EPA STRIVE Programme 2007–2013

CLASSIFICATION OF FRESHWATER SPONGE COLLECTION

Classification of Freshwater Sponge Collection at EPA Kilkenny

A Small Scale Study under the Science, Technology, Research and Innovation for the Environment (STRIVE) Programme 2007–2013

End of Project Report available for download on http://erc.epa.ie/safer/reports

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The EPA STRIVE Programme addresses the need for research in Ireland to inform policymakers and other stakeholders on a range of questions in relation to environmental protection. These reports are intended as contributions to the necessary debate on the protection of the environment.

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PROJECT STATEMENT

The 10-week study to classify taxonomically the freshwater sponge collection was carried out at the Biology Laboratory of the Environmental Protection Agency (EPA) in Kilkenny.

Species records from the study will be donated to the National Biodiversity Data Centre (NBDC).

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Bryan Kennedy obligingly carried out scanning electron microscopy (SEM) on some samples and Martin McGarrigle is also acknowledged for kindly facilitating this work. The authors would like to thank the staff/Dermot Daly from the CRANN Advanced Microscopy Laboratory, CRANN, Trinity College Dublin for assistance with electron microscopy.

Michelle O'Neill, who had been on a FÁS placement, catalogued some of the earlier site records and Kevin Clabby provided eight additional sponge samples collected from outside the main study area.

Sierra McKenna tentatively identified 50 of the samples from the collection in 2010 for a UREKA project with University College, Dublin.

Karen Evans of the University of Liverpool, the only other person in these islands working on the freshwater sponge fauna, provided some helpful discussions.

Finally, Nigel Monaghan, Keeper and Leona McArdle, Technical Assistant at the Natural History Division of the National Museum of Ireland are gratefully acknowledged for providing a sample of the rare Irish sponge *Eunapius fragilis*, collected in 1910, from which a slide was prepared.

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Executive Summary

The main objective of the project was to classify taxonomically the sponge collection housed at the Environmental Protection Agency's Regional Inspectorate in Kilkenny.

Despite their role in filtering water, no significant work on Irish freshwater sponges has been published since 1920. The Porifera is a group (phylum) that includes some species that are difficult to identify: they are regularly encountered by freshwater biologists but the taxonomic expertise to identify them has been lacking at national level. Among the research recommendations of the Freshwater Working Group of the National Platform for Biodiversity Research is the production of national inventories for less well-known freshwater taxa such as freshwater sponges and an update on their distribution.

As part of an overall project on sponges in southern Irish rivers and streams, 266 sample slide preparations, as well as one from a canal, were permanently mounted, photographed and species identified. The samples had been collected during biological monitoring surveys carried out in the period 1984 to 2012. Species could be identified from the sample material at 230 different sites in 164 rivers.

Sponges were found in all the 12 hydrometric divisions in 11 counties that the main study area comprised. The survey of southern Irish rivers and streams revealed the presence of five species of freshwater sponge: *Ephydatia fluviatilis*, *Racekiela ryderi*, *Spongilla lacustris*, *Ephydatia muelleri* and *Eunapius fragilis* in order of decreasing frequency of occurrence. *Eunapius fragilis*, which is rare in Ireland, was recorded at just one site in the region – at Tallowbridge in the River Bride (Waterford). Previously this species had been recorded from one site in the study area – at Kilsheelan in the River Suir (Tipperary) in 1911 – in the company of *E. fluviatilis*, while in the present study the latter species together with *S. lacustris* was found there. The original Suir sample could not be located among the holdings of the Natural History Museum, but a sample also collected and identified by Jane Stephens from the River Owengar (Mayo) in 1910 was found and a slide prepared from a fragment of the sponge tissue was confirmed as *E. fragilis*. *Racekiela ryderi*, which has its main distribution in eastern North America and is largely absent from Europe, with only known rare recordings for the Hebrides, Faroes and Norway, was found to be of relatively common occurrence in south-western Irish rivers. *Ephydatia fluviatilis*, which was not previously recorded from the south-west, occurs in some of the rivers there and was the most common species found throughout the whole study area.

The current project has filled the void of taxonomic expertise in the freshwater sponge fauna field that was highlighted by the National Biodiversity Data Centre, as well as addressing a research recommendation of the Freshwater Working Group of the National Platform for Biodiversity Research. It is a good example of cooperation between research bodies, the Environmental Protection Agency (EPA) and University College Dublin (UCD), on a difficult taxonomic group.

The output from this work will now be used as part of an overall project which will examine the distribution of these sponge species in relation to water quality.

1 Introduction

1.1 Background

Sponges represent a natural resource for their functional role in natural processes of water purification in freshwater ecosystems (Manconi & Pronzato, 2008). However, in most water bodies populations of freshwater sponges are sufficiently sparse that they are unlikely to play a major role in ecosystem functions (Reiswig *et al*., 2010). Yet sponges can play a significant role in some flowing water ecosystems. A detailed study of a section of the River Thames in England estimated that sponges accounted for nearly 40 per cent of the total production by benthic animals (Mann *et al*., 1972).

Freshwater sponges, of which there are 45 known genera in six families, are members of the Porifera, a primarily marine phylum that successfully colonized, starting in the Mesozoic period, a wide variety of still- and flowing-water habitats (Manconi & Pronzato, 2008). The species occurring in Ireland are all members of the class Demospongiae, i.e. siliceous sponges and one family therein, Spongillidae.

Sponges mostly die back in winter, although in Ireland sponge tissue material has been collected in December (e.g. 12/12/2012). Gemmules (see Box 1 and Figure 3) are internal buds of sponges which are the result of asexual reproduction and which can be dormant through the winter. They can be resistant to desiccation, freezing and anoxia and then later develop into new sponge tissue. The other method of reproduction is when a piece of the mature sponge simply becomes detached and eventually grows into a new sponge (budding). The fact that sponges can have very variable shape, dimensions and colour makes their specific identification based on morphological features impossible (see Figure 1). The main diagnostic characteristic for identifying freshwater sponges is skeletal architecture.

Identification of species involves digesting sponge material in acid followed by microscopic examination of tissue fibres and skeletal elements, called spicules, to distinguish different species. The work for the current project involved preparing and mounting samples for microscopic examination and photographing the specimens for identification. It was carried out as a small-scale study under the EPA STRIVE Programme 2007–2013 in partnership with the School of Biology & Environmental Science, University College Dublin. The study, based in the Biology Laboratory at the Kilkenny EPA Inspectorate, was completed in a 10-week period split into two moieties (21 May–23 June and 5 November–7 December 2012).

Figure 1. Some of the growth forms of river sponges observed during the study.

1.2 Objectives and Aims

Among the research recommendations of the Freshwater Working Group of the National Platform for Biodiversity Research (2012) is the production of national inventories for less well-known freshwater taxa such as freshwater sponges and updates on their distribution.

The sole purpose of the 10-week study was to catalogue the sponge sample collection housed at the EPA Kilkenny. The main tasks were:

- Preparing and mounting samples for microscopic examination
- Photographing and archiving microscope slides
- Identifying samples to species level

Thus, the main objective was to classify taxonomically the sponge collection. There are five known species of freshwater sponges in Ireland but no significant work has been published since 1920. That study was started more than 100 years ago. The present study is based on sponge samples taken during biological monitoring from southern Irish rivers, chiefly in the period between 1992 and 1997, but the collection was started as early as August 1984 and finished as recently as December 2012.

2 Materials and Methods

2.1 Study Area

Samples were collected from rivers and streams in the 12 hydrometric areas (HAs) numbered 13–24 (see Figure 2) between 1984 and 2012, but chiefly in the period 1992–1997. Additionally eight samples, collected from HAs 12 and 36 in 1998, were kindly provided by Kevin Clabby, then senior biologist with the EPA. The main study area, from where the sponges had been collected, included part or all of the following 11 counties: Wexford, Carlow, Kilkenny, Laois, Kildare, Offaly, Tipperary, Waterford, Cork, Kerry and Limerick. More than one sample was collected at some sites, such as in the River Suir, where it was known, or considered, that more than one species occurred or where different colonies existed.

2.2 Laboratory Methods

Sponge samples collected in the field had been preserved in ethanol (96%). Sponge spicules were prepared for optical microscope examination by digesting the sponge tissue in concentrated nitric acid (min. 69%).[1] The procedures of Lauer and Spacie (1996) were followed with some slight modifications. Firstly, glass test tubes were placed into a test tube rack to ensure they would remain upright. Using an automatic dispenser (dispensette, easy calibration), 2 ml of nitric acid was dispensed into each test tube. A 20–30 mm piece of sponge tissue was cut and placed onto absorbent paper. It should be noted that nitric acid and ethanol can react violently and even explode when mixed in the wrong concentrations. The risk of explosion can be reduced when larger volumes of nitric acid are used relative to the preserved tissue sample, and dabbing the sponge sample on a paper towel to absorb the ethanol will further reduce the risk of violent reaction and explosion. With the fume cupboard turned on, the sponge sample was placed into nitric acid and there was an immediate evolution of nitric oxide. The samples were then left to digest over a 24-hour period.

After 24 hours, the nitric acid was pipetted out of each test tube. To reduce the risk of cross-contamination the pipette was rinsed several times in distilled water before being placed into the next test tube. After the nitric acid was removed, 2 ml of distilled water was added and the sample was left to settle for 10 minutes. This was repeated two times to ensure all the nitric acid was removed.

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[1] 

Nitric acid is highly corrosive and so the digestion process was conducted behind a shield in a fume cupboard while wearing safety gloves, eye protection and a laboratory coat.

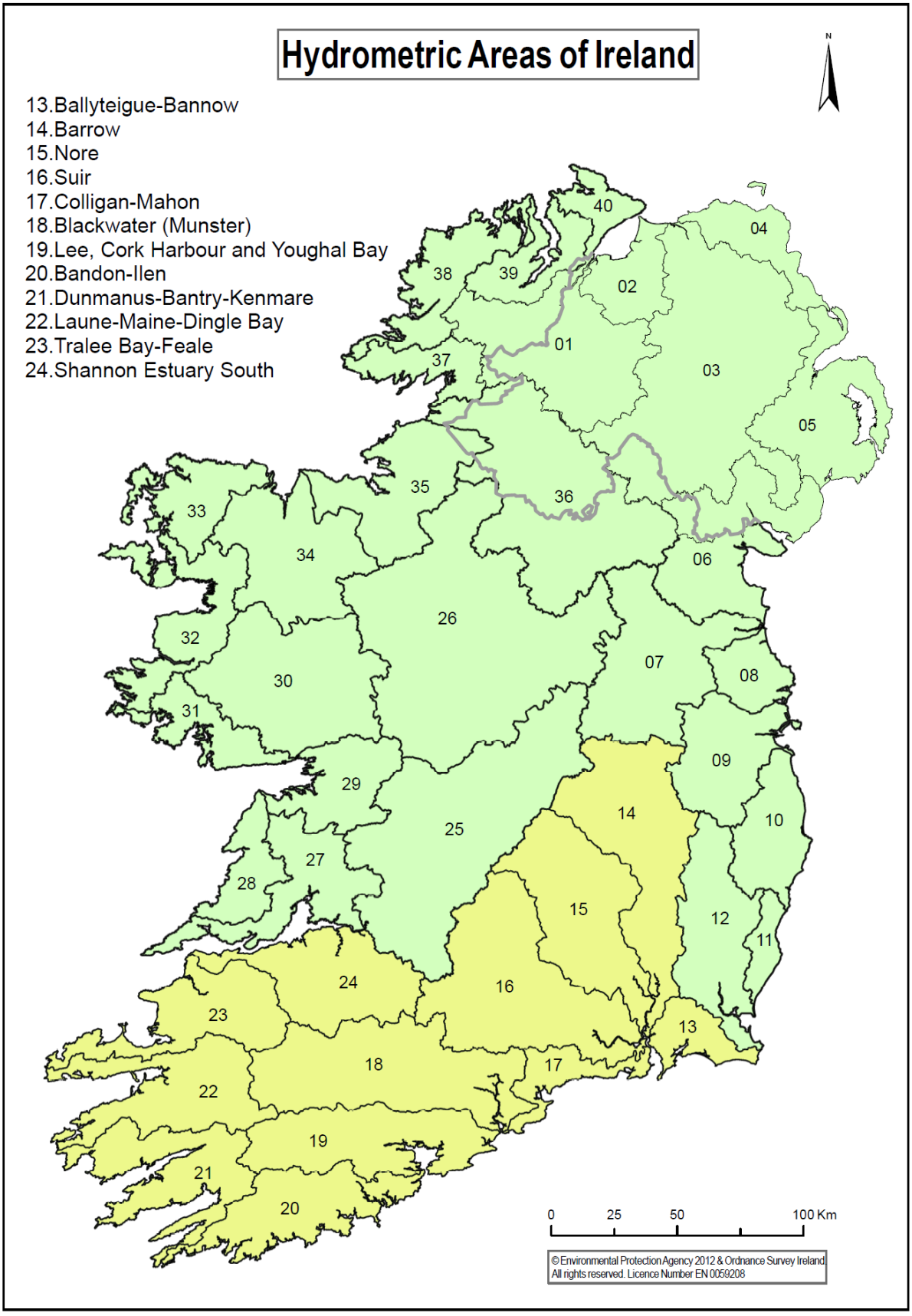


Figure 2. Map showing hydrometric areas (HAs) of Ireland with main study area (HAs 13–24) highlighted.

Finally, 2 ml of ethanol was added to each sample and after 10 minutes most of the ethanol was drawn off using a pipette, leaving the spicules behind. To make a permanent slide, a small drop of ethanol containing the spicules was placed onto a slide. Once the sample was dry a drop of mounting fluid (DPX mountant) was added and the coverslip was applied. The slide was then placed under a microscope (Nikon Eclipse 50*i*) and the species identified using an Irish key (Stephens, 1920) and European keys (Pronzato & Manconi, 2001; Manconi & Pronzato, 2002; Eggers & Eiseler, 2007). Peer-reviewed papers were also consulted (e.g. Økland & Økland, 1996; Dröscher & Waringer, 2007; Oscoz *et al*., 2009), as was the previous preliminary work conducted on part (50 samples) of this collection of Irish sponges (McKenna, 2010). At least one photomicrograph of each slide preparation was taken for archiving with the permanent slide collection.

Figure 3 shows the surface of a gemmule of *Ephydatia fluviatilis*. Box 1 gives a summary of the spicule characteristics used for identification of sponge species in the project, while Figure 4 shows examples of photomicrographs of some of the tissue slide preparations illustrating the spicules.

In addition to light microcopy, six digested samples, including one collected in 1910, were scanned at the CRANN Advanced Microscopy Laboratory, CRANN, Trinity College Dublin (Dermot Daly), with electron microscopy and images supplied to the project (Bryan Kennedy).

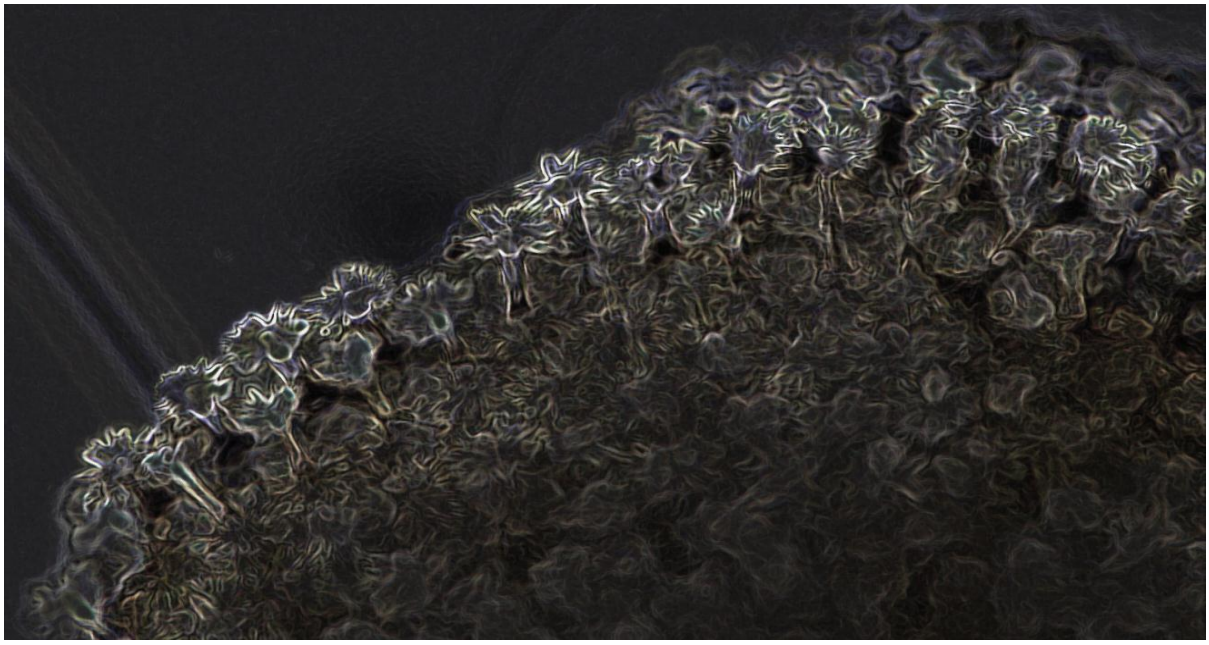


Figure 3. Photomicrograph (× 400) of gemmule surface of *Ephydatia fluviatilis* – River Cloghnagh, Kilkenny.

Box 1. Body and Gemmule Spicules of Freshwater Sponges

Sponges possess megascleres, microscleres and gemmule spicules and the following characteristics were used in identification of the five species during the project (see Figure 4).

Megascleres are large spicules 60–>200 micrometres (µm) in length and often function as the main support elements in the skeleton. These can be spiny or smooth. *Ephydatia muelleri* has spiny spicules (megascleres).

Microscleres are small spicules measuring 10–60 µm in length, scattered throughout the tissue and are not part of the main support element. These can be spiny or smooth, needle shaped, rod shaped, star shaped (asters) or dumb-bell shaped (birotules: having two wheel-shaped ends called rotules connected by a more or less cylindrical shaft). Some species, such as *Racekiela ryderi*, do not have microscleres.

Gemmules are internal buds of sponges, usually <0.5 µm in diameter, which are the result of asexual reproduction; these are formed by sponges for overwintering and can remain dormant through the winter. Gemmules usually contain gemmoscleres and their arrangement can be used to identify species, although some species can lack gemmoscleres. Other diagnostic characteristics include the presence or absence of a tube surrounding the foraminal opening.

Ephydatia fluviatilis – Megascleres (222–450 × 5-18 µm) are needle-like, slightly curved to (rarely) straight. Megascleres are mostly smooth but some can be micro-spined and often deformities such as bulbous ends can be seen. Microscleres are absent and gemmuloscleres (20–32 × 6–24 µm) are present as birotules with smooth or spiny shafts; the rotules (disc-like structures) are slightly indented along the margins.

Ephydatia muelleri – Megasclere (178–310 × 7–15 µm) are needle-like and can be slightly curved to straight. Megascleres usually have numerous small spines along the entire length but can also be smooth. Microscleres are absent and gemmuloscleres are present as birotules. Birotules (8–15 × 13–20 µm) have short shafts with rotules deeply indented and wider than the length of the birotule.

Racekiela ryderi – Megascleres (170–320 × 5–12 µm) are curved to straight and can be heavily to very finely spined along entire length except at tips. Tips of megascleres can be pointed or rounded. Microscleres are absent. Gemmuloscleres are present as two types: short birotules (24–52 × 10-28 µm) and long pseudobirotules (35-70 × 10-20 µm). Birotule shafts can be smooth or spiny (1–8 spines) with large plate-like rotules that are slightly serrated. Pseudobirotules have spined shafts (1–8) with small pseudorotules that are curved into hooks.

Spongilla lacustris – Megascleres (210–380 × 5–16 µm) are slightly curved to straight, usually smooth but can also be slightly spined. Microscleres can be very abundant to rare (50–115 × 2–6 µm), slightly curved (almost boomerang-shaped) with dense spines along the entire length and pointed tips. Gemmuloscleres (50–120 × 5–7 µm) if present are slightly straight to strongly bent, with round tips and spines concentrated at the tips.

Eunapius fragilis – Megascleres (210–250 × 9–12 µm) are needle-like and smooth. Microscleres are absent. Gemmoscleres (95–120 × 5–7 µm) are rod-shaped with either pointed or rounded tips. The gemmoscleres are covered in spines which are often more abundant at the tips.

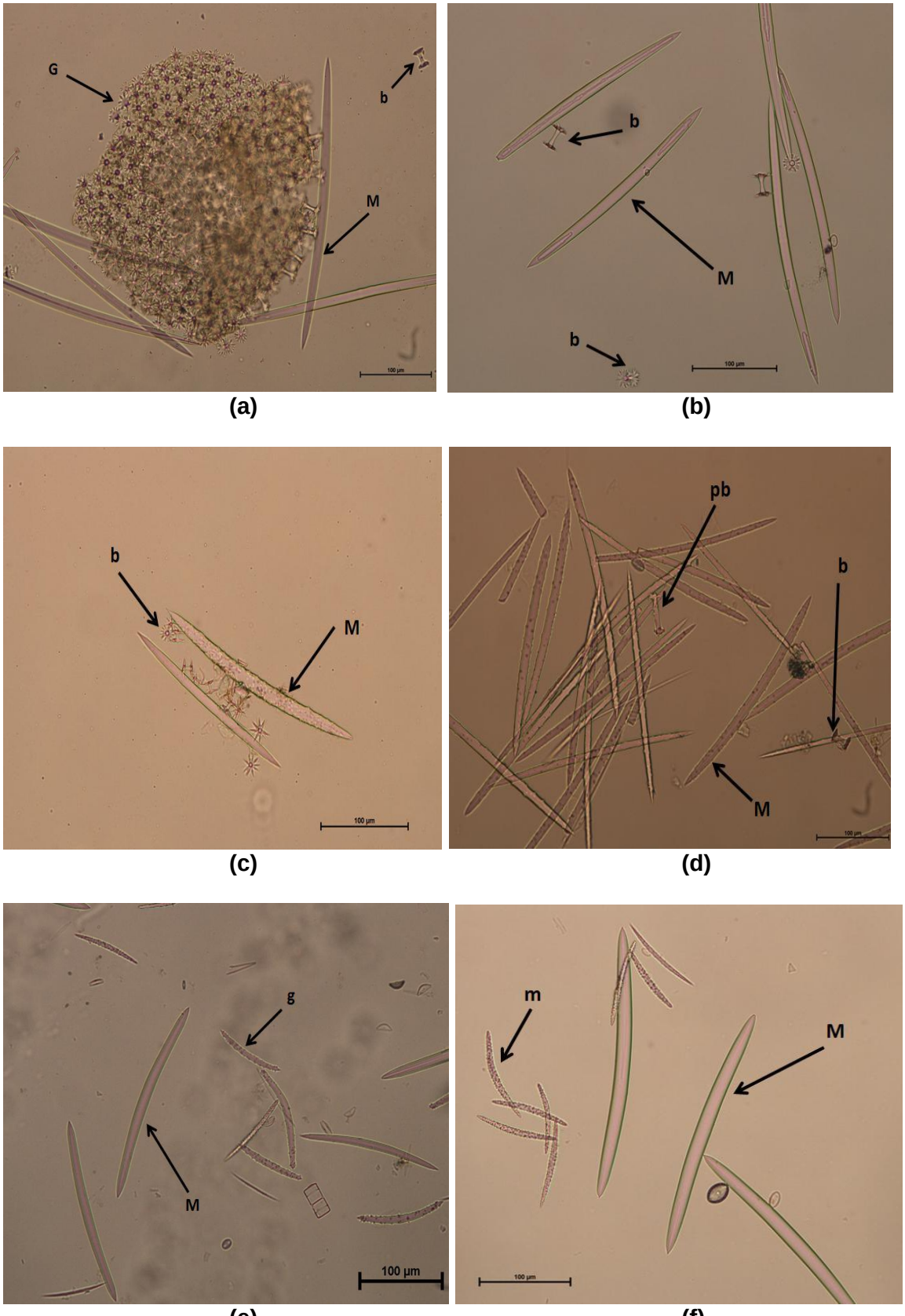


Figure 4. Photomicrographs of sponge spicules (G = gemmule, g = gemmosclere, M = megasclere, m = microsclere, b = birotule, pb = pseudo-birotule) from the study: (a) *Ephydatia fluviatilis*, (b) *Ephydatia fluviatilis*, (c) *Ephydatia muelleri*, (d) *Racekiela ryderi*, (e) *Spongilla lacustris*, (f) *Spongilla lacustris*.

3 Results

Sponge tissue samples were prepared for microscopic examination by making permanent slide mounts. Photomicrographs were taken of slide preparations as necessary and catalogued. A total of 267 tissue sample slides were prepared – 266 river/stream and 1 canal – permanently mounted, photographed and species identified. The samples had been collected during biological monitoring surveys carried out in the period 1984 to 2012.

The spicule dimensions of the species found in the study are given in Box 1 and are largely similar to those previously recorded for Irish specimens (Stephens, 1920). As only one specimen of *E. fragilis* was recorded in the present study the dimensions for that species are obviously limited.

Sponges were found in all the 12 hydrometric divisions in 11 counties that the main study area comprised. The species' locations with dates of collection are given in the Appendix. In some cases the species could not be specifically identified because of the lack of gemmoscleres or other identifying features in the sample.

The survey of southern Irish rivers and streams revealed the presence of five species of freshwater sponge: *Ephydatia fluviatilis*, *Racekiela ryderi*, *Spongilla lacustris*, *Ephydatia muelleri* and *Eunapius fragilis* in order of decreasing frequency of occurrence.

- *Ephydatia fluviatilis* was recorded at 119 sites of 78 rivers in all 11 counties of main study area and Cavan.
- *Racekiela ryderi* was recorded at 47 sites of 39 rivers in 3 counties of main study area.
- *Spongilla lacustris* was recorded at 37 sites of 28 (including 1 canal) rivers in all 11 counties of main study area and Cavan, Leitrim and Monaghan.
- *Ephydatia muelleri* was recorded at 28 sites of 18 rivers in all 11 counties of main study area and Cavan.
- *Eunapius fragils* was recorded at 1 site of 1 river in 1 county of main study area.

While *Racekiela ryderi* shows the second largest number of site and river occurrences, it is restricted in geographic range with just a single record from outside the Cork–Kerry region.

Some sites had been surveyed more than once, and multiple samples were taken at some sites. Species could be identified from the sample material at 230 different sites in 164 rivers. There were 16 sites where determination at the species level was not possible but the material examined indicated either *Ephydatia fluviatilis* or *Spongilla lacustris*; these are listed in the Appendix as Indeterminate (indet).

The following summarises the occurrence of the five species in the project area:

- *Ephydatia fluviatilis* (L.): A relatively common and widely distributed species in rivers.
- *Spongilla lacustris* (L.): A widely distributed species but less common in rivers than in lakes, as the specific name implies.
- *Racekiela ryderi* (Potts): A common species in parts of the project area, particularly in the south-west, with outliers in other soft-water rivers.
- *Ephydatia muelleri* (Lieberkühn): An uncommon species with an apparent rare occurrence outside the Barrow catchment.
- *Eunapius fragilis* (Leidy): A very rare species in southern Irish rivers.

Figures 5–9 show macroscopic growths with microscopic images for some of the species in the study.

Figure 5. Sponge, *Spongilla lacustris*, growing on duck mussel, *Anodonta anatina*, with gemmules visible, on right posterior ventral part of shell, in lower right-hand side – Grand Canal, Laois + Photomicrograph of tissue slide.

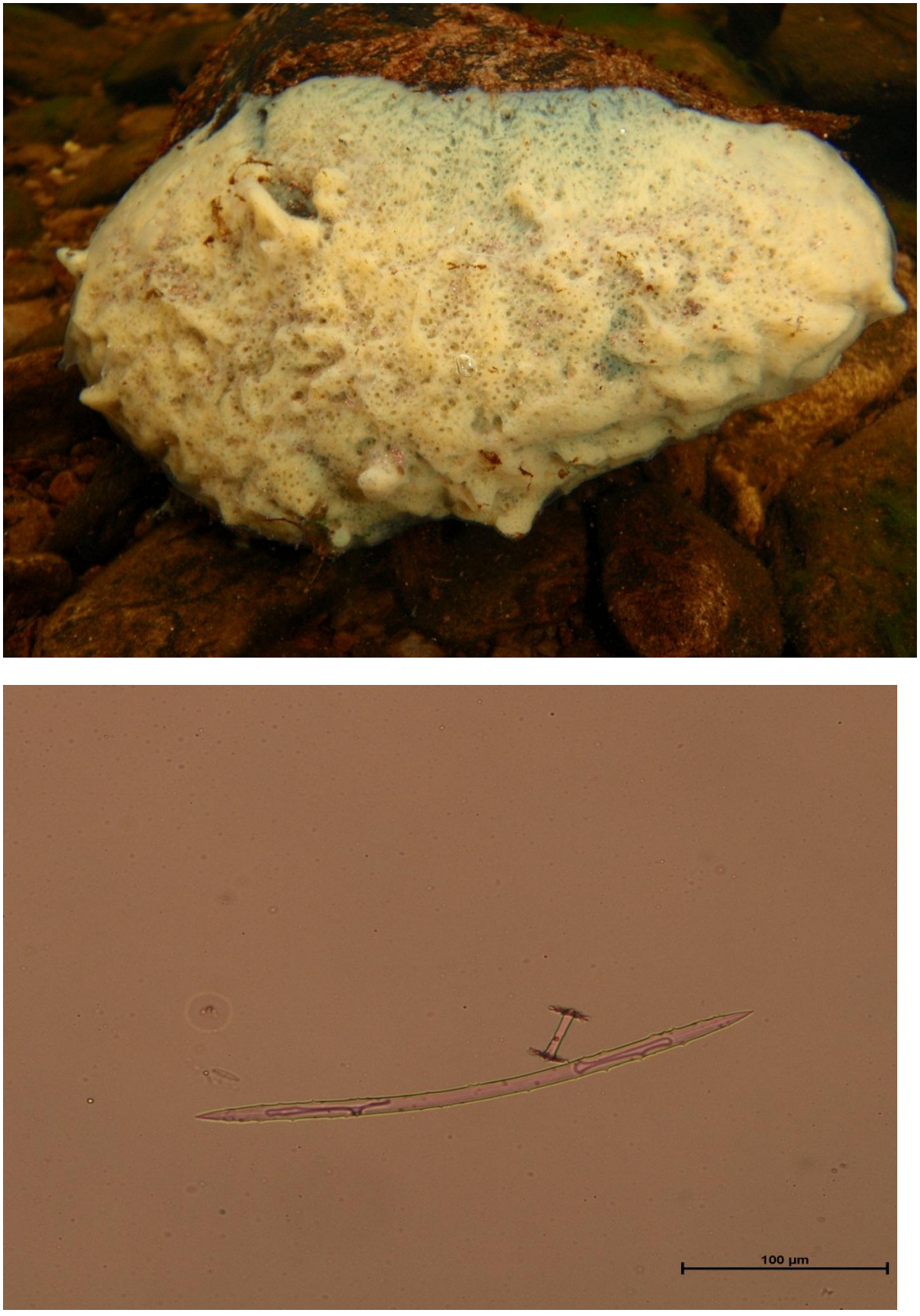


Figure 6. Sponge, *Racekiela ryderi*, growing on rock – River Sheen, Kerry + Photomicrograph of tissue slide.

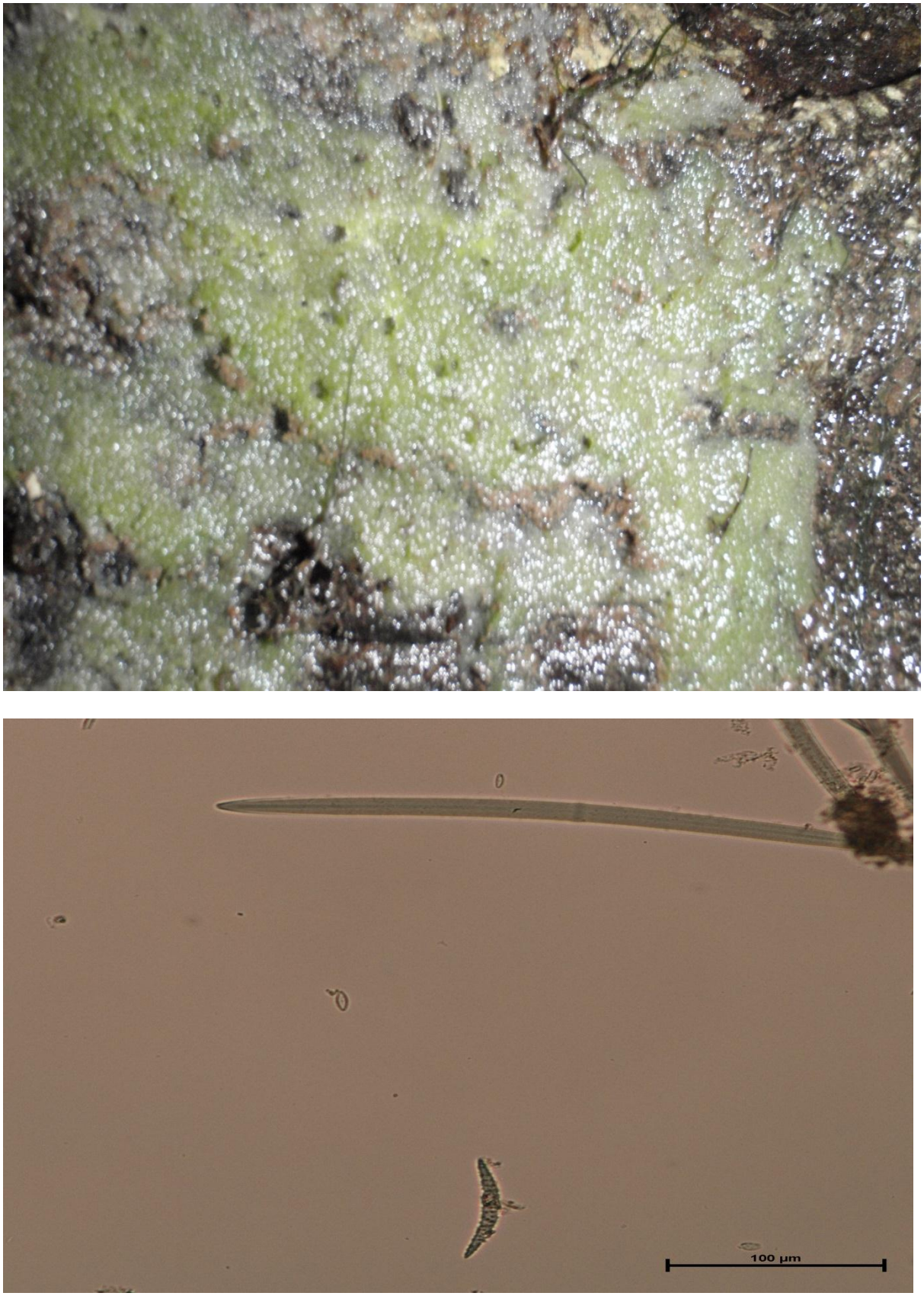


Figure 7. Sponge, *Spongilla lacustris*, growing on rock – River Suir, Tipperary (Winter growth – December) + Photomicrograph of tissue slide.

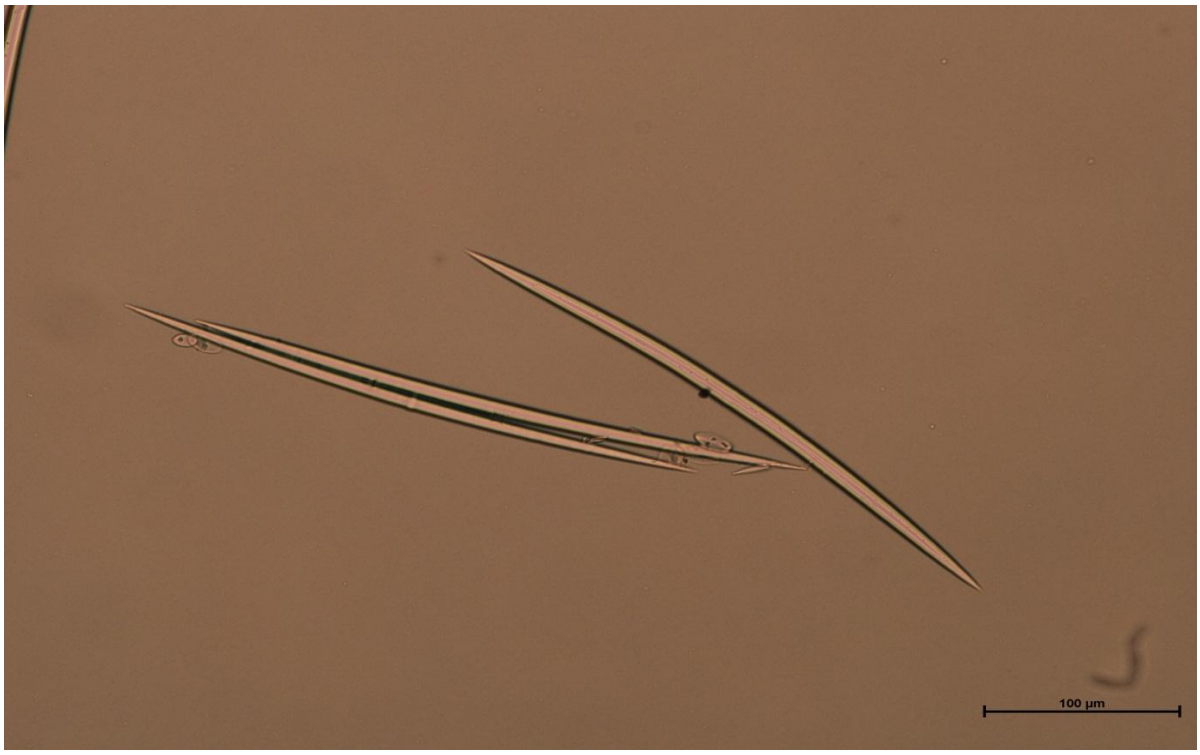
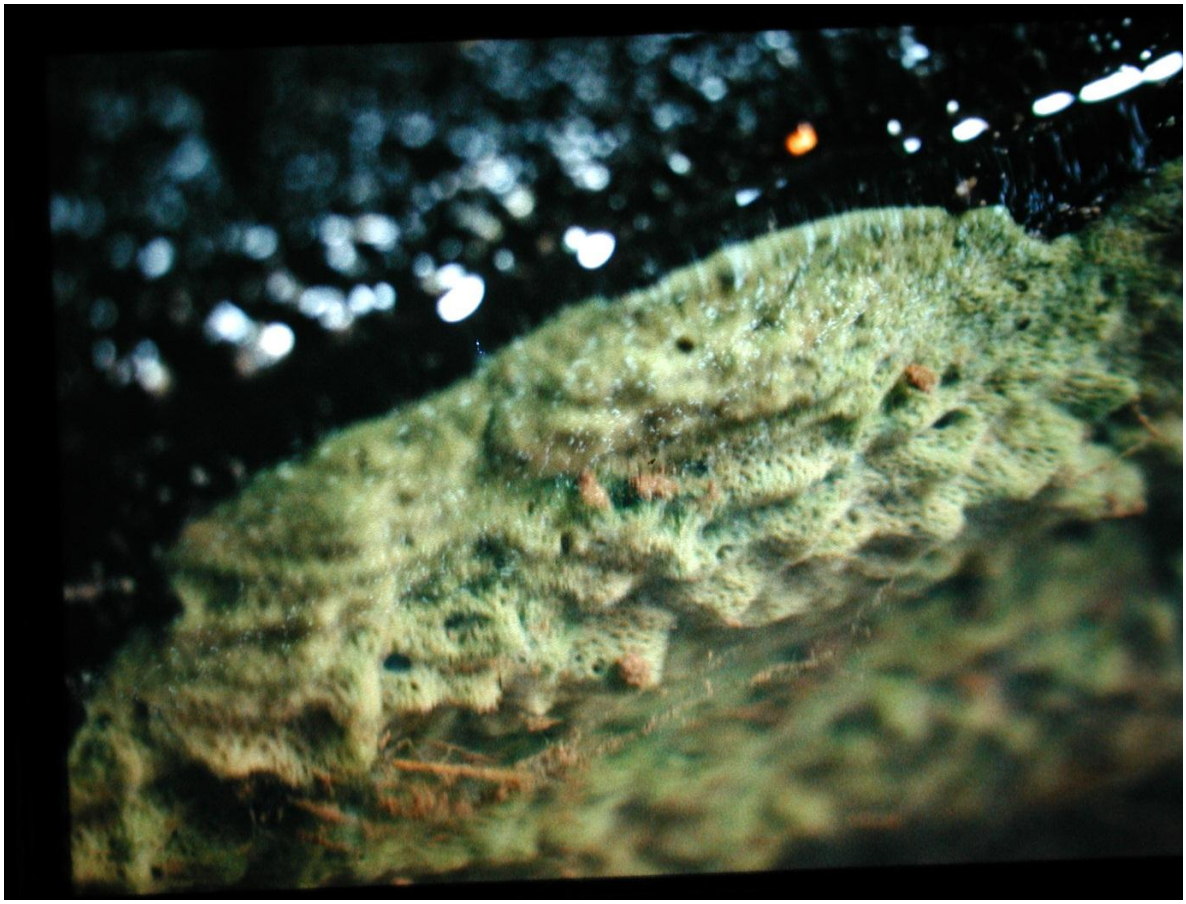


Figure 8. Sponge, *Ephydatia muelleri*, growing on rock – River Daingean, Offaly + Photomicrograph of tissue slide.



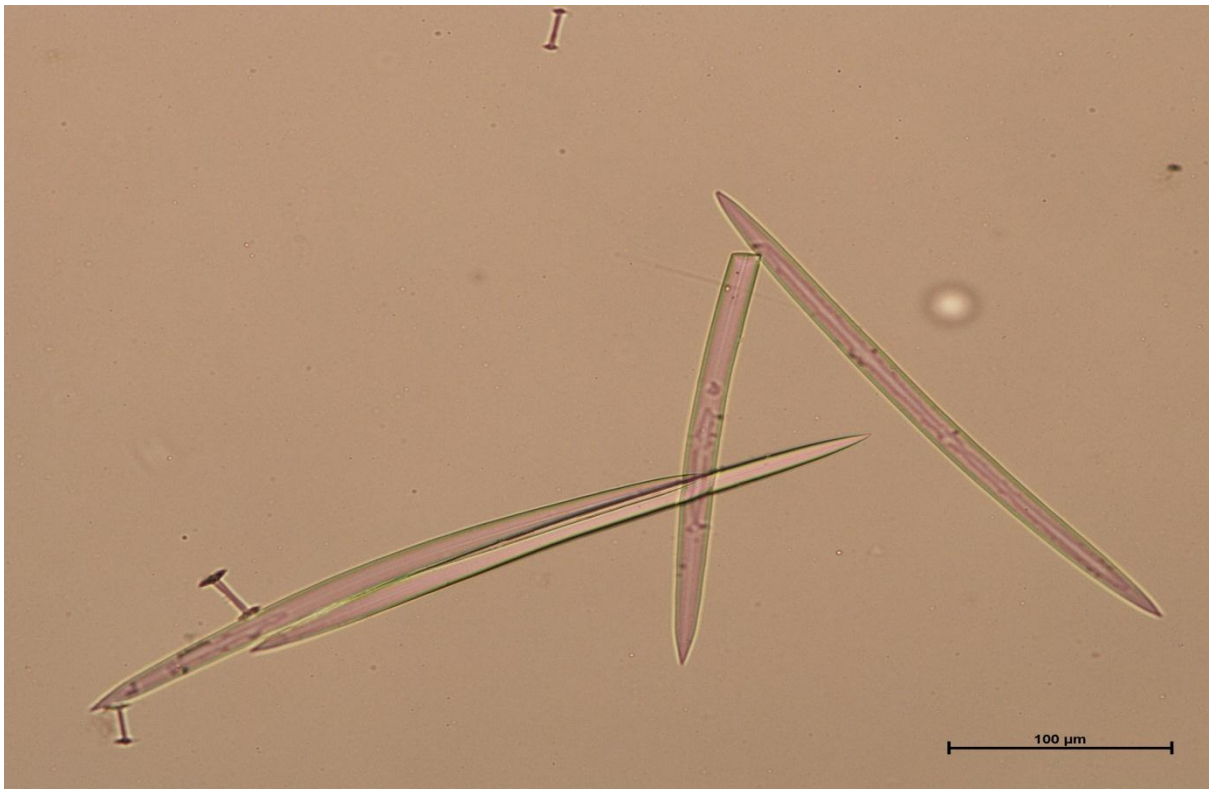


Figure 9. Sponge, *Ephydatia fluviatilis,* growing on upright under bridge - River Mahore, Limerick + Photomicrograph of tissue slide.

4 Discussion and Conclusions

Freshwater sponges filter large amounts of water – e.g. it has been estimated that a finger-sized sponge can filter more than 125 litres in a day (Frost, 1980) – and, like pearl mussels, play an important role in filtering and recycling water. Sponges can be found growing on all submerged hard surfaces, and *Ephydatia fluviatilis* was even found growing on a clay bank of the River Suir just downstream of Knocklofty Bridge in September 1993. Freshwater sponges can control the presence of the zebra mussel (Lauer & Spacie, 2004) and this was seen in 2012 in the Grand Canal at Tullamore, where sponges had covered some specimens of this invasive species (*Dreissena polymorpha*).

Jane Stephens, who worked on Irish sponges between 1905 and 1920, noted that in some years sponges were absent where previously found. This observation was also made during this study, e.g. *E. fluviatilis* was common in 2009 (24/09/2009) at a site in the Clonakilty Stream (Cork) but absent three years later (26/09/2012).

In the River Suir at Kilsheelan, Jane Stephens recorded *Ephydatia fluviatilis* and *Eunapius fragilis* while in the current study *Ephydatia fluviatilis* and *Spongilla lacustris* were recorded. In the earlier study only a few small specimens were found at the location (Stephens, 1920). In contrast with that site, Stephens (1912) had discovered large patches of *E. fragilis* in the Owengar River, just downstream of Doo Lough, in County Mayo. A search of the Natural History Division of the National Museum of Ireland's sponge collection did not locate the Suir sample. A preserved specimen of the Owengar sponge, collected in October 1910 (NH:1910.462.2.), was, however, located and a fragment of that sample was kindly provided by Mr Nigel Monaghan and Dr Leona McArdle from which a permanent slide was prepared (Figure 10).[2] A microscopic image of the specimen, collected in the River Bride almost a century later, is shown for comparison in Figure 11. This species, which has an almost worldwide distribution, is very rare in Ireland and has been found in only five widely scattered localities in the north, west and south of the country (Stephens, 1920). Surprisingly, Stephens (1920) did not record *S. lacustris* from the Suir at Kilsheelan and it must be concluded that it was not present there when she searched. She states that in 1911 when *E. fragilis* was recorded there, only a few small specimens were found. In the present study multiple samples were taken of sponge material at the Kilsheelan site.

[2] In Jane Stephens' time the spicules would have been drawn, by her friend and colleague Eileen Barnes, using the *camera lucida* method.

Compared with the earlier study, published in 1920, the following main points may be made:

- *Ephydatia fluviatilis* was of commonest occurrence while *Spongilla lacustris* was encountered most often in the earlier study.
- *Ephydatia muelleri* was previously only known from three locations, the Erne and Tolka rivers as well as Caragh Lake (Stephens, 1920). Though not common, it has been collected from 28 sites of 18 rivers during the surveys for the present project.
- *Ephydatia fluviatilis* was not previously recorded from the south-west.

Regarding the first of these points it can be noted that lakes were sampled more in the earlier study while rivers/streams, with the exception of one canal, were exclusively surveyed in the present one. As their specific names would signify, *S. lacustris* and *E. fluviatilis* show a preference for lakes and rivers respectively. In respect of the other two points, Jane Stephens did refer to parts of the country not being adequately covered and says 'In particular, the midlands have been neglected, and further work in parts of the north and in the south would add to our knowledge of the distribution of the various species' (Stephens, 1920). She had difficulty travelling to certain areas – a reference no doubt to the unstable situation following the 1916 Easter Rising and leading up to the start of the War of Independence.

Racekiela ryderi was found to be widespread with a westerly distribution in rivers and streams of lower ionic strength. Previously, Stephens (1912, 1920) had also found *R. ryderi* (=*Heteromeyenia ryderi*) to be widely spread in the country in non-limestone areas. This species, which has its main distribution in eastern North America and is largely absent from Europe, with only known rare recordings (=*Anheteromeyenia ryderi*) for the Hebrides, Faroes and Norway (Økland & Økland, 1989), was found to be of relatively common occurrence in south-western Irish rivers. *Ephydatia fluviatilis*, which was not previously recorded from the south-west (Stephens, 1920), occurs in some of the rivers there and is the most common species found throughout the study area.

Light microscopy is usually sufficient for the morphological analysis of sponge spicules, but in some cases the discrimination between certain species, e.g. *Spongilla lacustris* and *Eunapius fragilis*, may be difficult (Richelle-Maurer *et al*., 1994) and identification of the samples by classical morphological studies of their skeleton spicules using scanning electron microscopy (SEM) is often performed in parallel (Roovere *et al.*, 2006). The project reported on here did not have access to SEM facilities but Mr Bryan Kennedy (EPA) kindly had some of the specimens scanned at the CRANN Advanced Microscopy Laboratory, Trinity College Dublin and two-dimensional images generated (Figure 12).

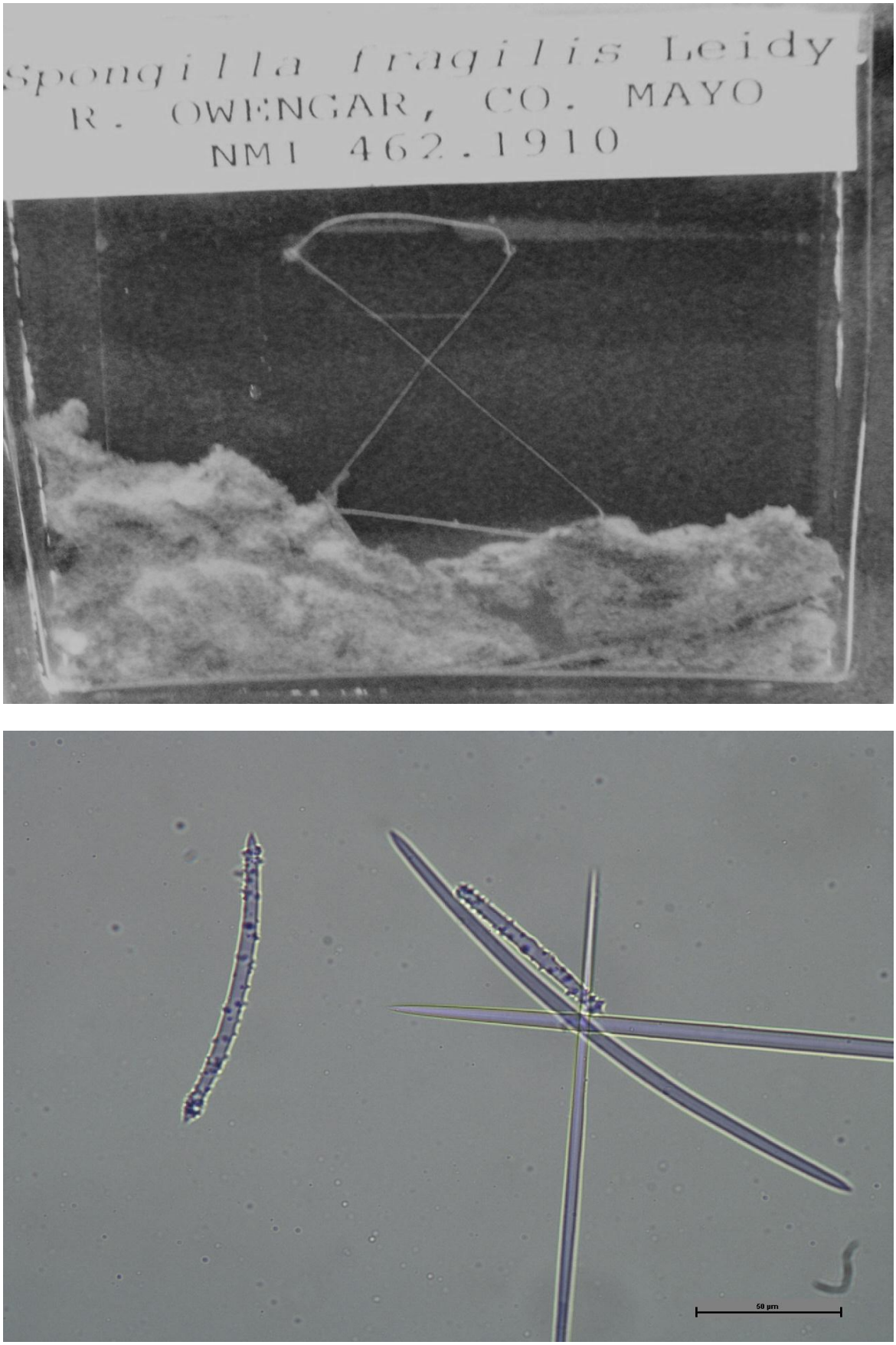


Figure 10. Sponge, *Eunapius fragilis*, from Owengar River, Mayo, collected by Jane Stephens in October 1910 (NH: 1910.462.2) + Photomicrograph of tissue slide.

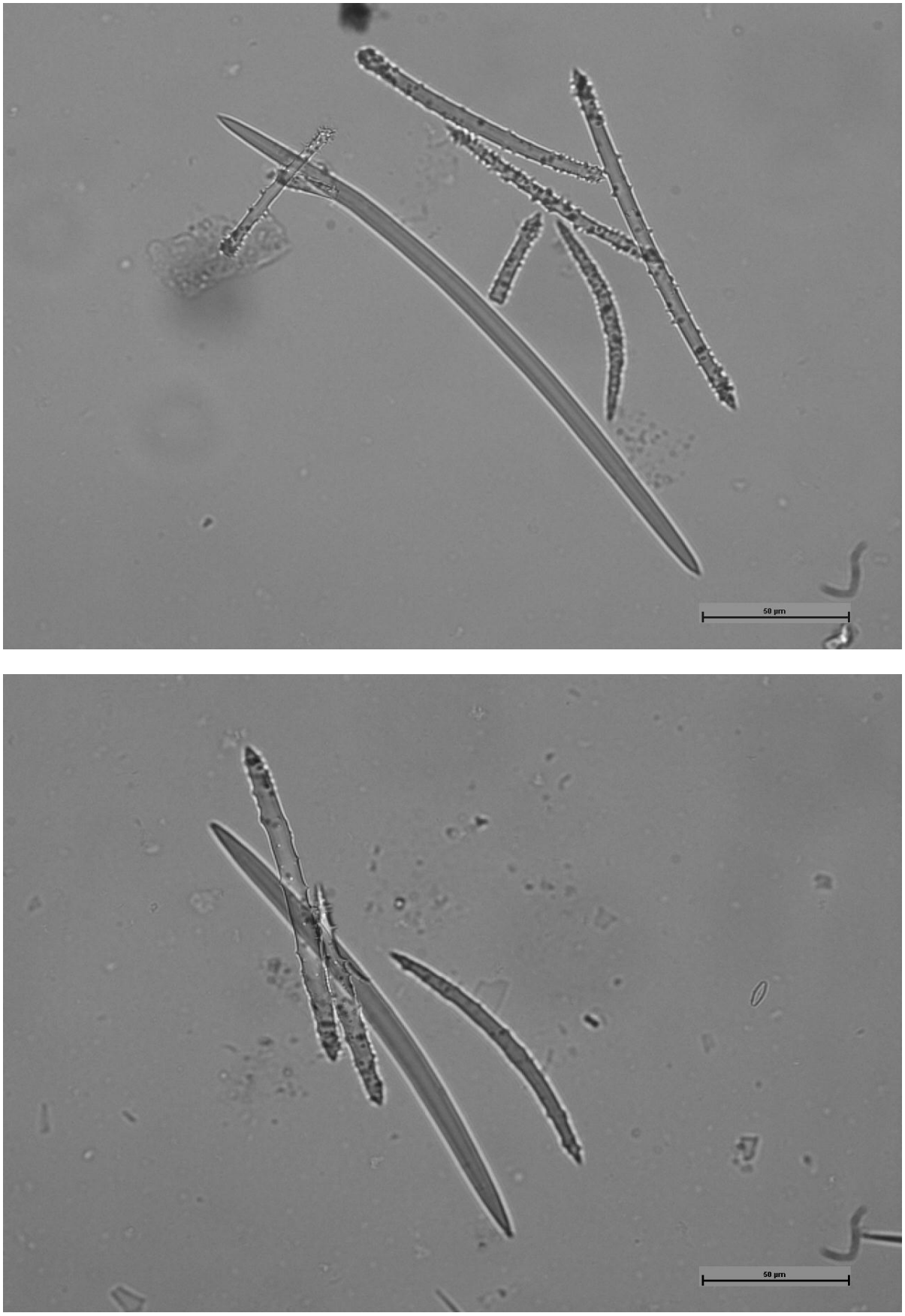


Figure 11. Photomicrographs of sponge, *Eunapius fragilis*, from River Owengar – October 1910 (top) and River Bride – 30 August 2006 (bottom).

The main objective of the project – to classify the sponge collection taxonomically – has been achived. The next step will be to plot the geographical distribution of the species in southern Irish rivers and streams and to establish water quality relationships (Lucey & Cocchiglia, in preparation).

The National Biodiversity Data Centre (NBDC) held its 3rd Annual Recorders' Event on 20 and 21 August 2009 under the title *Ireland's Freshwater Habitats – priorities for biodiversity data*. A key objective for hosting the recorder's event was to identify and agree national priorities for freshwater biodiversity data for the following five or so years. One of the priorities identified from the discussions was to 'Promote networking among taxonomists and researchers on the more difficult groups (for example, sponges)'. The project reported on here is a good example of such networking and cooperation, between the Environmental Protection Agency (EPA) and University College Dublin (UCD), on a difficult taxonomic group.

It has been noted that relatively little is known about some aspects of Ireland's freshwater biodiversity and that a particular example is the freshwater sponges: these are regularly encountered by freshwater biologists but on a national level we lack the taxonomic expertise to identify them (NBDC, 2010). This study has gone some way to rectifying this situation.

The 10-week study entailed identifying sponge samples from 266 southern Irish river sites (in the south-east, south, south-west and mid-west and from one canal location). The output from this work will now be used as part of an overall project which will look at the distribution of these sponge species in relation to water quality.

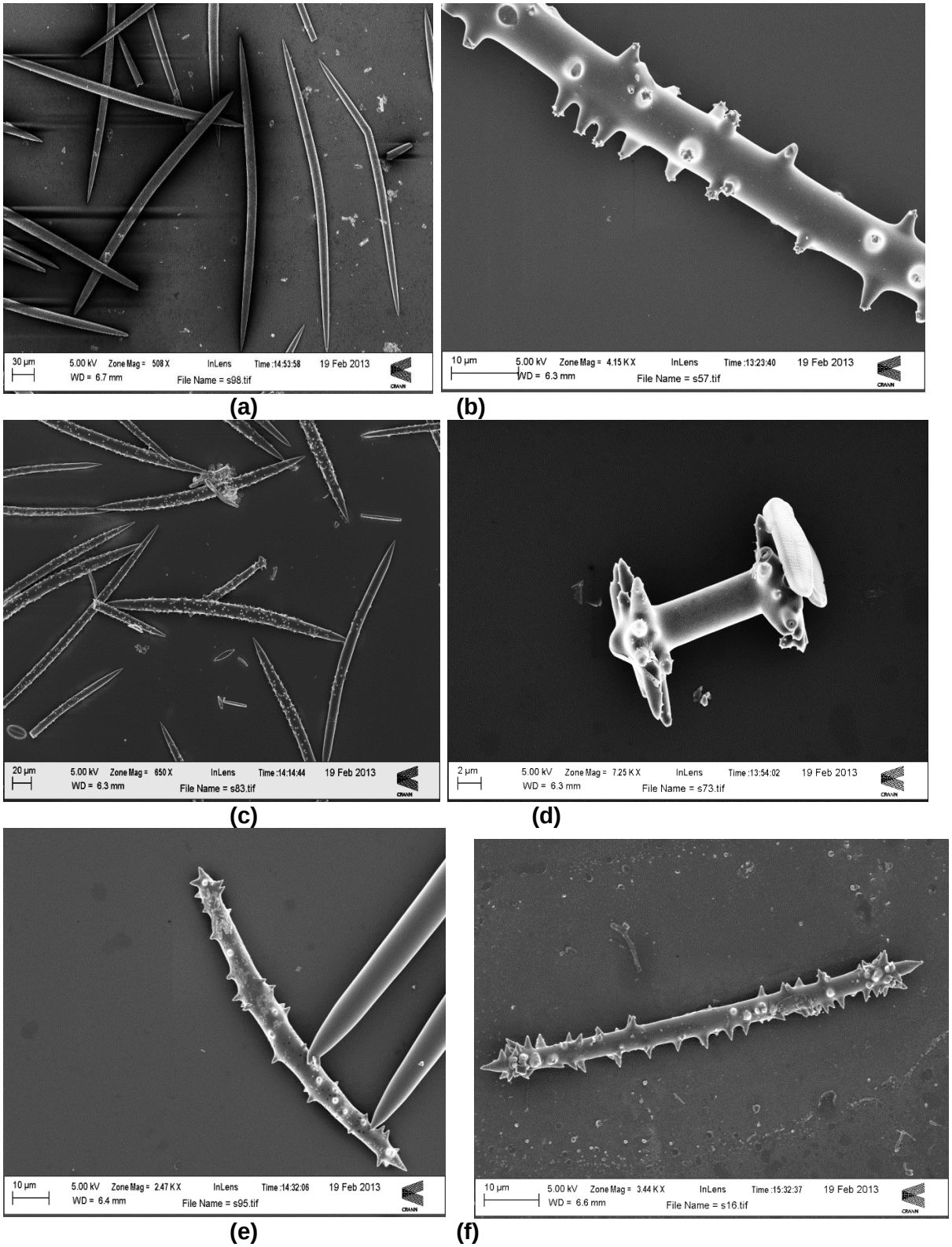


Figure 12. Scanning electron micrographs (SEMs) of specimens showing (a) megascleres from Awbeg, (b) shaft of megasclere from Barrow, (c) megascleres and gemmoscleres from Bride, (d) birotule from Laune with rapheless valve of a diatom (*Achnanthidium minutissimum*) attached to rotule, (e) gemmosclere and megascleres from Moyle, (f) gemmosclere from Owengar (all images Bryan Kennedy/CRANN).

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Appendix: Species and Locations. Dates of collection of sponge species. Hydrometric numbers marked with an asterisk (*) denote areas outside main study.

Species/ Date	River/Stream	Location	County	Hydrometric Area
Ephydatia fluviatilis				
12/08/1992	Fishmoyne	Br SE of Kilfithmone Crossroads	Tipperary	16
22/07/1992	Ara	Br NE of Lacken	Tipperary	16
17/09/1993	Suir	Kilsheelan Br (a)	Tipperary	16
17/09/1993	Suir	Kilsheelan Br (b)	Tipperary	16
17/09/1993	Suir	Kilsheelan Br (c)	Tipperary	16
05/08/1993	Arra	Br East of Cullenagh House	Limerick	24
05/08/1993	Deel	d/s Castlemahon Br	Limerick	24
29/07/1993	Cushina	Br E of Monavane House	Offaly	14
29/07/1993	Daingean	Br E of Mount Lucas House	Offaly	14
23/07/1993	Aherlow	Killardry Br	Tipperary	16
05/08/1993	Deel	Br near Killaready	Limerick	24
27/07/1993	Owenass	Owenass Br	Laois	14
19/08/1993	Suir	d/s Black Stream LHS	Tipperary	16
29/06/1993	Lerr	Lerr Br	Carlow/Kildare	14
24/06/1993	Stradbally	Br nr Camac Aqueduct	Laois	14
19/08/1993	Suir	Br nr Suirville House (New Bridge)	Tipperary	16
13/08/1993	Aughnacrew	Br u/s Pollmounty R confl	Wexford	14
05/08/1993	Deel	Br nr Balliniska	Limerick	24
17/09/1993	Suir	N of Churchtown House	Tipperary/Waterford	16
16/09/1993	Suir	Knocklofty Br (a)	Tipperary	16
16/07/1993	Morningstar	Athlacca Br	Limerick	24
16/09/1993	Suir	Knocklofty Br (b)	Tipperary	16
16/09/1993	Suir	Twomile Br (Sir Thomas Br)	Tipperary/Waterford	16
15/09/1993	Suir	Br at Ardfinnan	Tipperary	16
16/09/1993	Suir	Newcastle Br	Tipperary	16
20/09/1993	Barrow	Graiguenamanagh Br	Carlow/Kilkenny	14
16/09/1993	Suir	Knocklofty Br (c)	Tipperary/Waterford	16
17/09/1993	Suir	N of Churchtown House	Tipperary/Waterford	16
13/07/1993	Mahore	Hospital Br	Limerick	24
08/07/1993	Burren	Br NW of Ballycrogue	Carlow	14
14/07/1993	Camoge	Cloghansoun Br	Limerick	24

Species/ Date	River/Stream	Location	County	Hydrometric Area
Ephydatia fluviatilis (ctd.)				
08/09/1993	Lismakerry Stream	Br ESE of Tomdeely	Limerick	24
30/08/1993	Gowran	Br N of Goresbridge (South Channel)	Kilkenny	14
02/09/1993	Tully Stream	Kilberrin Br	Kildare	14
08/09/1993	Greanagh	Coolagh Br	Limerick	24
15/09/1993	Suir	2 km u/s Cahir	Tipperary	16
15/09/1993	Suir	Cahir Br	Tipperary	16
16/09/1993	Suir	Fords nr Ballyveera	Tipperary	16
02/09/1993	Tully Stream	Br nr Tully House	Kildare	14
15/09/1993	Suir	Ford 1.8 km d/s Cahir Park Br	Tipperary	16
16/09/1993	Suir	Marlfield u/s Clonmel	Tipperary/Waterford	16
31/08/1993	Figile	Kilcumber Br	Offaly	14
15/08/1994	Barrow	Graiguenamanagh Br	Carlow/Kilkenny	14
30/09/1994	Maigue	Br in Croom	Limerick	24
17/08/1994	Grange Stream (Guillie)	Br nr Killabban	Laois	14
01/12/1994	Owenageeragh	Blubell Br	Cork	18
29/09/1994	Maigue	Fort Br	Limerick	24
14/07/1994	Bandon	Br nr Desert Station	Cork	20
14/07/1994	Blackwater (Bandon)	Blackwater Br	Cork	20
28/07/1994	Barnakyle	Br SE of Clarina	Limerick	24
09/08/1994	Maine	Br NW of Currans	Kerry	22
19/07/1994	Arigdeen	Lisselane Br	Cork	20
16/08/1994	Barrow	Fenniscourt Lock	Carlow	14
24/11/1994	Bride (Waterford)	Br S of Rathcormack	Cork	18
28/09/1994	Loobagh	Riversfield Br	Limerick	24
30/11/1994	Bride (Lee)	Br at Crookstown (LHS)	Cork	19
11/09/1995	Goul	Maynebog Br	Laois	15
01/09/1995	Clogh	Slatt Br	Kilkenny	15
24/08/1995	Nore	Lismaine Br	Kilkenny	15
22/08/1995	Nore	Nore Br, SE of Roscrea	Tipperary	15
22/08/1995	Nore	New Br, WNW of Borris-in-Ossory	Laois	15
22/08/1995	Nore	Quaker's Br	Laois	15
25/08/1995	Nore	Ballylinch Br	Kilkenny	15
11/09/1995	Goul	Br 2km NW of Johnstown	Kilkenny	15
20/09/1995	Erkina	Clarneyball Br	Laois	15
31/08/1995	Dinin	Massford Br	Kilkenny	15
22/06/1995	Mountrath	Rushin Br	Laois	15

Species/ Date	River/Stream	Location	County	Hydrometric Area
Ephydatia fluviatilis (ctd.)				
20/09/1995	Erkina	Br nr Black Island	Kilkenny	15
17/07/1996	Farneybridge	Farney Br	Tipperary	16
29/08/1996	Barnakyle	Br SE of Clarina	Limerick	24
08/10/1997	Blackwater (Munster)	Keale Br	Cork	18
07/10/1997	Blackwater (Munster)	Ballyhooly Br	Cork	18
18/09/1997	Awbeg (West)	Scart Bridge	Cork	18
08/10/1997	Blackwater (Munster)	1.5 km d/s Ballymaquirk Br	Cork	18
18/09/1997	Awbeg	Br d/s Scart Br	Cork	18
25/09/1997	Finisk	Modelligo Br	Waterford	18
19/09/1997	Awbeg	Br in Doneraile	Cork	18
19/09/1997	Awbeg	Kilcummer Br	Cork	18
20/06/1997	Funshion	Ballynahow Br	Cork	18
20/06/1987	Funshion	Downing Br	Cork	18
01/07/1997	Dungourney	Ballynona Br	Cork	19
16/07/1997	Ballymahane	Ballymahane Br	Cork	20
16/07/1997	Brinny	Downdaniel Br	Cork	20
24/08/1998	Cloghnagh	Br u/s Dinin R confl	Kilkenny	15
05/08/1998	Ballyroan	Sallagh Br	Laois	15
25/08/1998	Dinin North	Massford Br	Kilkenny	15
27/08/1998	Kings	Newtown Br	Kilkenny	15
26/08/1998	Dinin	Dinin Br	Kilkenny	15
01/09/1998	Tinacross	Tinnacross Br	Wexford	12*
24/09/1998	Cavan	Br u/s Annalee R confl	Cavan	36*
21/07/1999	Drish	2nd Br u/s Longfordpass Br	Tipperary	16
07/07/1999	Moyle	Br u/s Anner R confl	Tipperary	16
10/08/1999	Dungourney	Br d/s Dungourney	Cork	19
27/09/2000	Glenaboy	Br u/s Bride R confl	Waterford	18
01/09/2000	Bandon	Baxter's Br	Cork	20
24/08/2000	Powerstown	Br SE of Powerstown	Kilkenny	14
02/10/2001	Dunhill	Br W of Dunhill Lodge	Waterford	17
07/09/2001	Gully	Colooney Br	Laois	15
29/06/2001	Brownstown Stream	Br S of Brownstown	Kilkenny	15
09/09/2002	Dissour	Br in Killeagh	Cork	19
10/09/2002	Glashaboy	Dunbulloge Br	Cork	19
26/07/2002	Blackwater (Kilmacow)	Br to W of Mullinavat	Kilkenny	16
13/08/2002	Owenduff	Taylorstown Br	Wexford	13
05/09/2002	Suir	Thurles Br	Tipperary	16
04/09/2002	Suir	Twoford Br (Main Channel)	Tipperary	16
30/08/2002	Outeragh Stream	Br u/s Suir R confl	Tipperary	16
26/09/2002	Aherlow	Killardry Br	Tipperary	16

Species/ Date	River/Stream	Location	County	Hydrometric Area
Ephydatia fluviatilis (ctd.)				
21/08/2003	Blackwater	Lombardstown Br	Cork	18
14/08/2003	Clodiagh	Br E of Rathcardan	Tipperary	16
22/10/2003	Owenboy (Cork)	Ballea Br (Lower)	Cork	19
03/09/2003	Caha	Caha Br	Cork	20
21/06/2004	Dunhill	Br W of Dunhill Lodge	Waterford	17
21/06/2004	Dunhill	Ballyphilip Br	Waterford	17
08/09/2004	Arrigle	Br WSW of Bohilla	Kilkenny	15
29/08/2005	Clonmore Stream	Br u/s Suir R confl	Tipperary	16
28/06/2005	Errill	Eglish Br	Laois	15
21/07/2005	Cappanacloghy	Br E of Cromoge	Laois	15
21/07/2005	Dinin	Br in Castlecomer	Kilkenny	15
03/08/2006	Halfway House Stream	Br to NW of Halfway House	Waterford	16
01/09/2006	Owbeg	Br u/s Owbeg Br	Waterford	18
26/09/2007	Mahon	ENE of Seafield Ho	Waterford	17
25/09/2007	Dunhill	Ballyphilip Br	Waterford	17
30/07/2008	Killenaule Stream	Br 1 km S of Killenaule	Tipperary	16
25/09/2009	Clonakilty Stream	Br in Clonakilty	Cork	20
21/09/2010	Dinin	Massford Br	Kilkenny	15
23/09/2011	Barnakyle	Br SE of Clarina	Limerick	24
07/10/2011	Halfway House Stream	Br to NW of Halfway Ho	Waterford	16
05/07/2011	Killenaule Stream	Saucestown Bridge	Tipperary	16
17/07/2012	Tourig	Br SW of Tourig Hall	Kerry	18
25/09/2012	Roury	Inchnanoon Br	Cork	20
03/10/2012	Clonshire	Drehidnaman Br (LHS)	Limerick	24
Racekiela ryderi				
17/08/1993	Roughty	Morley's Br	Kerry	21
26/08/1993	Glashacooncore	Glashnacooncore Br	Kerry	23
27/08/1993	Glenacarney	Br u/s Feale R confl	Cork	23
24/08/1994	Sheen	Dromanassig Br	Kerry	21
05/10/1994	Lee	Foot-bridge d/s Inchigeelagh	Cork	19
05/10/1994	Lee	Br S of Gortafludig (Ford)	Cork	19
15/09/1994	Blackwater (Kerry)	Gearha Br	Kerry	21
24/08/1994	Sheen	Releagh Br	Kerry	21
07/09/1994	Finglas (Laune)	Cappagh Br	Kerry	22
25//08/1994	Adrigole	0.3 km u/s Adrigole Br	Cork	21
10/08/1994	Owenascaul	Br 1.6 km u/s Anascaul	Kerry	22
07/07/1994	Gearhameen	Br N of Cockow	Kerry	22

05/07/1994	Beheenagh	Br N of Gortderrig	Kerry	22
04/10/1994	Cullenagh Lake Stream	Br u/s Bandon R confl	Cork	20
13/09/1994	Inny (Kerry)	Ballynakilly Br	Kerry	21
10/08/1994	Owenalondrig	Br in Foheraghmore	Kerry	22
05/09/1994	Owenroe	1.1 km u/s Caragh R confl	Kerry	22
06/09/1994	Behy	Br d/s Coomaglaslaw L	Kerry	22
14/09/1994	Owreagh	Br W of Sneem	Kerry	21
15/09/1994	Owenshagh	Lauragh Br	Kerry	21
16/09/1994	Croanshagh	Croanshagh Br	Kerry	21
22/09/1994	Roury	Corran Br	Cork	20
04/07/1996	Mahon	Aughshemus Br	Waterford	17
11/09/1996	Feale	Wellesley Br	Kerry	23
02/10/1997	Sheen	Releagh Br	Kerry	21
01/10/1997	Croanshagh	0.6 km u/s Croanshagh Br	Kerry	21
17/09/1997	Clyda	Athnalacka Br	Cork	18
22/10/1997	Glan Stream	Br SE of Knockeens	Cork	21
10/07/1997	Glenlara	Ballyduane Br	Cork	18
07/07/1997	Owenanare	Priory Br	Cork	18
31/07/1998	Owgarriff	Owgarriff Br	Kerry	22
30/09/1997	Roughty	Ford NW of Kilgortaree	Kerry	21
19/08/1998	Smearlagh	Br u/s Lyracrumpane R confl	Kerry	23
02/09/1998	Owenascaul	Br 1.3 km d/s Anascaul	Kerry	22
01/09/1998	Owenmore	0.5 km d/s L Gal	Kerry	23
15/09/1998	Ferta	Deelis Br	Kerry	22
17/09/1998	Owenreagh	Br u/s Upper Lake	Kerry	22
05/10/2000	Ownagappul	Slieve Br	Cork	21
14/11/2000	Coomhola	Br nr Knockanecosduff	Cork	21
31/08/2000	Blackwater (Bandon)	Br NW of Teenah	Cork	20
27/07/2000	Glashawee	Br u/s Allow R confl	Cork	18
30/08/2001	Clydagh	Clydagh Br	Kerry	23
07/08/2002	Shournagh	Br ENE of Coolmona	Cork	19
20/06/2002	Owenmore	Br at Boherboy	Kerry	23
08/08/2003	Blackwater (Kerry)	1.5 km u/s Blackwater Bridge	Kerry	21
08/10/2003	Adrigole	Adrigole Br	Cork	21
06/09/2005	Feale	Wellesley Br	Kerry	23
28/07/2005	Owveg	Owveg Br	Kerry	23
02/10/2007	Cloghane (Owenmore)	0.5 km d/s L Gal	Kerry	23
18/09/2009	Behy	Br d/s Coomaglaslaw L	Kerry	22
07/09/2012	Sheen	Releagh Br	Kerry	21
21/08/2012	Coomhola	Br nr Knockanecosduff	Cork	21

Spongilla lacustris				
01/08/1984	Nore	Waterloo Br	Kilkenny	15
15/08/1988	Dawn	Br NW of Stonehouse	Waterford	16
20/08/1992	Clodiagh (Tipperary)	Ballyoughter Br	Tipperary	16
17/09/1993	Suir	Kilsheelan Br (d)	Tipperary	16
17/09/1993	Suir	Kilsheelan Br (e)	Tipperary	16
05/08/1993	Deel	Br nr Killaready	Limerick	24
24/06/1993	Grand Canal	Aqueduct over Stradbally River	Laois	14
01/07/1993	Greese	Br nr Geese Bank	Kildare	14
07/07/1993	Black (Borris)	Dunroe Br	Carlow	14
01/07/1993	Greese	Moone Br	Kildare	14
02/09/1993	Tully Stream	Br u/s Nurney	Kildare	14
17/08/1994	Barrow	Tankardstown Br	Kildare/Laois	14
14/07/1994	Bandon	Br u/s Inishannon	Cork	20
13/07/1994	Caha	Poulnaberry Br	Cork	20
22/06/1994	Sullane	Ford u/s Laney R confl	Cork	19
29/06/1994	Finisk	Modelligo Br	Waterford	18
14/06/1994	Owennacurra	Br S of Lisgoold	Cork	19
13/09/1994	Cummeragh	Just d/s Derriana Lough	Kerry	21
22/09/1994	Leap Stream	u/s Br in Leap	Cork	20
27/10/1994	Blackwater (Munster)	Ballyduff Br	Waterford	18
22/08/1994	Barrow	Twomile Br	Laois	14
25/08/1994	Magannagan Stream	Derryconnery Br	Cork	21
15/09/1994	Clonee	Just u/s Inchiquin Lough	Kerry	21
06/09/1994	Caragh	1.2km u/s Caragh Br	Kerry	22
15/09/1994	Clonee	Casha Br	Kerry	21
18/08/1994	Barrow	Dunrally Br	Kildare/Laois	14
08/09/1994	Laune	Beaufort Br	Kerry	22
16/08/1994	Barrow	Royal Oak Br (RHS)	Carlow	14
05/10/1994	Lee	Dromcarra Br	Cork	19
23/08/1994	Drumoghty	Dawros Br	Kerry	21
24/08/1995	Nore	Threecastles Br	Kilkenny	15
25/08/1995	Nore	Brownsbarn Br	Kilkenny	15
23/09/1998	Erne	Derrin Br	Cavan	36*
15/10/1998	Finn (Monaghan)	Annamakiff Br	Monaghan	36*
12/10/1998	Cullies	Br nr Kilbrackan House	Cavan/Leitrim	36*
13/10/1998	Cullies	Br d/s Disert L	Cavan	36*
18/10/2007	Tintern Abbey Stream	Bridge nr Tintern Abbey	Wexford	13
05/10/2011	Dungourney	Ballynona Br	Cork	19
12/12/2012	Suir	Kilsheelan Bridge (LHS)	Tipperary	16
12/12/2012	Suir	Kilsheelan Bridge (RHS)	Waterford	16

Ephydatia muelleri				
31/07/1992	Rossestown	Kilclonagh Br	Tipperary	16
02/07/1993	Fushoge	Br u/s Barrow R confl	Laois	14
21/09/1993	Slate	Agar Bridge	Kildare	14
22/07/1993	Aherlow	2nd Ford d/s Br SW of Ardrahin	Limerick	16
21/09/1993	Slate	River Bridge (a)	Offaly	14
21/09/1993	Slate	River Bridge (b)	Offaly	14
08/07/1993	Burren	Garryhill Br	Carlow	14
02/09/1993	Tully Stream	Cloney Br	Kildare	14
03/09/1993	Esker Stream	Newtown Br	Offaly	14
04/09/1993	Figile	Ardna Br	Offaly	14
31/08/1993	Figile	Derrygarran Br	Kildare/Offaly	14
31/08/1993	Figile	Kilcumber Br	Offaly	14
31/08/1993	Figile	Cushaling Br	Kildare/Offaly	14
29/06/1993	Athy Stream	Br nr Glenbawn	Kildare	14
19/08/1994	Barrow	Kilnahown Br	Laois	14
16/08/1994	Barrow	Milford Br	Carlow	14
30/09/1994	Maigue	Br in Croom	Limerick	24
23/08/1995	Nore	Tallyho Br	Laois	15
23/08/1995	Nore	Poorman's Br	Laois	15
24/08/1995	Nore	Fennessy's Mill (Ossory Br)	Kilkenny	15
30/08/1996	Owvane	Br u/s (SE of) Loghill	Limerick	24
08/07/1997	Allow	John's Br	Cork	18
01/10/1998	Rag	Br nr Killywilly House	Cavan	36*
24/09/1998	Annalee	0.2km d/s Cavan R confl	Cavan	36*
25/08/2000	Barrow	Cardinal Moran Br	Carlow	14
12/07/2001	Laune	Laune Br, d/s L Leane	Kerry	22
13/08/2002	Owenduff	Bridge nr Carnagh House	Wexford	13
Eunapius fragilis				
30/08/2006	Bride (Waterford)	Tallowbridge	Waterford	18
Indeterminate (indet) ***Ephydatia fluviatilis/Spongilla lacustris***				
22/06/1993	Pollanassa	Br SW of Ballynacooly	Kilkenny	16
02/09/1993	Slate	Ford Bridge	Kildare	14
17/09/1993	Suir	1.5 km u/s Carrick on Suir	Tipperary	16
17/09/1993	Suir	N of Churchtown House	Tipperary/Waterford	16
21/09/1993	Slate	Bridge nr Spencer Bridge	Kildare	14
08/09/1993	Ahacronane	Br SW of Barrigone	Limerick	24
03/09/1993	Figile	u/s Barrow R confl	Offaly	14
18/09/1997	Awbeg (East)	Br NW of Pruntus	Cork	18
19/06/1997	Sheep	O'Brien's Br	Cork	18
18/06/1997	Funshion	Killee Br	Cork	18

25/07/1997	Bride (Lee)	Br at Killumney	Cork	19
28/08/1998	Kings	Ennisnag Br	Kilkenny	15
20/09/2002	Loobagh	North Br d/s Kilmallock	Limerick	24
14/10/2004	Laune	Beaufort Br	Kerry	22
22/07/1993	Aherlow	New Bridge	Tipperary	16
08/07/1997	Allow	Leader's Br	Cork	18