Paper in:

Collections and climate change research:  
*Flustra foliacea* (L.) (Bryozoa) in the  
Natural History Museum, London  

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1. Introduction  
2. Climate change research and museum collections  
3. Why bryozoans?  
4. *Flustra foliacea* – a ‘canary’ for the temperate north Atlantic coasts  
5. The Collections  
6. Conclusions  
7. Acknowledgements  
Appendix 1: *Flustra foliacea* collection at the Natural History Museum  
Appendix 2: Work protocol chart  

1. Introduction  

Museums give pleasure by evoking wonder and awe. They allow us to explore the  
world, making other cultures and places real and tangible. In this ever changing world they  
give users a strong sense of place, identity and belonging based on the past. Museums have  
a considerable economic impact and stimulate science, creativity and industry. In this  
paper we focus on one of their major functions - providing evidence, ideas and opportunities  
for research and learning.  

Museum collections represent huge databases accumulated over time thus providing  
an historical perspective for modern surveys. As old collections become used and studied,  
old information is corrected in a continuous validation process that represents an  
important component of our general knowledge. Collections are especially important for  
groups that are either too big (i.e. some invertebrates), difficult to collect like those living  
in extreme locations, or groups that may have various restrictions in their collecting such  
as happens with many vertebrate taxa.  

Biological collections are cornerstones in such diverse areas as environmental  
monitoring, public health, taxonomy and systematics and even in national security
(Ponder et al. 2001, Hellberg et al. 2001, Hoffmaster et al. 2002, EPA 2002). They play a crucial role in biodiversity loss, biological invasions, biogeography and evolution studies. Without these collections many species would be unknown, even more so in the light of present day biodiversity loss as a consequence of the rapid environmental degradation of the 21st century.

In spite of their intrinsic value, museum collections also pose many problems to the user. Among these is the lack of detailed information in older lots, or the use of obsolete locality names. Museum collections are usually arranged taxonomically thus posing problems for ecological studies. Other problems include temporal and spatial gaps, several types of sampling biases, not enough specimens to evaluate species variability, etc. In many cases collections are not completely databased, though more and more museums dedicate large resources to this end. Indeed, in recent years, natural history museum collections have changed in order to meet the challenges of interdisciplinary studies. Many have become fully digital and are freely available on the Internet providing endless opportunities for research.

An important role is played by curators and collections managers. Their collaboration with researchers in using the collections is indispensable especially at a time when museum resources become compromised. Though extra resources are always difficult to secure, researchers try to help by directing some funds in their proposals to help collections management and databasing. On another hand, curators and collections managers could start being more open towards accepting a certain amount of destructive sampling, especially in cases when expected results merit such exceptions or when not so perfect material is available. As bulk sampling replaces the traditional specimen collecting, these collections can be used as a source of unsorted material for ecological studies while those not so perfect specimens can be used for destructive sampling.

Another aspect where museum collections still lag behind is in their integration with other databases. This is especially important at a time of heightened awareness concerning observed rapid changes – it would be extremely useful if museum collections could be integrated as much as possible with meteorological data, geological reconstructions and library records. Unfortunately field books and collecting records are often kept separated from the collections they refer to which means extra steps in order to retrieve that data.

An area where museum collections have been an invaluable resource is palaeobiology. Such research usually implies sampling many geographic locations throughout the time interval of interest as well as knowledge about depositional conditions, good preservation, etc. The use of palaeobiological collections have been the source of many breakthroughs in science and several seminal works were based on them (Sepkoski and Koch 1995, Jackson et al. 1999).

2. Climate change research and museum collections

Among others, climate change research can highly benefit from the study of natural history collections. The rapid changes documented today call for data from as many
species and locations as possible if we are to understand the underlying mechanisms of responses and adaptations at organisms’ and populations’ level. Besides, collections can also help find reliable organisms to be used as early indicators of environmental impact.

During the last decade and overwhelming evidence have been gathered showing how present day climate trends are already affecting the physiology, distribution and phenology of many species in a consistent way (Raven et al. 2005, Feely et al. 2009). Such data is being used to predict future changes and work out possible remediation and adaptation policies. Although natural variation is certainly responsible for many of these trends, human-induced climate is the most parsimonious explanation for others. The best evidence for these comes from intensive monitoring programs over an extended period (Fleming and Tatchell 1995, Phillips et al. 1998, Boyce et al. 2010) as well as studies comparing present day distributions and past data (Cresswell and McCleery 2003, Cruz-Motta et al. 2010, Genner et al. 2010, Hawkins et al. 2013). Results show how important long term data sets are to identify vulnerable species and communities (Harrington et al. 1999). Unfortunately, such data sets are relatively rare, and new baseline monitoring programs are very much needed. In the absence of these programs museum collections can play an extremely important and decisive role. Most climate studies use either mathematical models or experimental observations in order to predict future changes and advise policy makers. Natural history collections, recent and fossil alike, can bring a different perspective into this research. Indeed, the millions of specimens stored in museums document past (whether millions of years, hundreds or just decades) ecological conditions and how species adapted to these conditions and their turnover. The study of museum material allows us to document changes in biodiversity and abundances through time and space, and analyze how species answered environmental impacts. Museum collections are already being used to monitor geographic range extensions, the establishment or extinction of local populations, to track the advance of exotic species and the replacement of native fauna and flora, etc. Because museums are easily accessible to the non-specialist, collections should also be used to illustrate taxa so non-scientists can help monitoring ‘newcomers’ thus playing a major role in the assessment of the conservation status and level of threat of many species. The amount of new information that collections can provide is overwhelming and they can serve as a real baseline to help make more informed and accurate predictions. Of course, in order to correctly distinguish subjective changes in collecting effort and objective changes in species ranges and abundances (McCarthy 1998) we also need to develop better quantitative methods that allow us to extract the correct information from existing collections taking in account that collecting effort was not the same over time.

With over 70 million specimens, from mammoth skeletons to microscopic slides, a leading research institution and one of the most important world repositories of natural history collections, the Natural History Museum, London (NHM) is a good example of how museum science has changed during the last several decades.

The aim of this paper it is to describe the collection of Flustra foliacea (L.) (Bryozoa) collection housed at the NHM, and its potential use in climate change studies.
3. Why bryozoans?

Current data show that ongoing changes in ocean chemistry will severely impact marine taxa with unpredictable consequences for abundance, diversity and species distribution (Raven et al. 2005, Riebesell 2008, Feely et al. 2009, IPCC 5th Assessment Report 2013) as well as food chains and ecosystems. Taxa that mineralize calcium carbonate skeletons should be especially affected by such trends. In order to better understand observed events and be able to deal with its consequences we need more studies of the short and long term consequences of ocean acidification. Especially important are data on the combined effects of $CO_2$ and temperature.

The Phylum Bryozoa has an enormous potential concerning studies of ocean acidification impacts that have somewhat been overlooked even though they play an important role in benthic temperate ecosystems (Ryland 1970). Bryozoans can be found both in the ocean and in freshwater and they are well preserved and abundant as fossils with a record extending from the early Ordovician (some 488 million years ago) (Pohowsky 1978, McKinney and Jackson 1989, Jones 2006, Taylor et al. 2013). Important bio constructors both in past and recent environments, they are fundamental components of the marine sessile epifauna (Scholz and Krombein 1996, Massard and Geimer 2008). Many encrusting and erect species coexist, often in direct competition for space and food forming the so-called ‘biomats’ that may cover large areas thus increasing substratum complexity and the diversity of sessile communities (Stebbing 1971a). Bryozoan diversity of shapes, forms and growth strategies makes them a good subject study in benthic temperate ecosystems and an easy subject for inferences of environmental regimes.

Recently, a series of important studies using bryozoans as model systems for bio-invasions, larval dispersal and marine speciation, palaeotemperature proxies, sources of bioactive (antifouling and antimicrobial) compounds and acidification impact in calcification patterns became available. Being sessile organism, they are first to be impacted by pollution and stress. Several species have been successfully used as indicators in coastal pollution and changes in bottom communities. A few studies also indicate that particularly sensitive species could be used in programs monitoring the effects of ocean acidification and as early indicators of switches in carbonate regimes (Smith 2009, Smith and Garden 2013).

Several bryozoans studies have focused on skeletal mineralogy, growth rates and carbonate production (Smith et al. 1992) as well as their use to monitor seasonal changes in temperature and primary productivity in northern latitudes (Bader 2001, Bader and Schäfer 2005, Schäfer and Bader 2008). Bryozoans’ skeleton composition can be calcite, aragonite or a combination of both elements. They exhibit a wide range of $\text{MgCO}_3$ (0 – 14 wt%) which was shown to be at least partially phylogenetically controlled (Smith et al. 2006, Taylor et al. 2009). Studies on dead skeletons have shown that their mineralic composition (LMG-/HMG-calcite, aragonite) conditions the way acidity effects them. Besides, different species will react to acidification impact differently and at different stages in their life cycle. As a result, more sensitive species could be used as ‘sentinels’
thus providing early warning signals of acidification impacts in shelf communities (Smith 2009, Smith and Garden 2013). Bryozoan species with bi-mineralic skeletons could be good candidates for this ‘canary’ status. Many of them form thickets on the seafloor being important ecologic elements and carbonate producers in mid-latitude northern- and southern-hemisphere shelf communities.

Bryozoan zooid morphology has been previously used in inferences of environmental regimes. Several species have easily discriminated growth bands that provide a reliable tool to calculate growth rates. These data have been used to infer relationships between growth and environmental parameters such as temperature and productivity levels (Menon 1975, Okamura 1987, Bader and Schäfer 2005). Zooid morphology was also shown to be temperature dependent in a few species (O’Dea and Okamura 1999, 2000a, 2000b, O’Dea and Jackson 2002, O’Dea 2005, Lombardi et al. 2006, Amui-Vedel et al. 2007).

4. *Flustra foliacea* – a ‘canary’ for the temperate north Atlantic coasts

*Flustra foliacea* (Figure 1) is a cheilostome bryozoan widely distributed in the North Atlantic Ocean, on both American and European coasts where it plays an important ecological function as habitat structuring. It often forms dense thickets dimensionally structuring the sea floor and providing habitat to a rich epifauna (Stebbing 1971a, Bitschovski et al. 2011, Bitschovski 2013). This is a widespread species on gravel grounds in the UK coast and many other regions around the temperate western North Atlantic (i.e. Normandy, Steingrund, Helgoland, etc.) but rare in several adjacent seas (i.e. Baltic, White, Kara and Barents seas). It is also quite abundant in the Bay of Fundy (US/Canada east coast) where recent coastal surveys show a notable range expansion during the last decades. This is interpreted as one of the possible reasons for the changes in community structure observed in the area (Kenchington et al. 2007). Interesting enough, *Flustra foliacea* may also be present in the Commander Islands, Bering Sea, (A. Grischenko pers. comm.) which if true would probably represent the remnants of an old distribution.

The first reference to *Flustra foliacea* was in 1665, when it was observed and its zooids described by Robert Hooke. In 1758, Linnaeus included it in the 10th edition of *Systema Naturae* under the name of *Eschara foliacea* which was later changed to its current name (Hansson 1999). *Flustra foliacea* is the type species of the genus (Ryland 1969).

This species is an important producer of biologically active metabolites. Flustramine E from North Sea colonies interferes with the development of *Botrytis cinerea* and *Rhizotonia solani*. Furthermore, Deformylflustrabromine was shown to be relatively toxic to the cell line HCT-116 (human colon cancer). This contributes to make *F. foliacea* a potential subject for medical studies (Lysek et al. 2002, Liebezeit 2005, Sala et al. 2005, Sharp et al. 2007) and an emblematic species.

Being mostly a cold temperate species, *F. foliacea* grows mainly during spring and summer producing annual growth rings. Colonies grow usually on shells and small rocks being usually restricted to sub littoral waters though they can be found deeper. They start
as flat encrustations and its characteristic loose fronds form from the second year onwards. They are produced when two growing lobes of the colony meet, and the two edges that make contact begin growing upwards, forming a bi-laminar, upright frond. This initial frond starts to branch and colonies may live up to 12 years growing in bushy clumps. Flat, lobate fronds can reach up to 20 cm and are flexible due to the small amount of calcium carbonate. Most of the zooecium walls are chitinous (Fish and Fish 2011).

This species exhibits considerable morphologic variability along its distribution area (Figure 1) ranging from bush-like forms with shorter, wide fronds in the Atlantic open coastal areas to thin, elongated (often ribbon-like) branches in marginal habitats. Besides, specimens from open ocean areas of the western Atlantic usually present up to four well-developed spines on the zooids top. This could be a possible response to predation by nudibranchs (quite abundant in Atlantic coastal settings) as it was shown for Membranipora membranacea (Harvell 1984, 1992). In adjacent seas (i.e. Baltic, Barents Sea) spines are either absent or weekly developed.

Growth-check lines are more pronounced in the colony younger parts. Easily observable with naked eye and under an optical microscope, this was used by Stebbing (1971b) to study monthly growth rates in material collected from South Wales. Results indicated a linear growth pattern in height and similar growth rates at all ages. Later on, experimental data showed the existence of a direct relationship between temperature and growth rates.
and an inverse relationship with zooecia average size (Menon 1975). These data were used as the basis to use zooid profiling and seasonal growth cycles in *Flustra foliacea* as a tool to infer environmental regimes, especially temperature changes, in perennial bryozoan skeletons (O’Dea and Okamura 1999, 2000a, 2000b, O’Dea 2005). This is the so-called MART technique that has also been used as a tool in palaeoclimate studies (O’Dea and Jackson 2002).

The wide distribution and conspicuousness of *Flustra foliacea* makes it a good subject for climate change studies. Being a bi-mineralic species it can be used as an indicator in coastal north Atlantic areas. Fortunato *et al.* (2013) found higher variability in zooid frontal index and density in Baltic colonies than in North Sea ones. Despite similar carbon and carbonate contents in colonies from both areas, preliminary data from muffle oven tests show higher weight loss in Baltic colonies whereas Helgoland colonies show higher sensitivity to dissolution which may relate to the different phenotypes that inhabit these areas. On the other hand Schäfer *et al.* (2014) found that Baltic colonies exhibit a higher content of organic matter at the expense of carbonate. This may be seen as another way of fulfilling the skeleton main function, i.e. support, being also a better protection for the calcitic matrix in a higher corrosive environment. Similar conclusions were made by Rodolfo-Metalpa *et al.* (2010) in relation to *Myriapora truncata* grown under naturally acidic conditions in the Mediterranean Sea. Preliminary results from colonies grown under different temperature and CO$_2$ levels show changes in calcification levels (Fortunato unpub data).

Data using zooid profiling to infer environmental regimes, especially temperatures, have given somewhat contradictory data. Indeed, whereas *Conopeum seurati* shows a clear relation between temperature regimes and zooecia area rather than its linear size (O’Dea and Okamura 1999), *Pentapora fascialis* zooid sizes seem to be more sensitive to temperature regimes than zooid area (Lombardi *et al.* 2006) whereas *Cryptosula pallasiana* colonies collected during early summer had longer and wider zooids than those collected in January but colonies grown under different temperature regimes presented significantly longer and wider zooids at lower temperature values (Amui-Vedel *et al.* 2007). In order to understand how MART technique can be better used in climate change studies we need a baseline which can be obtained analyzing specimens from one single species collected since pre-industrial times and kept in museum collections. Such information can then be used to detect changes in growth rates and calcification levels that could eventually be related to ongoing environmental changes. Furthermore it could be used to calibrate experimental data and build inferences for future changes.

*Flusta foliacea* can fulfil this role. It is often washed up on beaches after storms and following high tides and is conspicuous enough to be frequently collected and in relatively large amounts. (It was often mistaken by collectors in the past as a plant and deposited in herbaria). As a result there are now excellent collections dating from the late 1600s onwards and housed in several museums. The collections housed in the NHM are extensive (Figure 2) and have recently been utilised to study *F. foliacea* growth patterns and calcification levels over the last couple of hundred years.
Figure 2. Two pages of the Catalogue.
5. The Collections

The NHM collections present a unique opportunity to make direct comparisons between specimens of *Flustra foliacea* collected in different parts of the North Atlantic basin since pre-industrial times. Especially important is the fact that they allow comparisons across populations inhabiting different environments thus being ideal for both MART (O’Dea and Okamura 2000a, 2000b) and calcification rates studies. The collections were made over 300 years spanning a period from pre-industrialization through today and can thus be used to build up a temporal baseline necessary to assess eventual temperature changes in the ocean during the last several centuries. Zooid parameters will be used to obtain estimates of the temperature regime at the time the colony was alive. Data will then be used in ongoing climate change studies and related ocean acidification impact in carbonate builders to help assess observed morphologic changes and its relation to environmental factors.

Appendix 1 lists the *F. foliacea* collection stored at the NHM. A total of 111 samples were examined by stereo- and scanning electron microscopy. Figures 3-7 illustrate some of the most interesting lots. The museum also has a wet sample from the Canadian Atlantic coast which was not included in this study but is included in the database for future reference.

Due to their unique value, colonies from historical collections were studied only by stereomicroscopy. Images were captured and analyzed using the image capture system (Zeiss AxioCam High-resolution digital system, Figure 8) available in the Zoology department. Zooid sizes in calibrated digital images were measured using morphometric software.

Work followed a protocol (Appendix 2) developed earlier by Fortunato *et al.* (2013). The work proceeded the following way:

1. All lots were initially surveyed and entered in a database. Besides of the BM access number, each lot received a unique ID that will be used for further work. Besides of the information in the label, other information about the lots (i.e. colony morphology type, number of colonies in the lot, preservation condition, etc.) was registered in the database. All lots were photographed including the BM label and the ID.

2. If more than one colony was available in the lot, one of them was chosen, preferably one with a starting point well recognized by its incrusting attachment. In this colony a long branch was chosen for further work so a bigger time span would be followed. This colony was further identified in the original lot by another label specifying that it was studied by the author.

3. Measurements were done following a profile running from the growing tip of the branch and moving downwards towards its base following the sequential generations of budding zooids (Figure 9). Three zooids in each sequential generation along the profile were measured.

4. The following parameters were measured, counted or checked in every studied colony branch: maximum zooid length and width, presence and location of spines, number of
spines if present, number of ovicells per generation, number of avicularia per generation, number of generations in the branch. Geminated zooids, zooids with avicularia and ovicells were not used for measurements. If they occurred in the area of measurements, they were skipped and other nearby zooids used. Towards the base of the branch, in the encrusting area, it was difficult to find complete zooids; besides zooids were often heavily deformed and overgrown. For this reason measurements were either not taken in this area or zooids outside the transect were used if available.

5. Zooid density, collected every 4.6 mm intervals along the profile starting from the growing tip, was measured as the number of zooids in an area of 0.88 mm².

6. Two indices were calculated: index of zooid frontal area (length x width) and zooid shape (length/width) following O’Dea and Jackson (2002). The presence and frequency of ovicells was used to assess the reproductive status of the studied colonies.

Specimens from recent cruises and kept at the museum were studied through Scanning Electron Microscopy (LEO 1455). In this case, a selected colony was washed and dried to clean it as much as possible. This colony was then mounted, photographed and the photograph used to navigate in the electron microscope to help set up a transect along which SEM photos were taken for later zooid measurements. The same protocol of work described above for digital images was used here (see Appendix 2).

The present material will allow the study of growth rates in colonies collected during the 18th, 19th and 20th centuries. Compiled information will be compared with data from

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**Figure 3** (left). Flustra foliacea: two lots received from the Cambridge Botany School. Catalogue information places collection date around 1761.

**Figure 4** (below). Flustra foliacea: lot collected probably around late 1700s.
recently collected specimens and those grown in experiments with varying CO$_2$ and temperature levels. Through the comparison of growth rates and calcification levels in older material and modern colonies we aim to be able to detect and analyze eventual changes in growth and calcification in *Flustra foliacea* which could be paralleled with the accelerated acidification trends documented for the western North Atlantic since pre-
industrial times. Temperature related data obtained using the MART methodology will be used to calibrate our experimental based inferences concerning temperatures and environmental regimes. Results will finally be integrated in a database aimed to use *F. foliacea* as an environmental indicator of temperatures (and acidification levels) in the Northern Hemisphere coastal areas. This will represent an important contribution to the use of bryozoans as key elements to monitor changing oceanic pH and its effects in temperate northern shelf areas aiming to a better understanding of ocean acidification impact in benthic marine systems and its biodiversity.

6. Conclusions

Despite the growing amount of research using natural history museum collections (Suarez and Tsutsui 2004, Barnet *et al.* 2008, Pinto *et al.* 2010), this practice is not yet common. Natural history museum collections can and should be used more frequently as sources of information to complement ongoing surveys. They will add a temporal dimension necessary for a better understanding of ongoing trends and preparation for future changes. Furthermore, the use of natural history collections in high impact research areas such as climate change and global warming, medical and epidemiological surveys, invasive species monitoring and even national security will reinforce the need for the maintenance of these wonderful places, i.e. museums, thus justifying every penny spent...
on them and their staff. In the end, we all stand to gain, science and society alike, from this practice.

7. Acknowledgments

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References


Bader, B. 2001. Modern Bryomol-sediments in cool-water, high-energy setting: the inner


Appendix 1. Lots of *Flustra foliacea* in the Natural History Museum, London (NHMUK) collections. The left-hand numbers are the current study IDs. Locality information in parentheses was deducted by the first author (HF) from the data on the labels. Information and notes marked (*) also from the first author.

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</tbody>
</table>

...and many more locations and collections...
69 1997.9.29.6 Britain
coll GLarwood; leaves have Scrupocellaria

70 1972.5.1.15 Charmouth (W Dorset) Britain
05/30/1972 on beach; coll AB Hastings

74 2003.6.3.2 Portmeirion, Wales
10/031996 strandline; coll KJ Tilbrook

75 1996.10.16.2 Clacton (Essex) UK
02/1985 washed on beach

76 06/03/1999 St S99/260, depth 125m, RV Scotia;
59°22.03' 59°20.0N/01°10.4-01°14.47W;
coll M Spencer Jones

77 2007.9.18 Leasowe, Wirral Peninsula UK
04/2006 strandline; coll T Knowles

78 1998.6.18.1 Southwold, Suffolk UK
02/14/1998 coll K Tilbrook

79 Bell rock, NE Scotland
06/01/1999 St S99/251, trawl, depth 58m, RV Scotia;
56°25.6-56°23.41N/02°10.13-02°3.95W;
coll M Spencer Jones

80 42.12.19.2 Britain
coll Johnston; V Gray B M Rad p.112, spec 'f'

81 47.9.24.143 Firth of Forth (River Forth, Scotland)
coll Johnston; '85.d'

82 47.9.24.144 Berwick Bay (Scotland)
coll Johnston; '85.e'

83 47.9.24.142 Firth of Forth (River Forth, Scotland)
coll Johnston; '85.c'

84 47.9.24.142 Firth of Forth (River Forth, Scotland)
coll Johnston; '85.b'

85 47.9.24.141 Tay River, Dundee Scotland
coll Johnston; '85.a'

86 47.9.24.145 Ireland
coll Johnston; '85.f'

87 42.12.7.24 Firth of Forth (River Forth, Scotland)
coll Busk

88 99.7.1.949 Firth of Forth (River Forth, Scotland)
coll Busk

89 34.10.24.6 Cleethorpes, Sheffield (South Yorkshire)
coll GR Vine; B-L 39

90 29.12.8.7-8-9 Calvados (Normandy, France)
coll Busk/ A Waters

91 97.5.1.428 Hastings (E Sussex)
coll Busk; (HF note: correct location name?)

92 99.7.1.296 Algoa Bay (South Africa?*)
coll Busk; (HF note: correct location name?)

93 1963.3.30.205 Cromer (N Norfolk)
09/1881 probably found on beach; coll SF Harmer

94 1963.3.30.256 Cromer (N Norfolk)
09/1881 found on beach; coll SF Harmer

95 98.4.2.4-6 off of Poole (Dorset)
part of 89.7.27.47

96 61.12.26.6 Britain
D Adler (P)

97 81.7.24.1 Kurrachee
(Manora Peninsula, Kurachi, Pakistan)
Mus. Kurrachee (P);
(HF note: correct location name?)

98 34.8.26.4 Dr Bother

99 99.7.1 Calvados (Normandy, France)
1816* coll Busk

100 2009.11.4.2 Padstow, Cornwall
1972 coll PJ Hayward

101 2011.4.2.5 Walkford, Dorset
strandline; coll M Spencer Jones

102 2009.2.3.5 Holme Titchwell, Norfolk UK
09/30/2010 coll M Holloway

103 2009.2.3.5 River Hayle, Cornwall England
04/2007 mouth of river; coll KJ Tilbrook

104 2009.2.3.5 Skarreklit, Bulbjerg Denmark
05/1962 washed on beach; coll A Cheetham

105 2009.2.3.5 east of Fair Isle (Scotland)
06/03/1999 St S99/257, depth 103m, RV Scotia;
59°14.54-59°17.95N/01°26.28-01°26.51W;
coll M Spencer Jones

106 2009.2.3.5 Mudeford, Dorset UK
12/26/2010 strandline, beach; coll M. Spencer Jones

107 1991 St 185, st ABBS dump, RV Scotia

108 1997 St 204, RV Scotia

109 05/30/1994 St 190

110 Bell rock (NE Scotland)
St S99/261, RV Scotia

111 2011.4.15.1 Snettisham, West Norfolk
03/22/2011 [TF6433] on beach; coll B Okamura

112 1966.2.8.1 Cheverie, Kings Co.,
Minos Basin, Nova Scotia
Appendix 2. Work protocol for digital imaging of *Flustra foliacea* collections housed at the Natural History Museum, London. All macro photographs used the same scale. Whenever possible, photos were done using the same magnifications.

**Sequential Procedure**

**Preparation of material**

1. Unique ID number given to each studied lot; ID is carried on to all further steps (SEM, photos, measurements, etc.).
2. Database lots, including labels’ information, number of colonies/lot, number of branches/colony. Includes a brief description of the colonies (general aspect (fresh, very old, fragile, etc.), morphology type (ribbon-like, bushy, mix type), coloration.
3. Macro-photograph of the lot including a scale and the original label. In the absence of a real scale a standard easily recognizable object (a coin) was used. The scale was used for all further photographs.
4. Choice of one colony for further work (when several available in the lot). Criteria for choice: colony aspect and preservation, presence of attachment area; branch size. Macro-photograph of this colony.
5. Choice and macro-photograph of a branch to measure in the colony used in (4). Longest branch was always chosen.

**Digital imaging**

6. Photograph of the whole colony chosen in (4) using the smallest available magnification.
7. Photograph of the branch chosen in (5) using a small magnification.
8. Position at the bottom of the branch, increase the magnification, start photographing along the transect in the middle of the branch, moving upwards. Successive photos overlap a few generations on both the bottom and the top so as to recognize them and be able to reconstruct the branch. Photos received a sequential number (including the colony ID). All photos have a scale. These photos will be used to count number of avicularia and ovicells, as well as presence, position and number of spines (if present).
9. Position at the tip of the branch, increase the magnification, start photographing going down the transect in the middle of the branch towards the attachment area. Photos receive a sequential number that includes de colony ID. These photos will be used to measure zooids parameters.