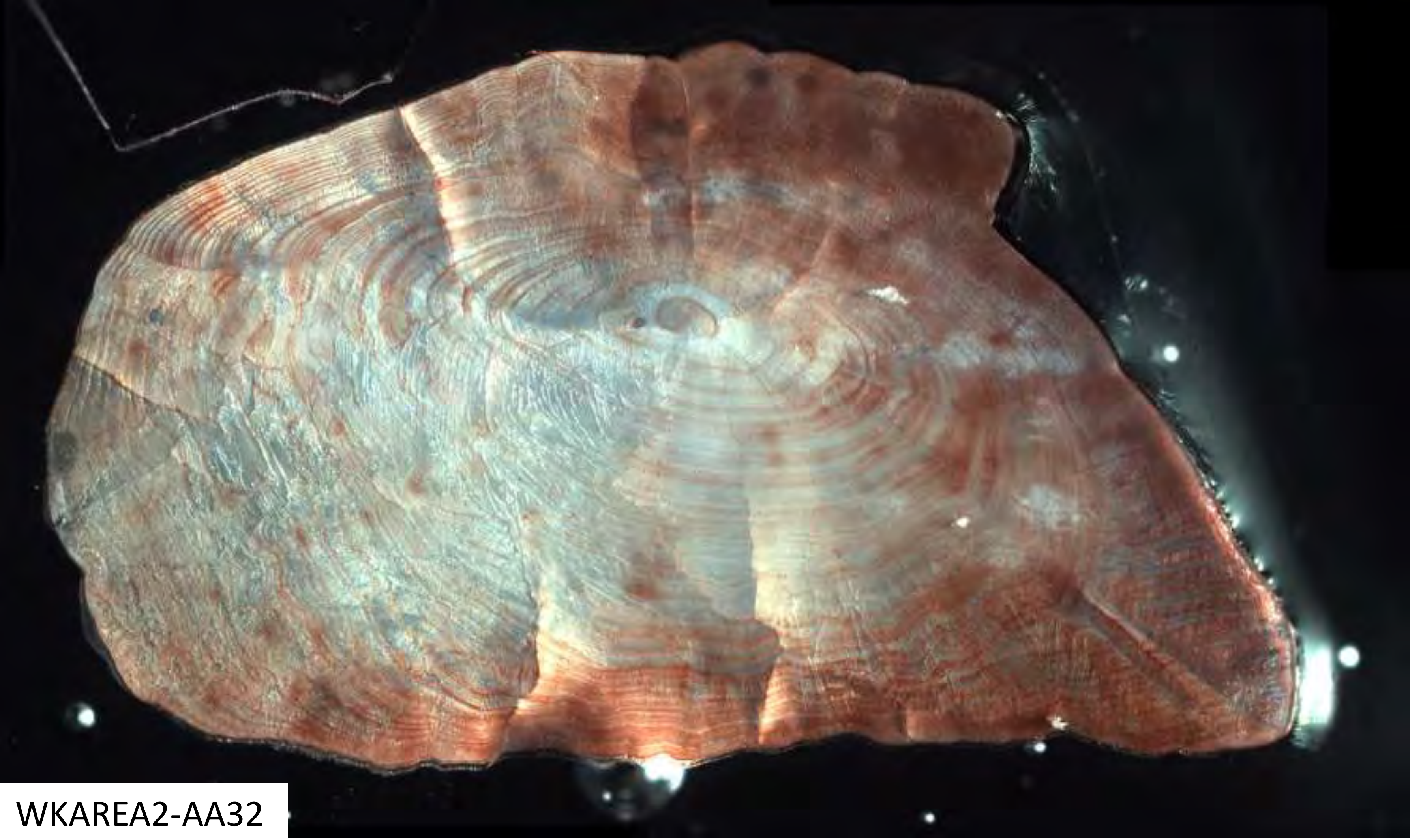
WKAREA2-AA30

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	404	92	female	ALC-marked and stocked as elver



WKAREA2-AA31

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Fardumeträsk	6-11	silver eel	762	793	female	stocked as elver in eel free lake



WKAREA2-AA32

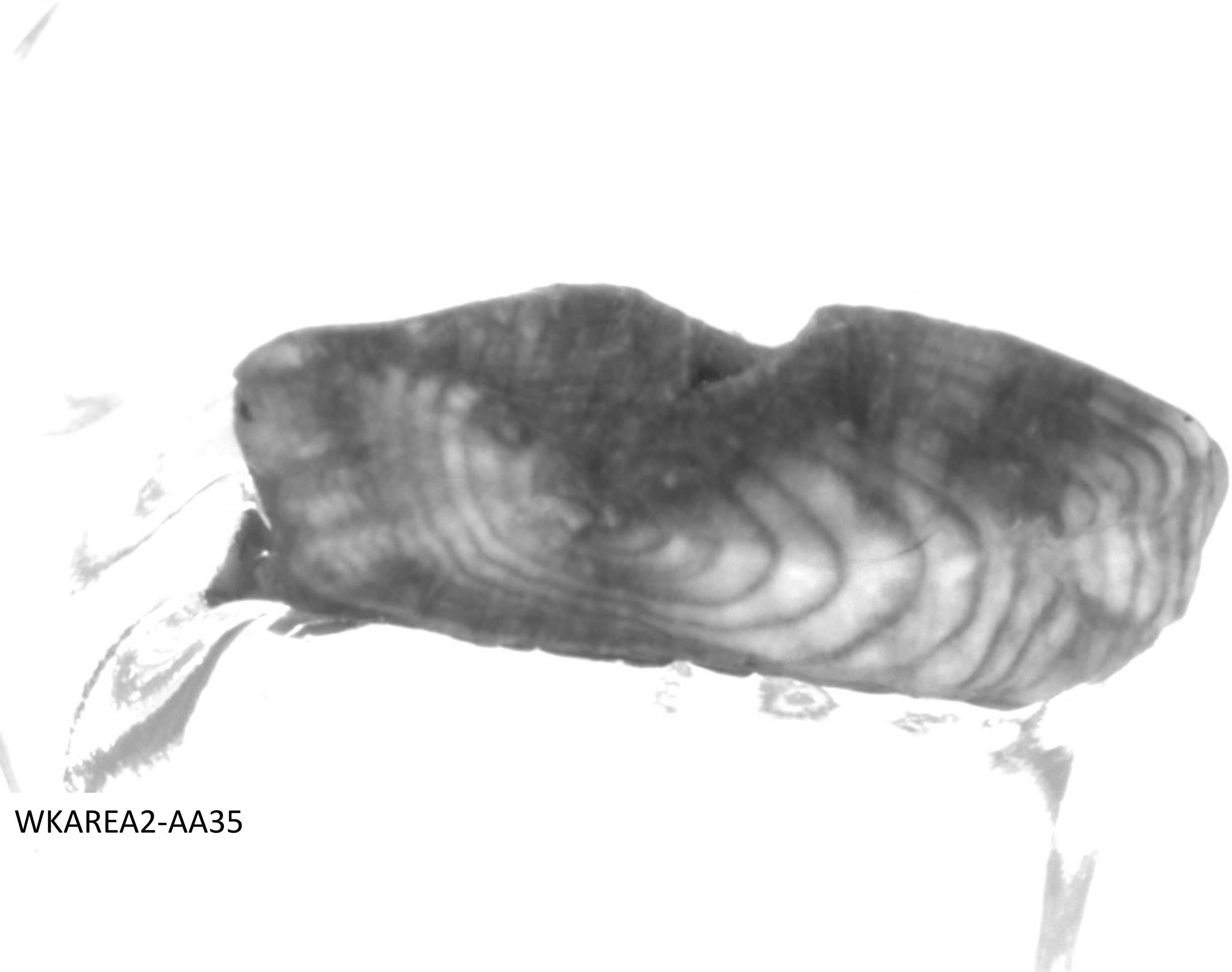
country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Ången	4	silver eel	1031	3003	female	stocked as yellow eel

WKAREA2-AA33

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	469	158	female	ALC-marked and stocked as elver

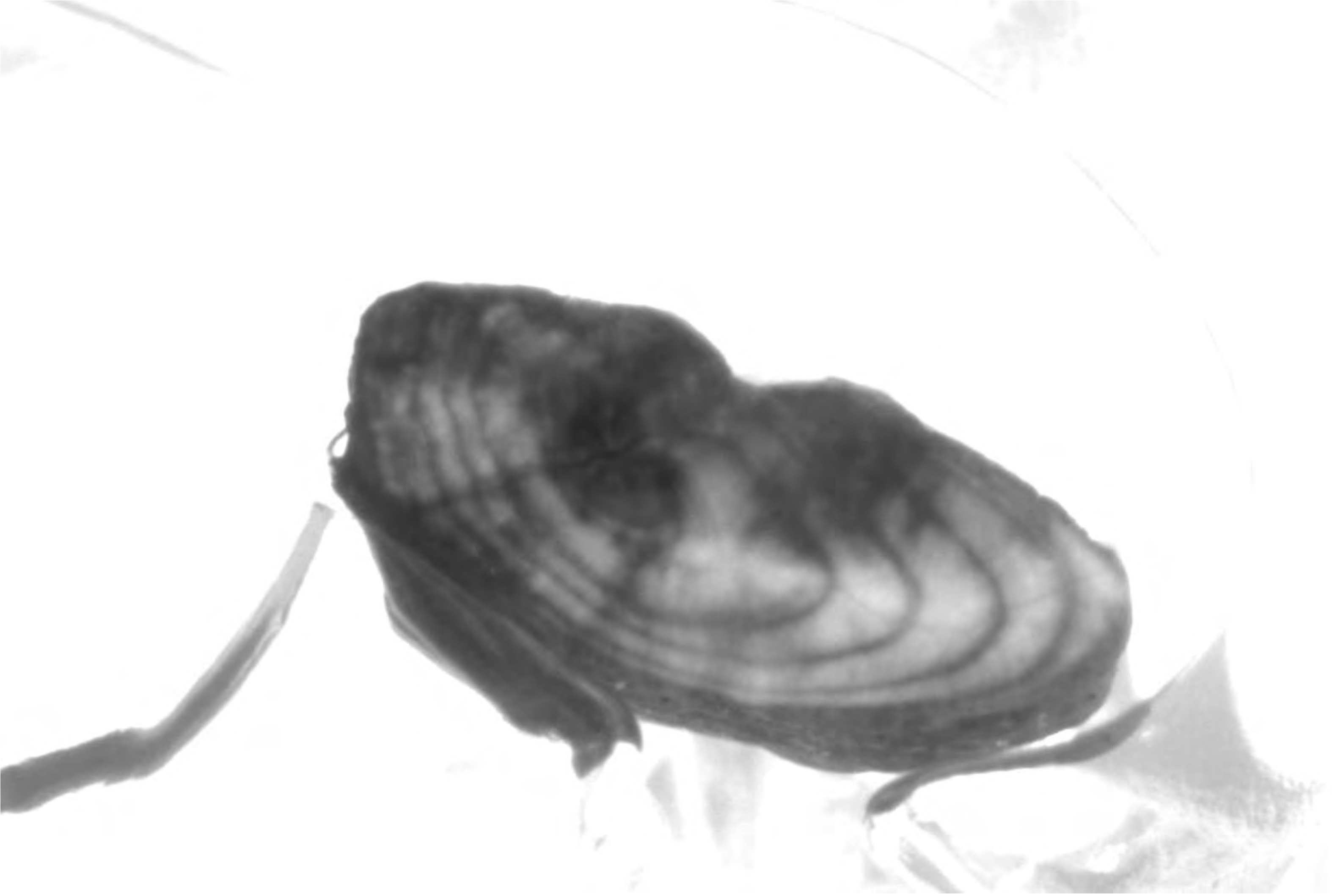
WKAREA2-AA34

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	701	548	female	ALC-marked and stocked as elver



WKAREA2-AA35

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	518	233	female	ALC-marked and stocked as elver



WKAREA2-AA36

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	6	yellow eel	410	103	female	ALC-marked and stocked as elver

WKAREA2-AA37

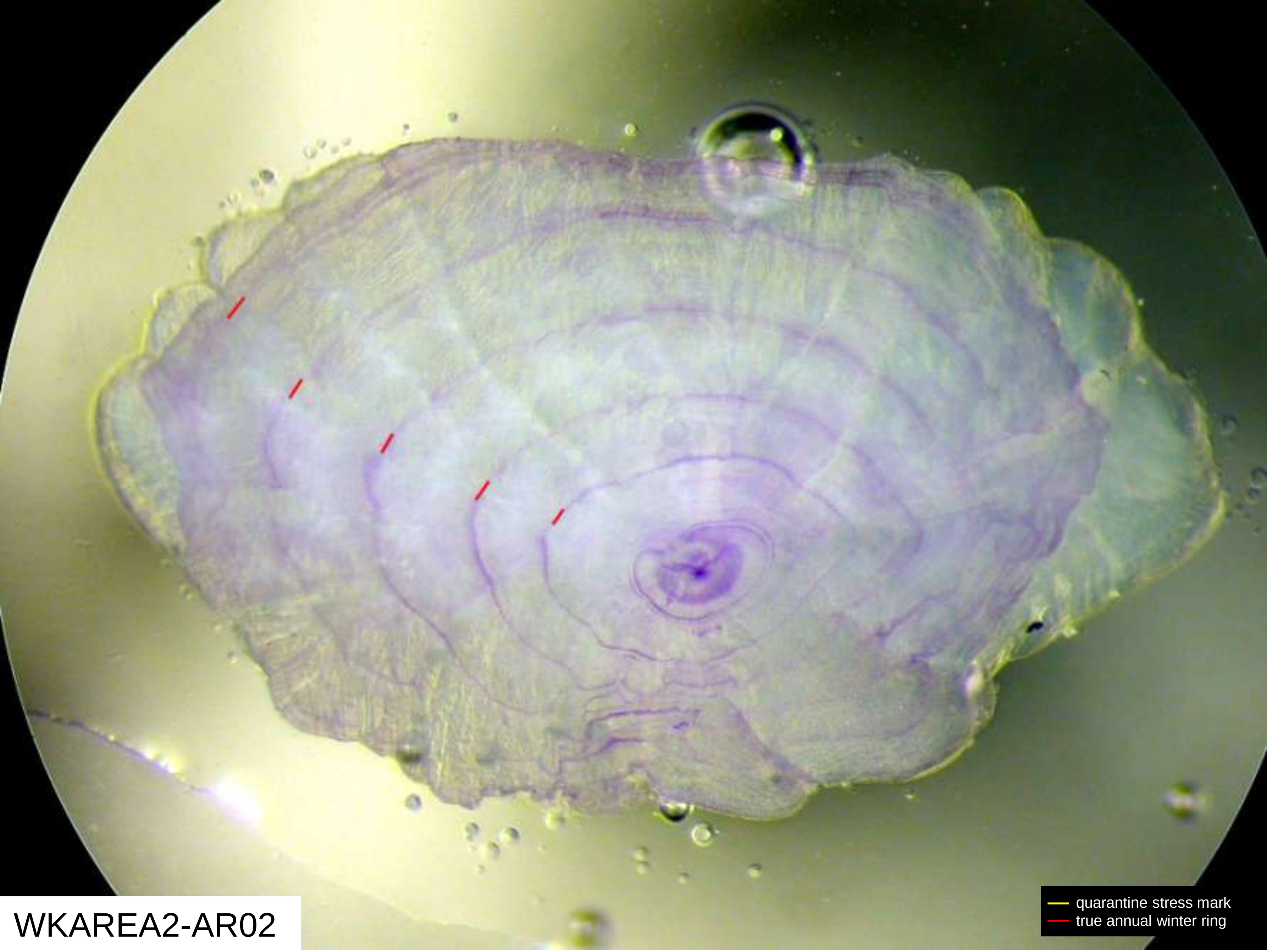
country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Fardume	3	silver eel	754	848	female	stocked

WKAREA2-AA38

country	location	month	life stage	length (mm)	weight (g)	sex	history
Sweden	Lake Mälaren	5	yellow eel	446	145	female	ALC-marked and stocked as elver

WKAREA2-AR01

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2007	7	yellow eel	355	female	OTC-marked and stocked as glass eel in 1999	8

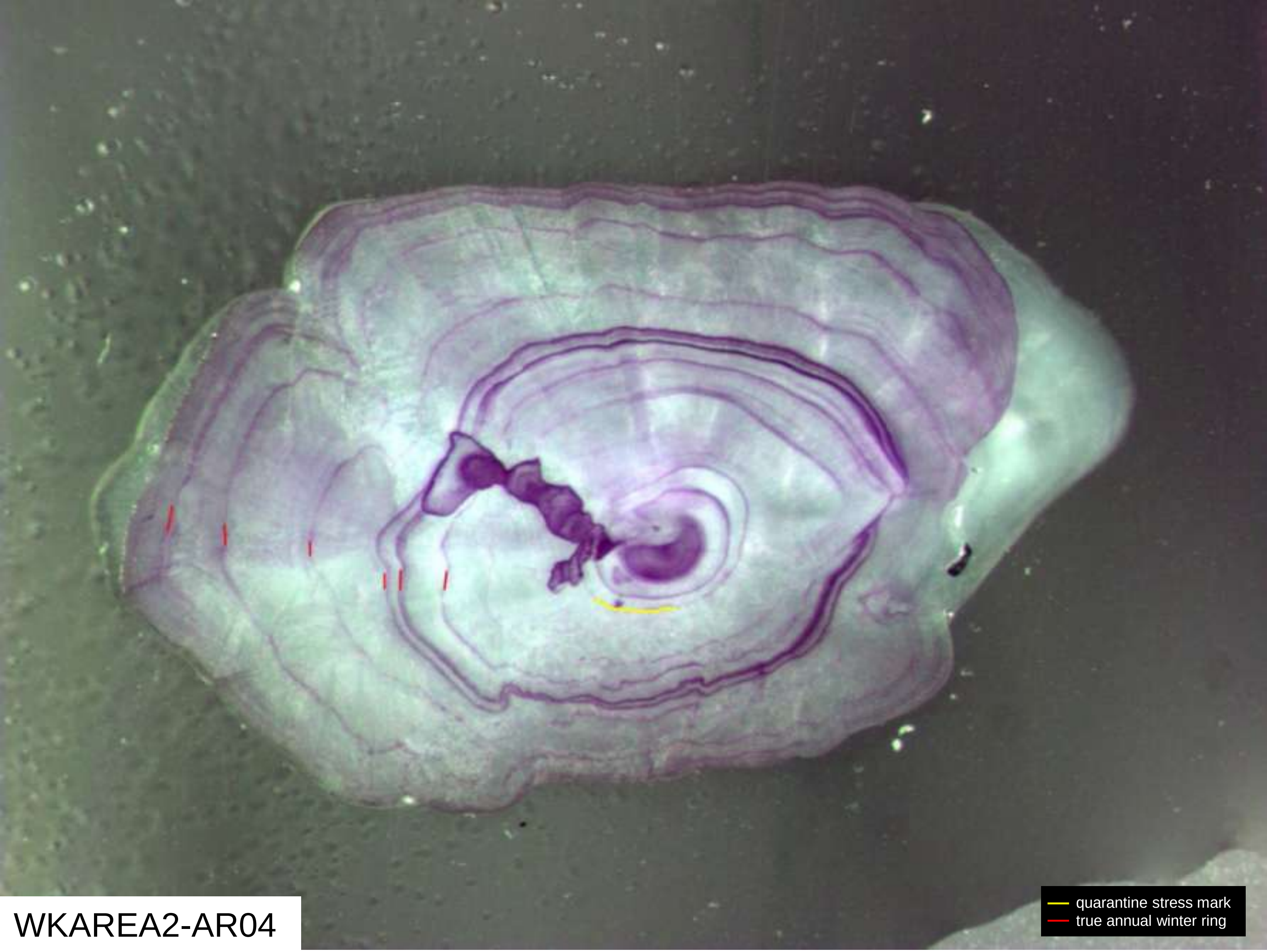


WKAREA2-AR02

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2004	7	yellow eel	444	female	OTC-marked and stocked as glass eel in 1999	5

WKAREA2-AR03

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2003	7	yellow eel	512	undetermined	OTC-marked and stocked as glass eel in 1999	4



country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2005	7	yellow eel	364	female	OTC-marked and stocked as glass eel in 1999	6

WKAREA2-AR05

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2008	7	yellow eel	320	female	OTC-marked and stocked as glass eel in 1999	9

WKAREA2-AR06

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2008	7	yellow eel	256	female	OTC-marked and stocked as glass eel in 1999	9

WKAREA2-AR07

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2004	7	yellow eel	147	female	OTC-marked and stocked as glass eel in 1999	5

WKAREA2-AR08

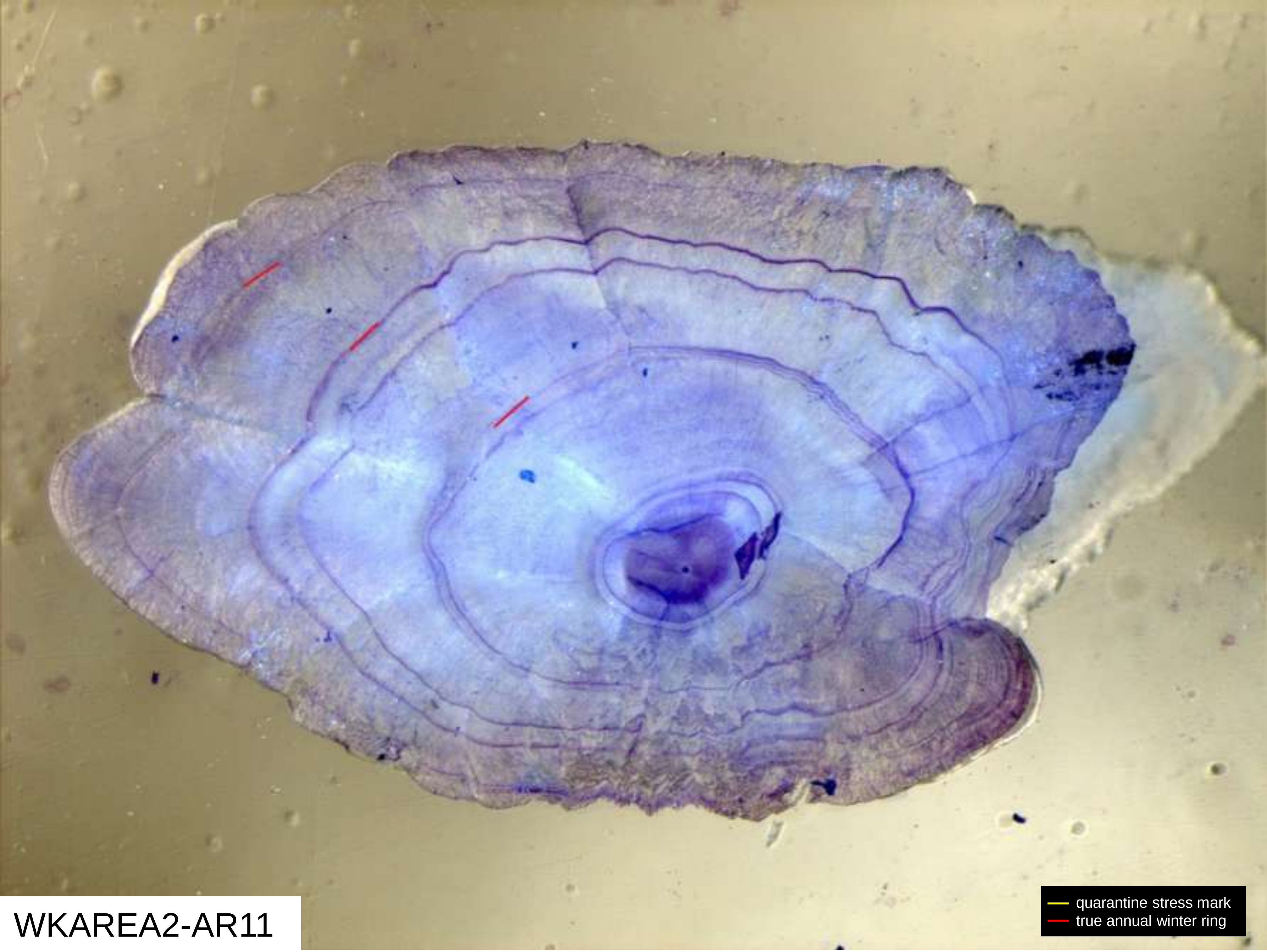
country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2004	7	yellow eel	563	female	OTC-marked and stocked as glass eel in 1999	5

WKAREA2-AR09

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2002	7	yellow eel	383	undetermined	OTC-marked and stocked as glass eel in 1999	3

WKAREA2-AR10

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2004	7	yellow eel	253	female	OTC-marked and stocked as glass eel in 1999	5



WKAREA2-AR11

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2002	7	yellow eel	376	undetermined	OTC-marked and stocked as glass eel in 1999	3

WKAREA2-AR12

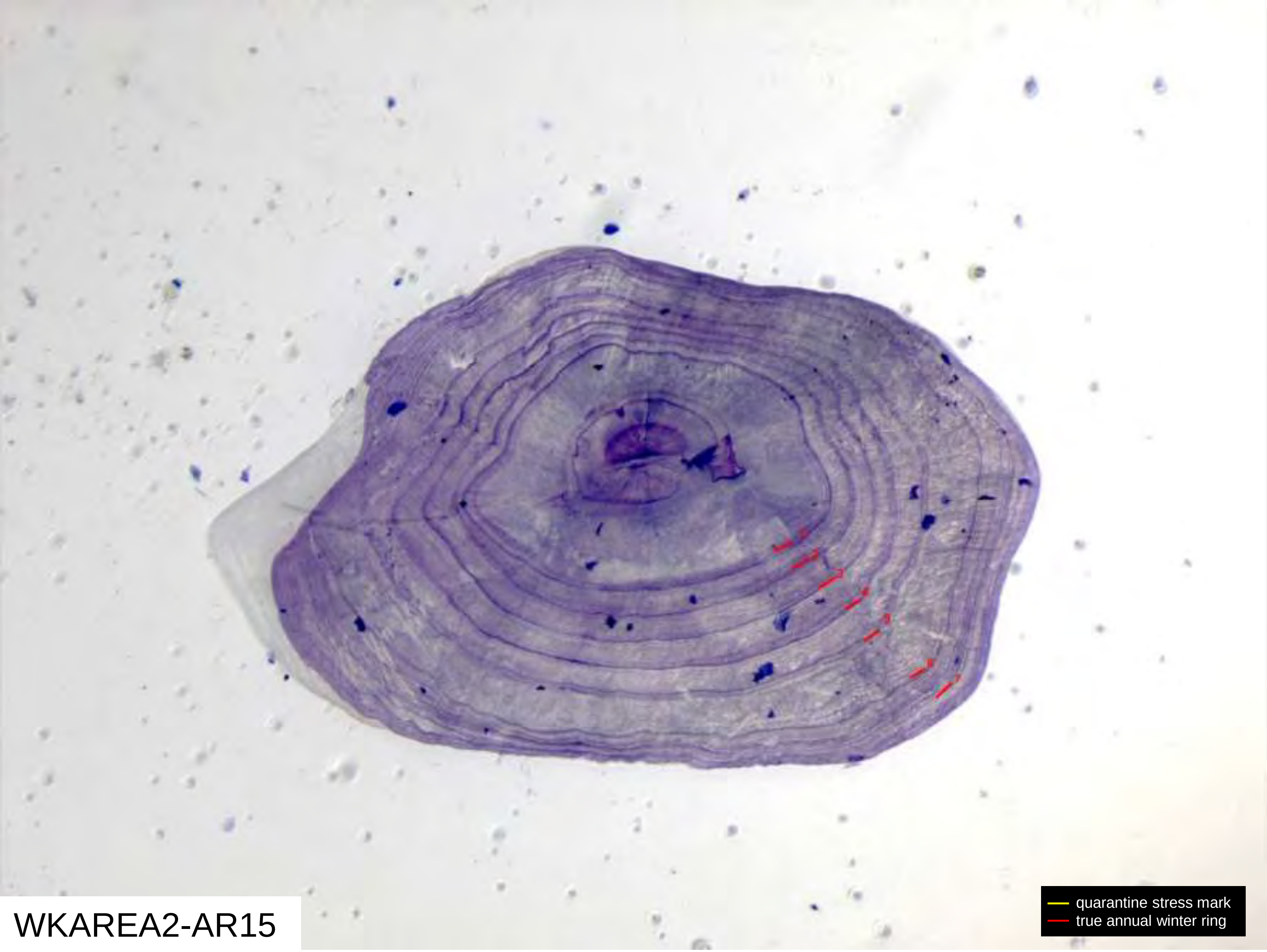
country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2002	8	yellow eel	145	undetermined	OTC-marked and stocked as glass eel in 1999	3

WKAREA2-AR13

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2008	7	yellow eel	495	female	OTC-marked and stocked as glass eel in 1999	9

WKAREA2-AR14

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2005	7	yellow eel	562	female	OTC-marked and stocked as glass eel in 1999	6

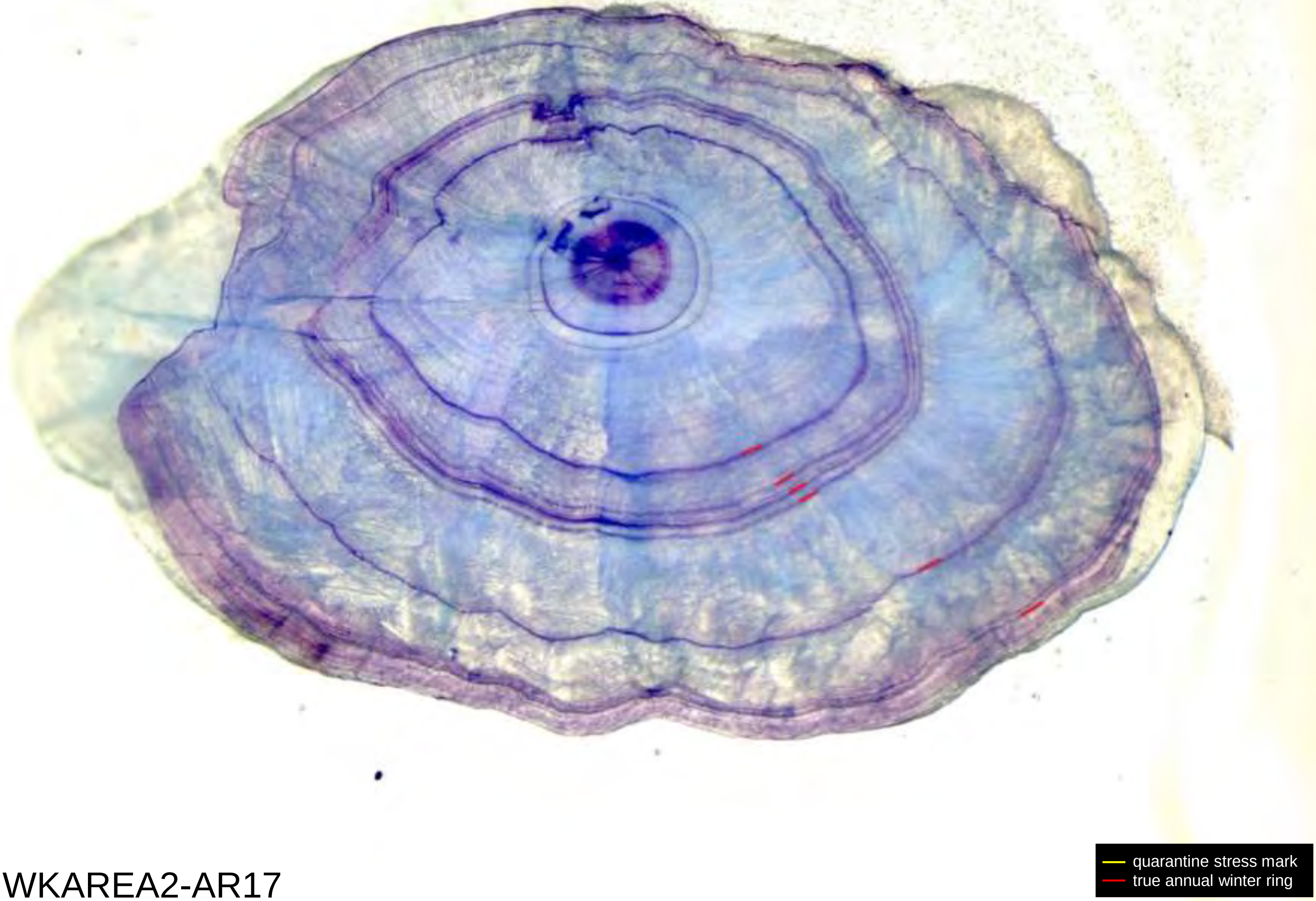


WKAREA2-AR15

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2006	7	yellow eel	225	female	OTC-marked and stocked as glass eel in 1999	7

WKAREA2-AR16

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2006	7	yellow eel	620	female	OTC-marked and stocked as glass eel in 1999	7



WKAREA2-AR17

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2005	7	yellow eel	365	female	OTC-marked and stocked as glass eel in 1999	6

WKAREA2-AR18

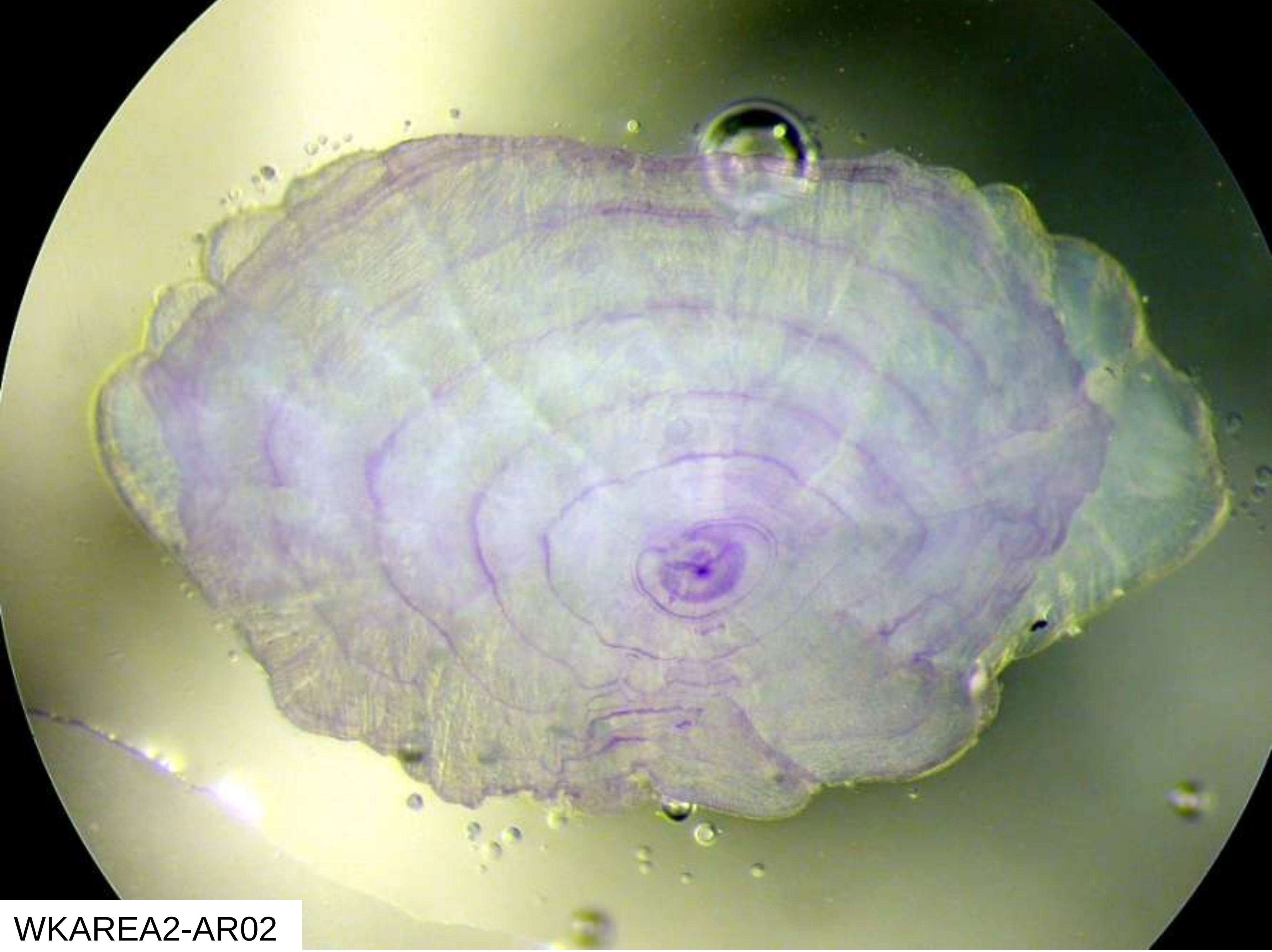
country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2006	7	yellow eel	358	female	OTC-marked and stocked as glass eel in 1999	7

WKAREA2-AR19

country	location	year	month	life stage	length (mm)	sex	history	actual age
Canada	Lac Morin, Quebec	2007	7	yellow eel	317	female	OTC-marked and stocked as glass eel in 1999	8

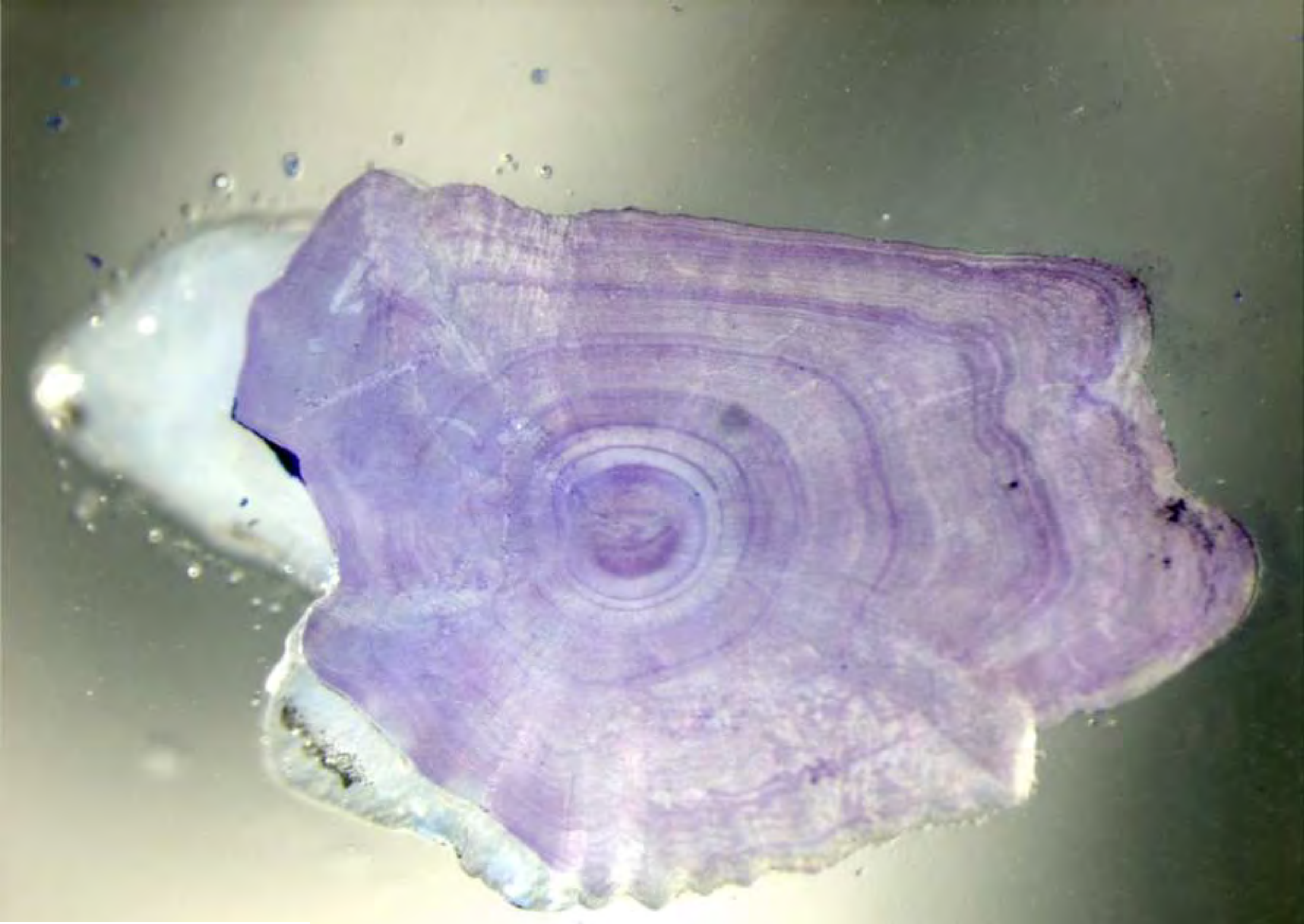
WKAREA2-AR01

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	355	female	OTC-marked and stocked as glass eel



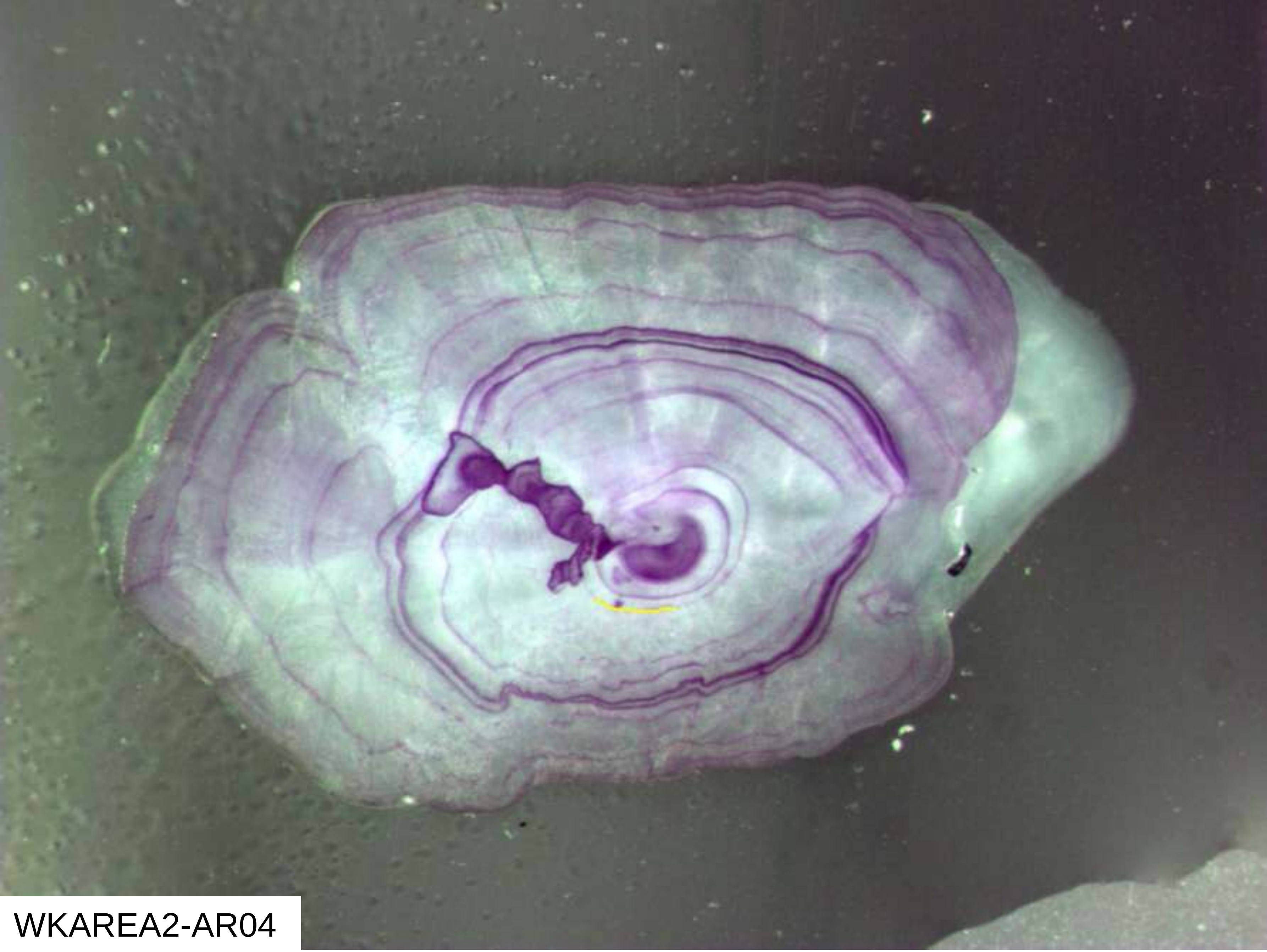
WKAREA2-AR02

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	444	female	OTC-marked and stocked as glass eel



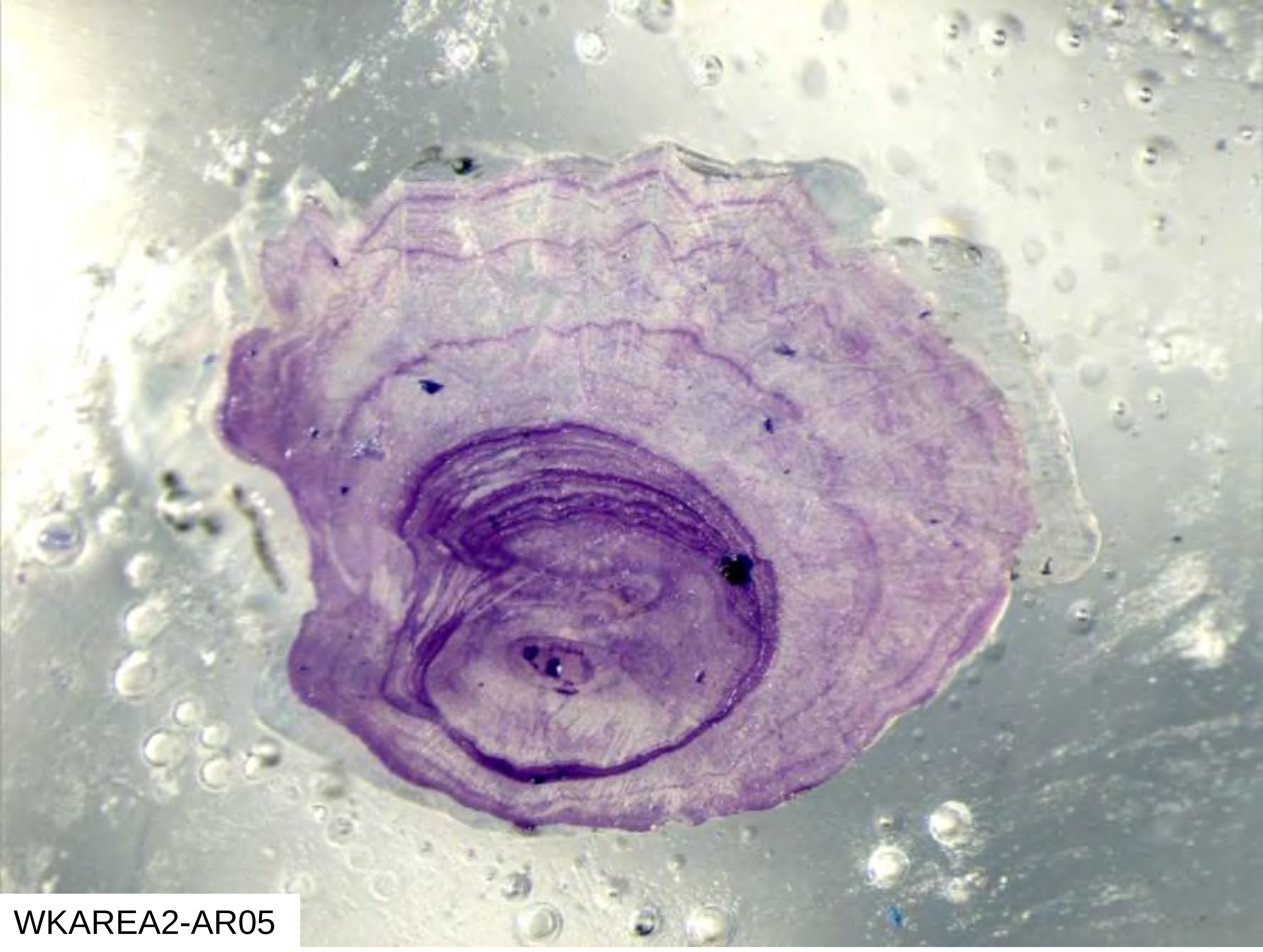
WKAREA2-AR03

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	512	undetermined	OTC-marked and stocked as glass eel



WKAREA2-AR04

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	364	female	OTC-marked and stocked as glass eel



WKAREA2-AR05

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	320	female	OTC-marked and stocked as glass eel

WKAREA2-AR06

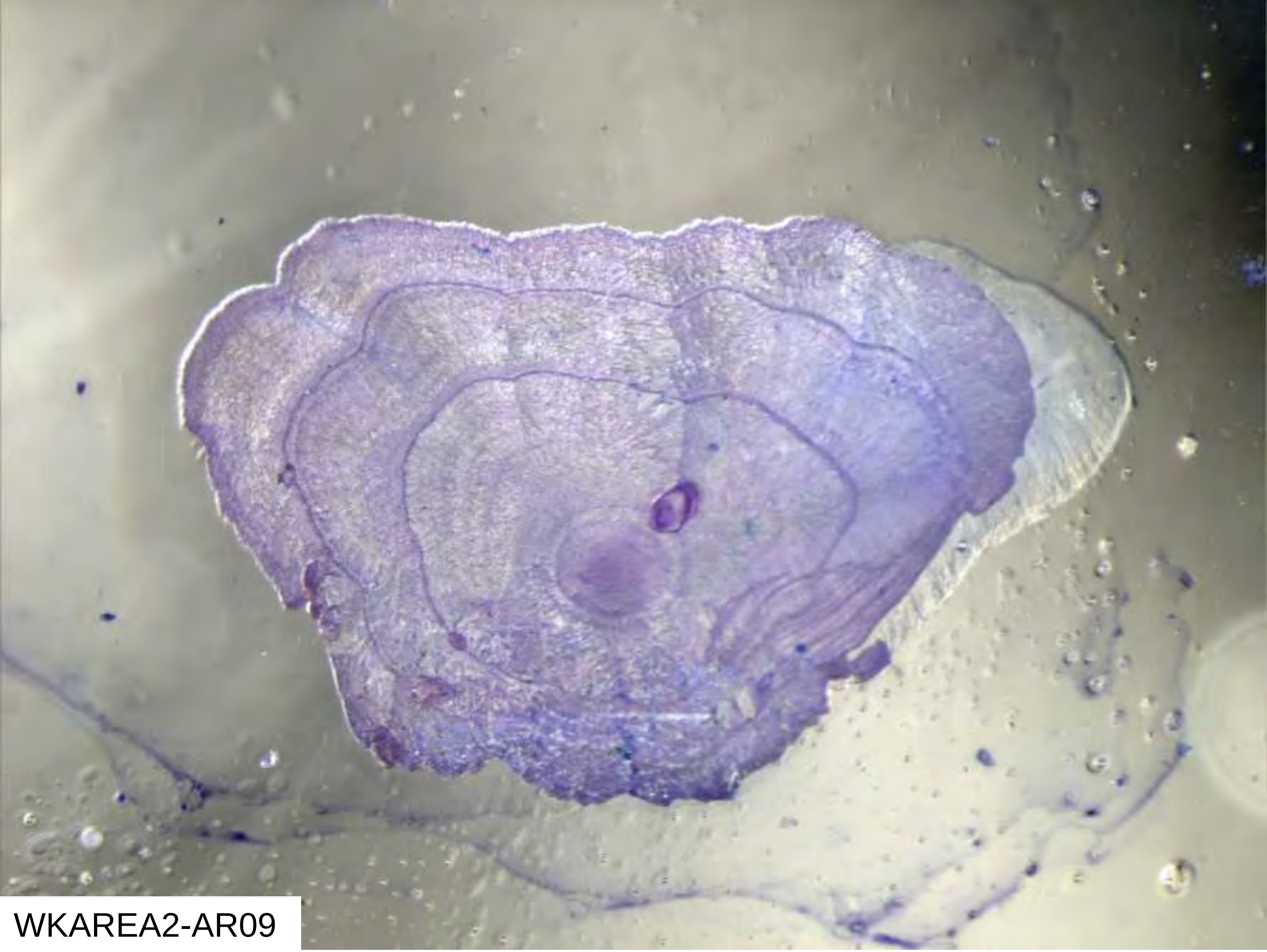
country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	256	female	OTC-marked and stocked as glass eel

WKAREA2-AR07

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	147	female	OTC-marked and stocked as glass eel

WKAREA2-AR08

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	563	female	OTC-marked and stocked as glass eel

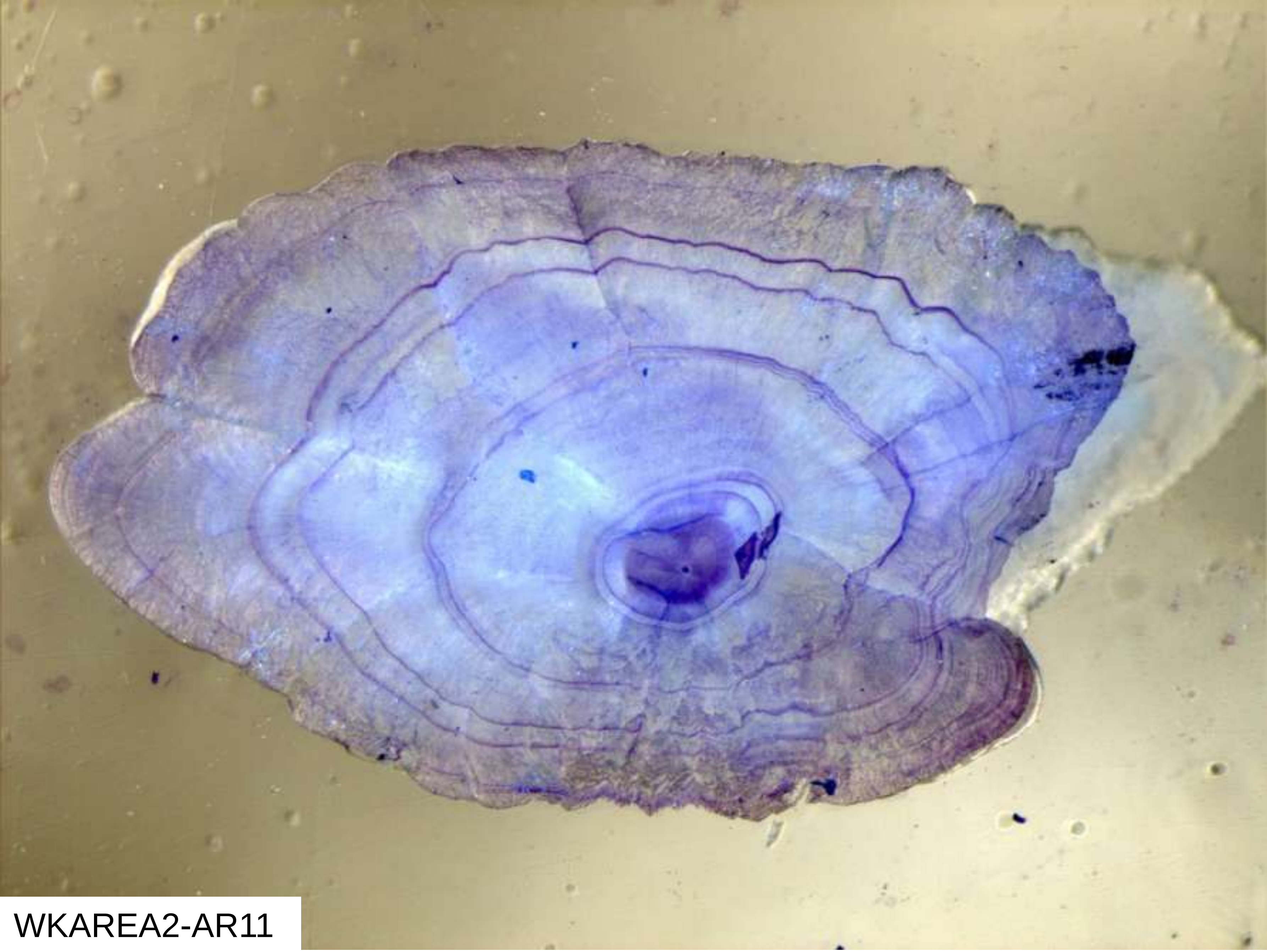


WKAREA2-AR09

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	383	undetermined	OTC-marked and stocked as glass eel

WKAREA2-AR10

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	253	female	OTC-marked and stocked as glass eel



WKAREA2-AR11

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	376	undetermined	OTC-marked and stocked as glass eel

WKAREA2-AR12

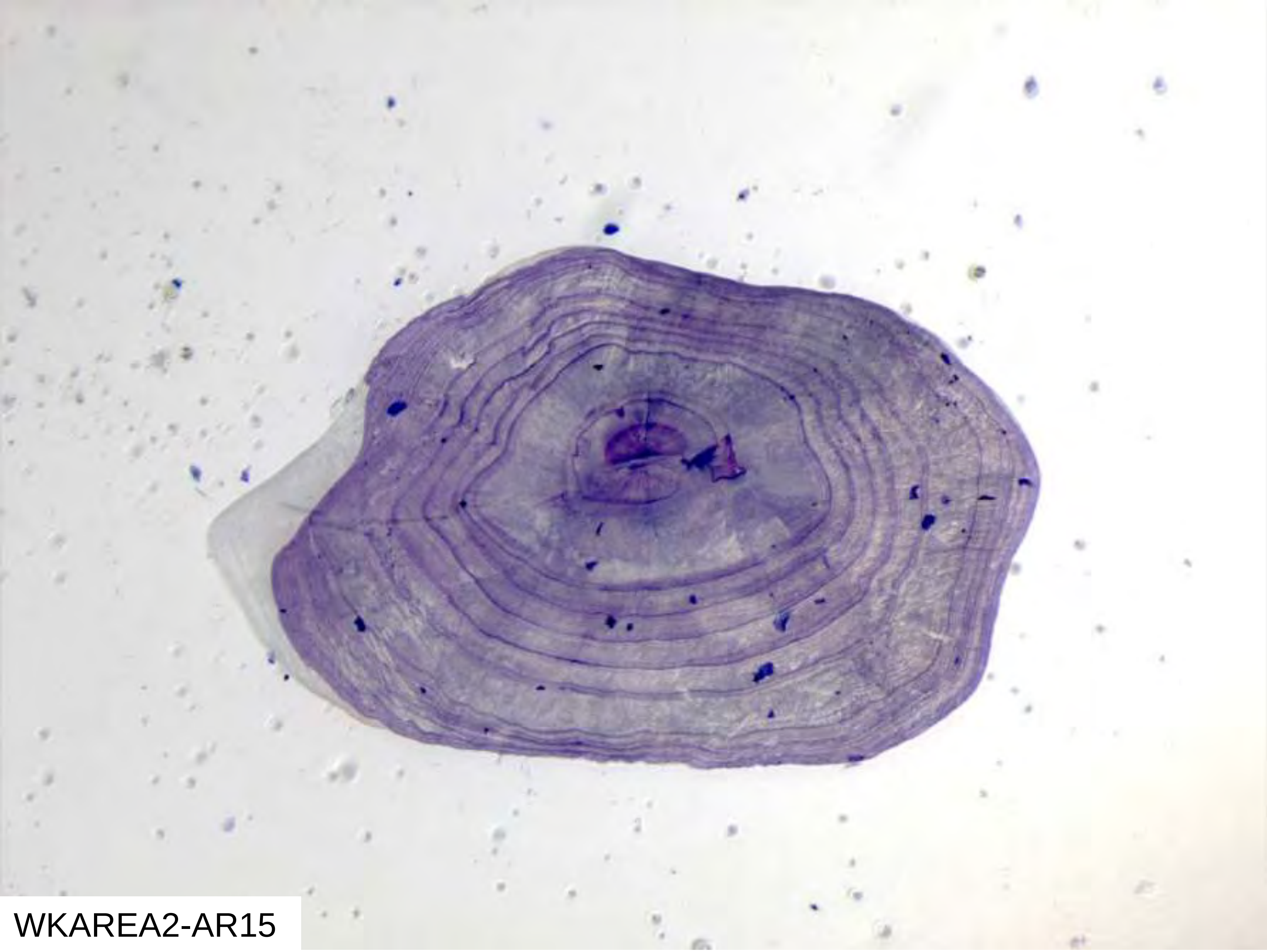
country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	8	yellow eel	145	undetermined	OTC-marked and stocked as glass eel

WKAREA2-AR13

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	495	female	OTC-marked and stocked as glass eel

WKAREA2-AR14

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	562	female	OTC-marked and stocked as glass eel

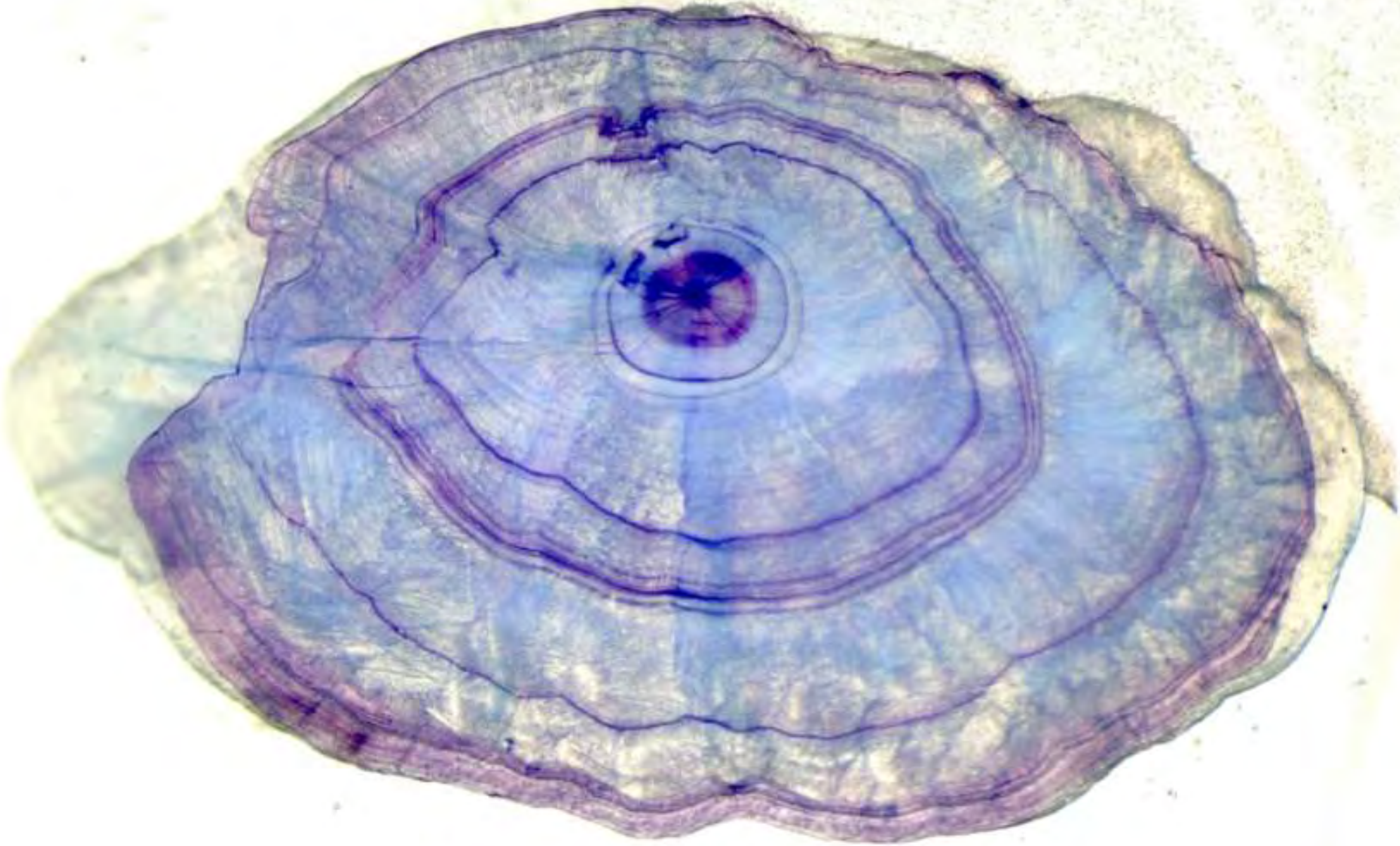


WKAREA2-AR15

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	225	female	OTC-marked and stocked as glass eel

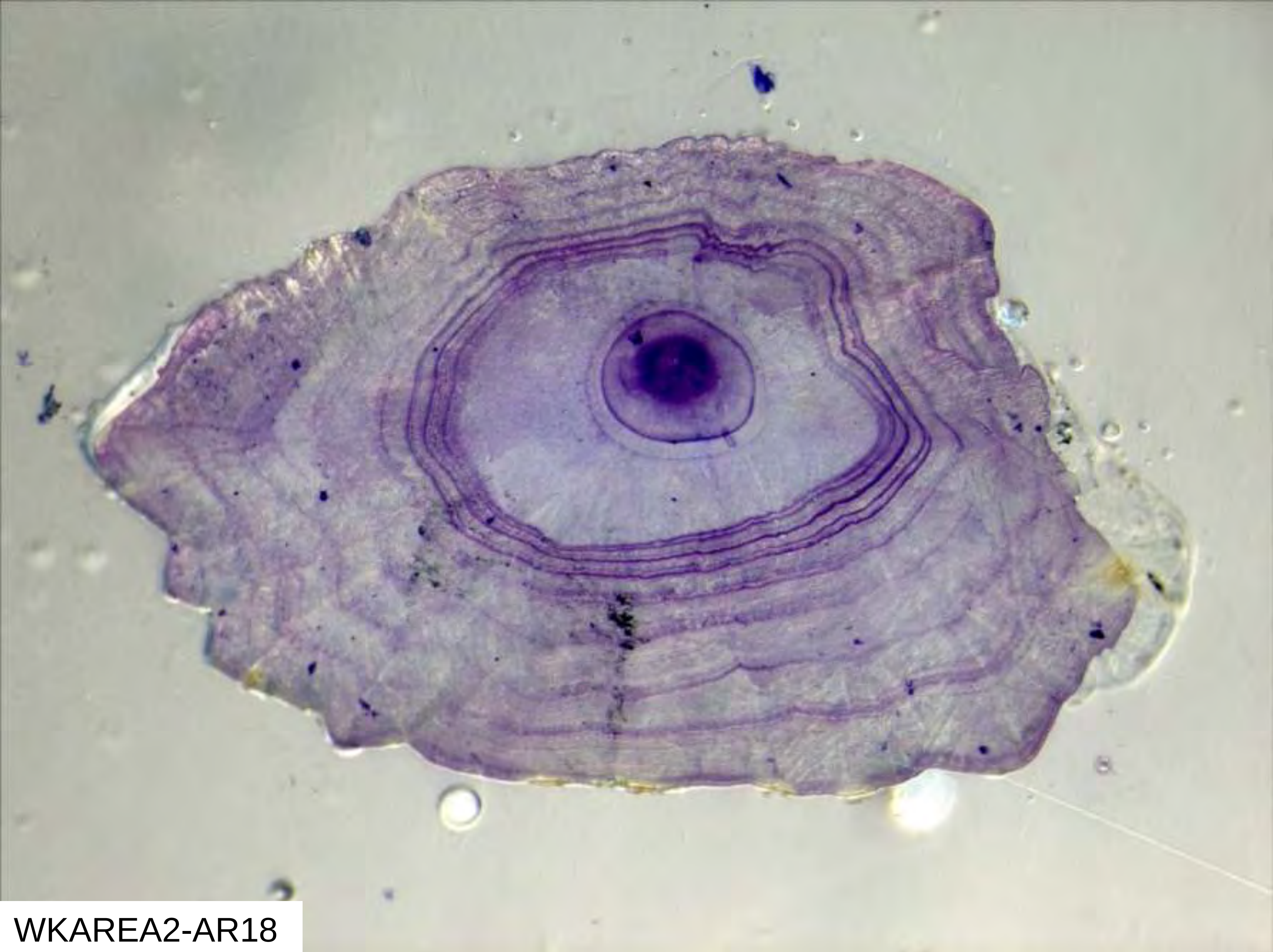
WKAREA2-AR16

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	620	female	OTC-marked and stocked as glass eel



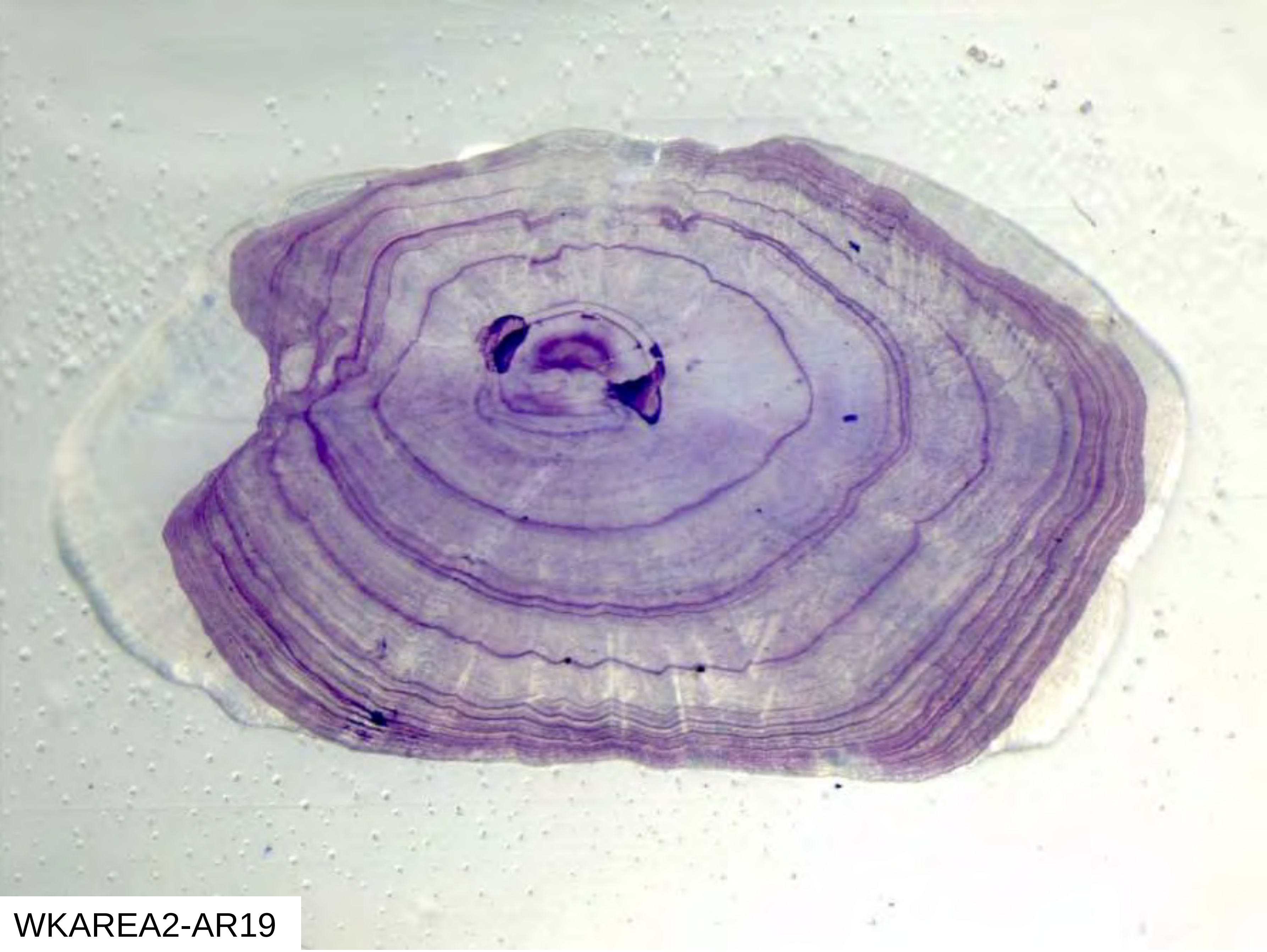
WKAREA2-AR17

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	365	female	OTC-marked and stocked as glass eel



WKAREA2-AR18

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	358	female	OTC-marked and stocked as glass eel



WKAREA2-AR19

country	location	month	life stage	length (mm)	sex	history
Canada	Lac Morin, Quebec	7	yellow eel	317	female	OTC-marked and stocked as glass eel