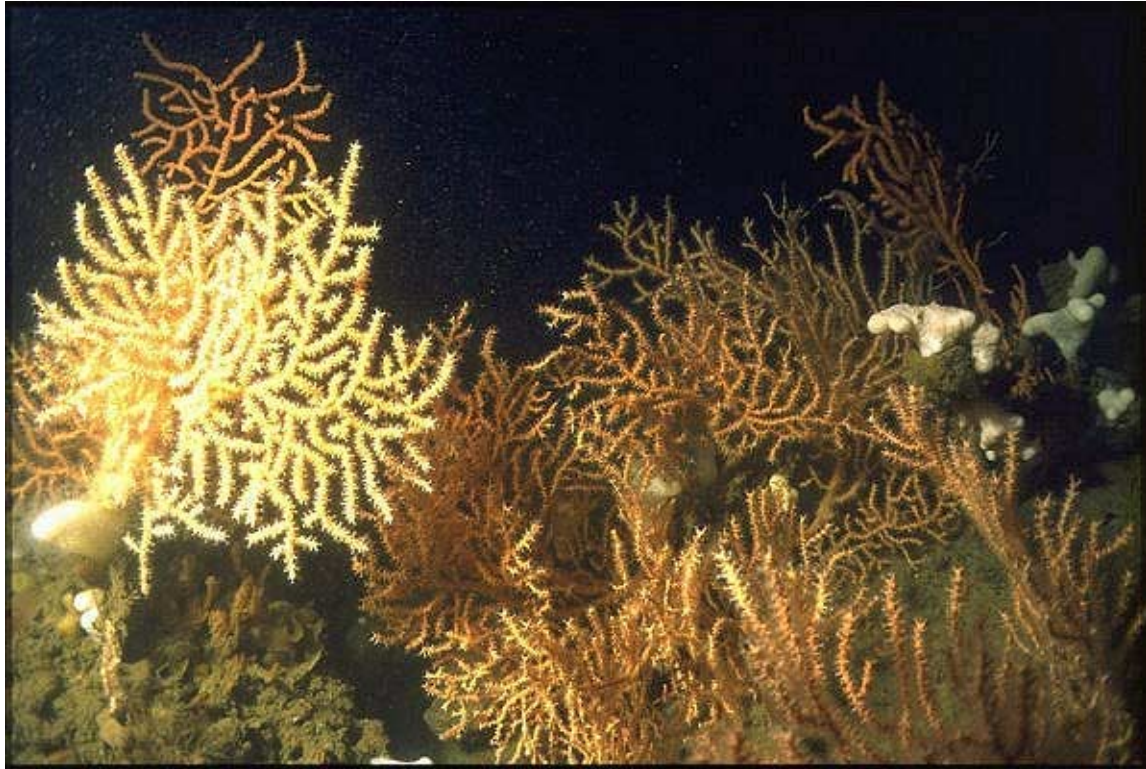


Reef Research

EUNICELLA VERRUCOSA REPRODUCTION STUDY



Determining the reproductive cycle of *Eunicella verrucosa*

**INTERIM REPORT
March 2003**

A REPORT TO CCW



Reef Research

1 Orchard Cottages, Coombe Barton
Shobrooke, Crediton, Devon EX17 1BS
Tel/Fax +44 (01) 363 775278 Mobile 07977 096738
E-mail: colin-m@marinebiologicalsurveys.co.uk
Web Site: <http://www.reef-research.org>
Contact: Colin & Lexie Munro, Project Directors

Determining the reproductive cycle of *Eunicella verrucosa*

**INTERIM REPORT
March 2003**

REEF RESEARCH

Report by:

Alexandra Munro & Colin Munro

Report Ref: RR Report 3/2003 ETR 07

CONTENTS

1. INTRODUCTION	1
2. WORK UPDATE	3
<u>Skomer sampling programme</u>	3
<u>East Tennants Reef sampling programme</u>	3
<u>Web Development</u>	3
<u>Conference presentations</u>	3
3. TYPES OF REPRODUCTIVE STRUCTURE OBSERVED	4
Gonad coils	4
Reproductive bodies	5
4. SUMMARY OF RESULTS TO DATE	6
<u>Skomer – September 2002</u>	6
<u>Lyme Bay – September 2002</u>	6
Female colonies	6
Male colonies	6
<u>Lyme Bay – February 2003</u>	6
Female colonies	6
Male colonies	7
5. DISCUSSION	12
6. SUMMARY OF OUTPUTS TO DATE	14
7. FORTHCOMING WORK	14
8. REFERENCES	14

1. INTRODUCTION

Our current research into the reproductive cycle of *Eunicella verrucosa* forms part of a wider project set up by Reef Research in 1994 to consider the autecology of UK *Eunicella verrucosa* populations. Our study involves the collection of long term data relating to colony growth, recruitment and mortality. The aims are to determine age-specific survivorship, mean colony growth rates and population growth – whether negative or positive. We consider such data fundamental to understanding the inter- and intra-population dynamics within temperate reef systems. The primary study area for this project is the East Tennants Reef, an offshore, subtidal reef within Lyme Bay, SW England, where we have established a permanent monitoring station in order to collect time series data for a discrete population of seafan colonies.

Through study of *Eunicella verrucosa*'s reproductive cycle we hope to provide key ecological information to inform future conservation and management strategies relating to the species. The main areas addressed in our study are:

1. determining the duration and pattern of *E. verrucosa*'s reproductive cycle (i.e. how frequently colonies spawn and what environmental cues (if any) influence the synchronisation of spawning);
2. determining the fecundity of individuals and populations, and the levels of larval survivorship and
3. determining the age / size at which colonies reach reproductive maturity.

There is no existing data on the reproductive ecology of *Eunicella verrucosa*. Research of other gorgonian species (Brazeau, D. A. & H. R. Lasker, 1989; Coma, R. *et al.*, 1995a; Coma, R. *et al.*, 1995b; Grigg, R. W., 1977; Lasker, H. R., 1990; Weinberg, S., 1979), suggests that there is considerable variation in reproductive ecology between different gorgonian species in the following aspects.

- average number of gametes produced per polyp
- whether spawning occurs in synchronous or non-synchronous events
- length of time to first reproduction (varies from 2-13 years)
- duration of oogenesis and spermatogenesis
- size of mature oocytes and sperm vesicles
- whether the species reproduces sexually, asexually or both
- length of time larvae spend in the water column prior to settlement
- whether fertilisation is internal or external
- ratio of male to female colonies within a population

Our study involves repeat sampling of tagged colonies from East Tennants Reef and Skomer MNR (Figure 1) through the monthly collection of branch clippings. On each collected branch clipping, five polyps are dissected to determine the number, size and type of reproductive structures present (Figure 2). Monthly sampling over a 15-month period should allow us to determine the duration of oogenesis and spermatogenesis and the size of mature oocytes and sperm vesicles. The study should also enable us to discover when spawning occurs in UK populations of *E. verrucosa*, whether spawning is a synchronous event, and which environmental factors (if any) influence the timing of spawning.



Figure 1 Diagram showing the sampling sites at Skomer, Pembrokeshire and East Tennants Reef, Lyme Bay.



Figure 2 Dissecting a branch clipping under a low power binocular microscope (© Rohan Holt)

This report details Reef Research's recent work on the reproductive cycle of the gorgonian octocoral *Eunicella verrucosa*. We summarise work conducted between September 2002 and March 2003 which has been enabled through the financial assistance provided by the Countryside Council for Wales' Species Challenge Fund.

2. WORK UPDATE

We are continuing to follow the methodology of our monthly sampling programme in order to understand the oocyte developmental cycle, and identify the spawning period for *E. verrucosa*. Prolonged periods of poor weather have prevented diving in some months at both Skomer and East Tennants Reef.

Skomer sampling programme

At Skomer, the marine team has continued to collect samples from a pool of 8 large seafan colonies at Bernies Rock and Bull Hole sites. Six colonies from within this pool were sampled in September; from each colony two clippings being taken from near the top and two from the side branches.

East Tennants Reef sampling programme

We have now tagged a total of six large colonies close to the existing seafan monitoring station on the East Tennants Reef and aim to collect clippings from each of these colonies on all sampling visits. In September all six large colonies were sampled. In February four of the six colonies were sampled. On each visit, a total of six 1 - 2cm clippings were taken from each colony; two from near the top, two from the side and two from the lower branches. We were concerned that monthly collection of branch clippings from the four medium colonies tagged on East Tennants Reef may cause excessive loss of tissue. Since sampling of both large and medium sized colonies is not considered essential to determine the oocyte developmental cycle it has been decided not to collect further samples from the medium seafans at present.

Following collection labelled clippings from Skomer and East Tennants Reef were placed in aerated, seawater tanks until microscopic examination was possible. From each clipping, five polyps were examined for the presence of reproductive bodies. The calyx surround each polyp was carefully excised to expose eggs or sperm vesicles between the mesenteries and developing gonad coils on the mesentery walls. Any eggs or sperm vesicles present were described, measured and counted. Developing gonad "coils" (see section 3) were counted and described according to colour (e.g. black speckled, translucent, creamy orange). Representative photographs were taken and line drawings made of all the different reproductive stages observed. We have been able to begin compiling a preliminary data summary of our findings to date.

Web Development

During October and November, when sample collection was not possible due to poor weather conditions, time was spent updating the Reef Research website at www.reef-research.org. Three new pages detailing the reproduction study have been added to the 'Research' section of the site. We have also added details of the Species Challenge Funding received through CCW to our '2002 Sponsors' page.

Conference presentations

In December we attended the Reef Conservation UK 2002 conference at the Royal Zoological Society in London to talk about the Seafan Monitoring work we have conducted to date.

All work undertaken since our last report to CCW is summarised in Appendix 1.

3. TYPES OF REPRODUCTIVE STRUCTURE OBSERVED

Reproductive structures observed to date vary in type and colour. We have developed a qualitative scheme by which reproductive structures are classified according to structure as 'gonad coils' and 'reproductive bodies'.

Gonad coils

We use the term 'gonad coils' to describe structures which have been observed attached to the mesentery walls within the gastrovascular cavity of individual polyps (see figures 3 & 4). The 'gonad coils' appear as a dense mass of tissue, which is often tightly coiled and can be coloured pale orange / pink, creamy or translucent with black, red or white speckling. We have been unable to determine discrete reproductive vesicles within the mass of gonadal tissue.

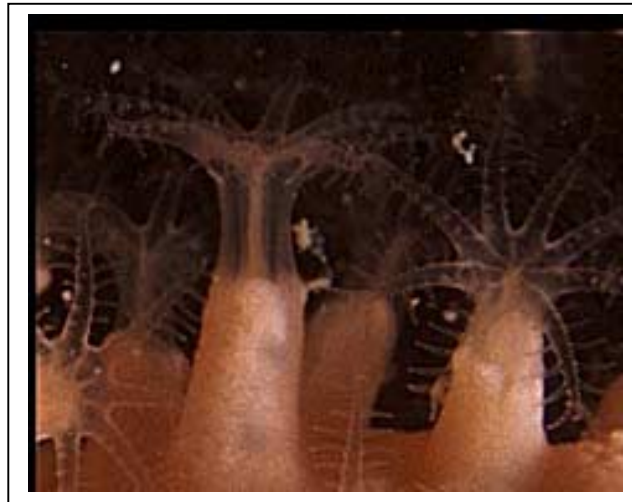


Figure 3 Close up image of *Eunicella verrucosa* polyps extending from calyces.

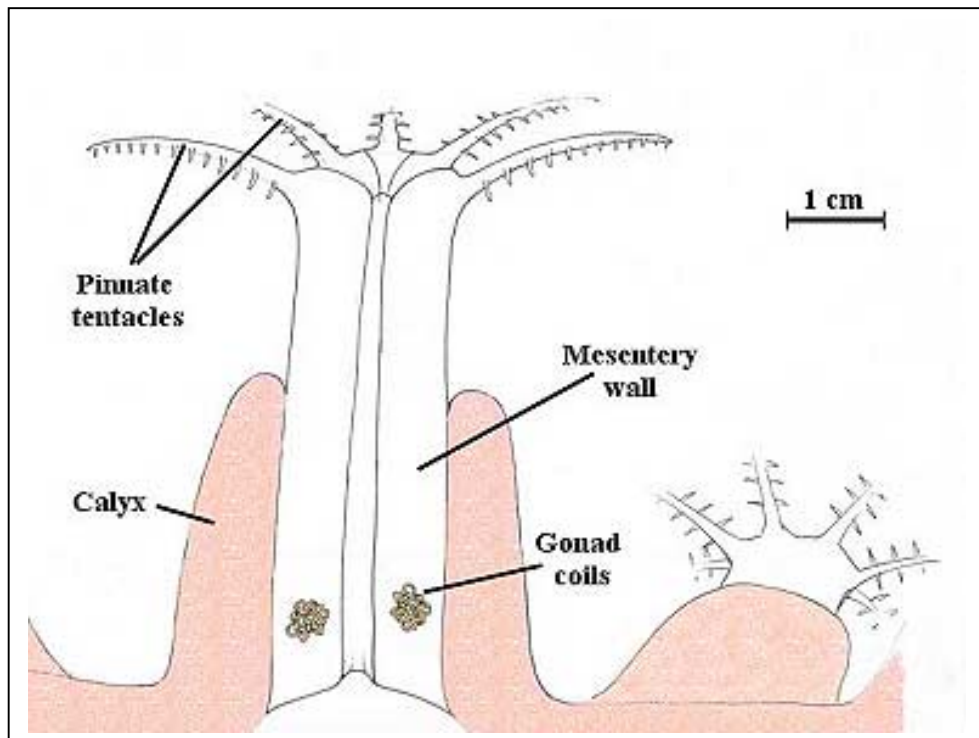


Figure 4 Diagram showing position of gonad coils on the mesentery walls of an *E. verrucosa* polyp

Reproductive bodies

In this study the term 'reproductive bodies' is used to describe discrete spherical bodies thought to be individual 'eggs' or 'spermaries'. We classify reproductive bodies according to diameter measured to the nearest 0.1mm. Broad size classes are as follows; very small, <0.1mm; small, 0.1mm - 0.2mm; medium, 0.2 – 0.4mm; large, 0.4 – 0.5mm and very large, 0.5 – 0.6mm. Reproductive bodies are also described by colour (red, orange, translucent/pink and translucent/white). Figure 5 shows translucent/white reproductive bodies. Red reproductive bodies are shown in Figure 6.

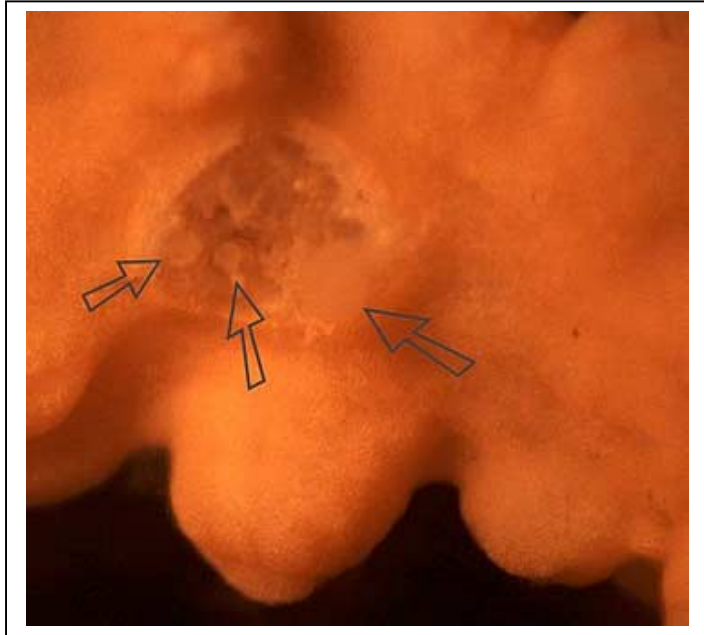


Figure 5. Translucent / white reproductive bodies, thought to be spermaries, are arrowed. The vesicle to the far right is 0.5mm diameter, that shown to the far left is 0.1mm diameter. Central vesicle measures <0.1mm diameter.

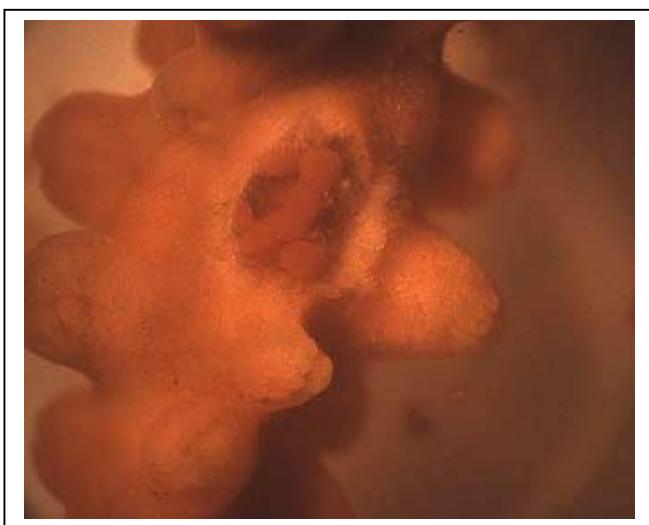


Figure 6. Reproductive bodies thought to be eggs are shown. Diameter of individual "eggs" observed in this polyp ranged from 0.3 – 0.4mm.

4. SUMMARY OF RESULTS TO DATE

Skomer – September 2002

Around Skomer sampling dives were conducted in September 2002 (24.09.02 [Bernies Rock]& 30.09.02 [Bull Hole]). A total of 120 polyps were examined for reproductive structures. As shown in Figure 7 (graph iii), a high proportion of polyps contained small gonadal structures and very small spherical reproductive bodies. Gonad coils were observed in the gastrovascular cavity of 74% (n = 84) of polyps examined. A total of 118 very small (<0.1mm diameter), orange, spherical bodies were removed from 22 polyps (18% of the total sample). For this size class the highest number of spheres removed from a single polyp was 13. Within the size range 0.1 – 0.2mm diameter, both red and orange spherical bodies were found. The highest number removed from a single polyp was 8, and in total 87 spheres within the 0.1 – 0.2mm size class were removed from 27 polyps (23% of the sample). The largest spherical bodies found in the September sample from Skomer were between 0.2 and 0.3mm diameter and both red and orange in colour. A total of 27 spheres within this size class were found in 14 polyps (i.e. in 12% of polyps dissected). The highest number of 0.2 – 0.3mm spheres removed from a single polyp was 4. No spherical bodies within the size classes 0.4-0.5mm and 0.5 – 0.6mm diameter were found in September.

Lyme Bay – September 2002

Sampling dives were conducted at East Tennants Reef on 13.09.02. A total of 180 polyps were examined for reproductive structures. Of these, 120 polyps were thought to be from female colonies and 30 from male colonies (at this stage we are still not certain of colony sex). We have excluded the results from one colony (L9) as we have insufficient data to even speculate on colony sex.

Female colonies.

As shown in Figure 8 (graph iii) gonad coils were observed in the gastrovascular cavity of 59 polyps (49% of the polyps examined – a far higher figure than had been found in previous months). A high proportion of the reproductive vesicles observed were in the size classes <0.1mm (n = 480) and 0.1 – 0.2mm (n = 201). No reproductive vesicles sized 0.4 – 0.5mm were found. In nine polyps very large red spheres (0.5 – 0.6mm) were found (16 large red spheres in total).

Male colonies

Figure 9 (graph iii) shows that low densities of reproductive vesicles were found in male colonies. In five polyps (17% of polyps sampled) a total of 11 vesicles within the size class 0.2 – 0.4mm were found. Coiled structures thought to be gonadal tissue were found within the gastrovascular cavity of 20 / 30 polyps (67% of polyps dissected).

Lyme Bay – February 2003

Sampling dives were made at East Tennants Reef on 06.02.03. Following sample collection a total of 150 polyps were examined for reproductive structures. Of these 120 polyps were thought to be from female colonies and 30 from male colonies.

Female colonies.

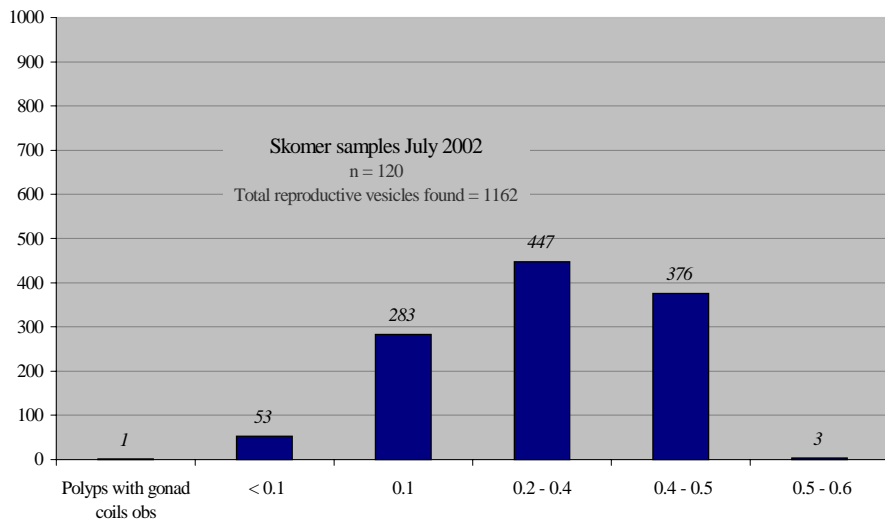
As shown in Figure 8 (graph iv) gonad coils were observed in the gastrovascular cavity of 57 polyps (48% of the polyps examined – a similar proportion of the sample to that found with gonad coils in September 2002). A high proportion of polyps (88%) contained very small (<0.1mm) spherical reproductive bodies; in total 812 female reproductive bodies in this size

class were found in 106 of the 120 polyps dissected. Slightly larger spherical bodies (0.1 - 0.2mm diameter) were also found in a high proportion (78%) of the female population sampled. More than half the polyps dissected contained spherical bodies in the 0.2 – 0.4mm diameter size class (427 spheres found in 61 polyps (51% of the sample)). The largest reproductive bodies observed were 0.3mm in diameter.

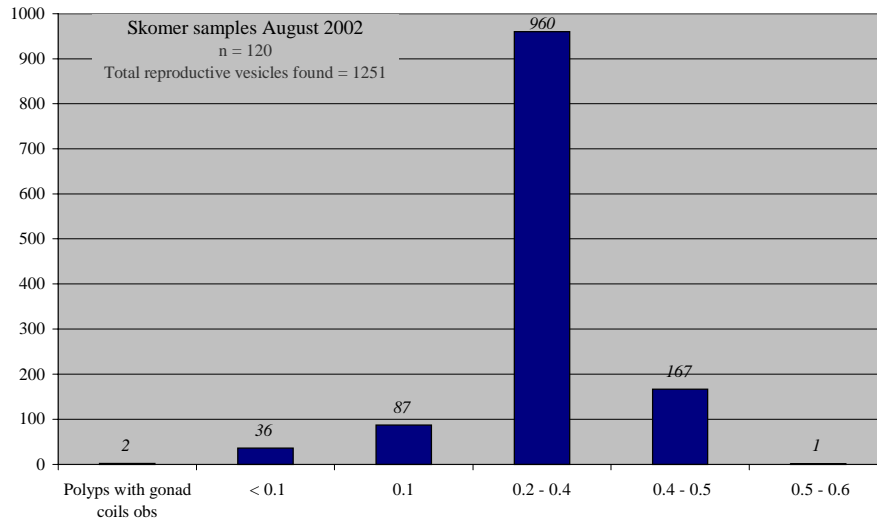
Male colonies

Figure 9 (iv) shows that gonad coils were observed within the gastrovascular cavity of 18 (out of a total of the 30) male polyps dissected, i.e. 60% of the sample. Discrete reproductive vesicles were found in the two smallest size classes, 17% of the sample (5 polyps) containing spheres <0.1mm diameter and 20% of polyps (6 polyps) containing spheres 0.1 - 0.2mm diameter. No reproductive bodies were observed in the size classes >0.2mm.

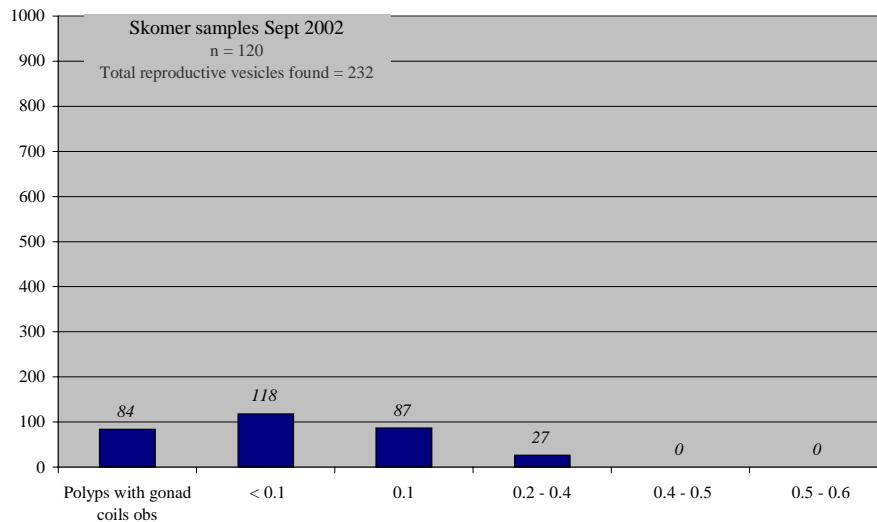
Figure 7. Graphs showing reproductive vesicles found in *Eunicella verrucosa* samples taken from Skomer population of seafans in July, August and September 2002



i) Graph showing distribution of reproductive vesicles by size class for samples collected July 2002

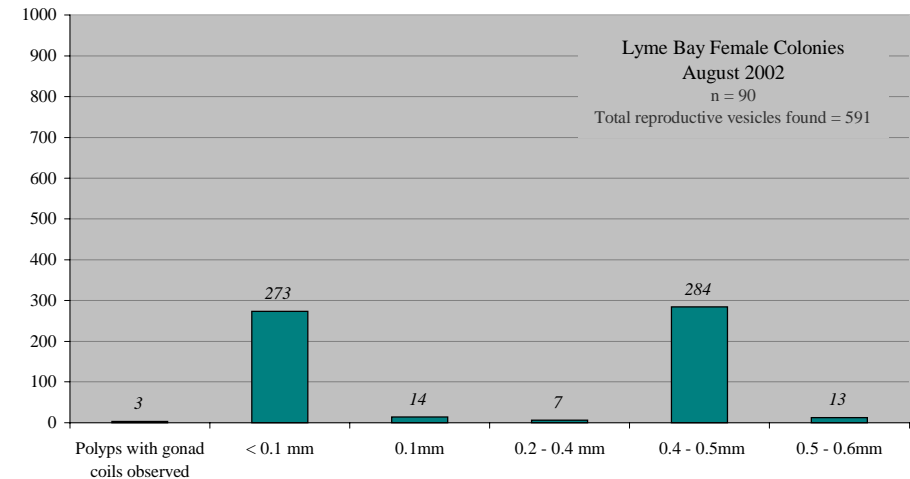
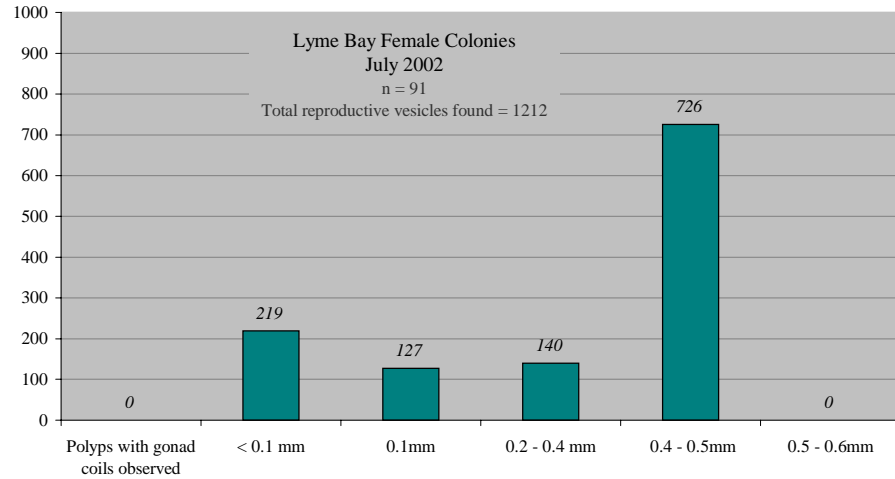


ii) Graph showing distribution of reproductive vesicles by size class for samples collected August 2002



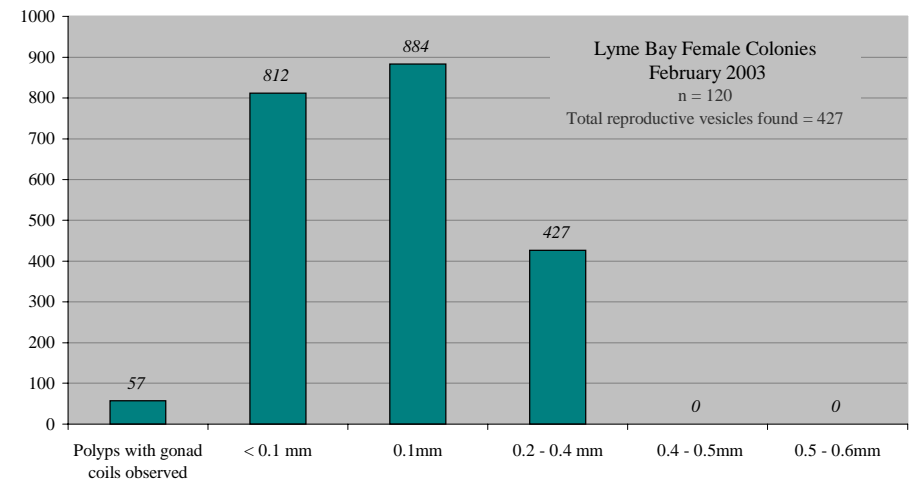
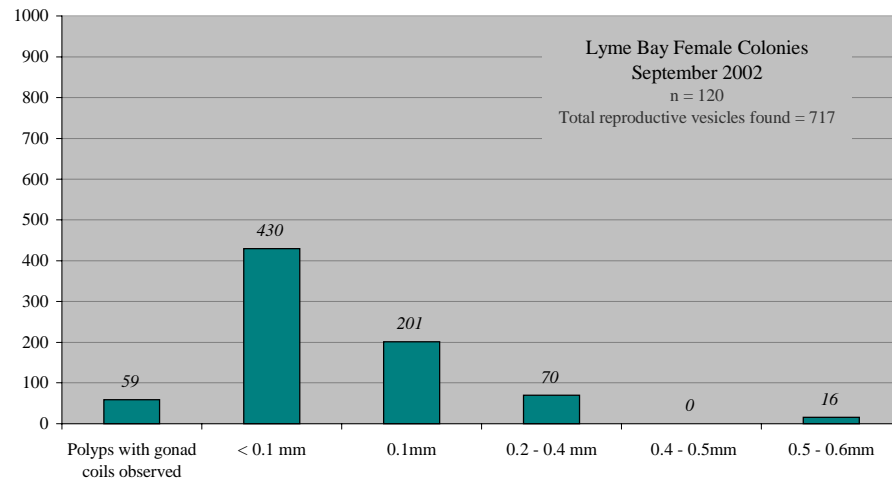
iii) Graph showing distribution of reproductive vesicles by size class for samples collected September 2002

Figure 8 Graphs showing reproductive structures found in female *Eunicella verrucosa* samples taken from Lyme Bay population of seafans July, August, Sept.2002 & Feb 2003



i) Graph showing distribution of reproductive vesicles by size class for samples collected July 2002

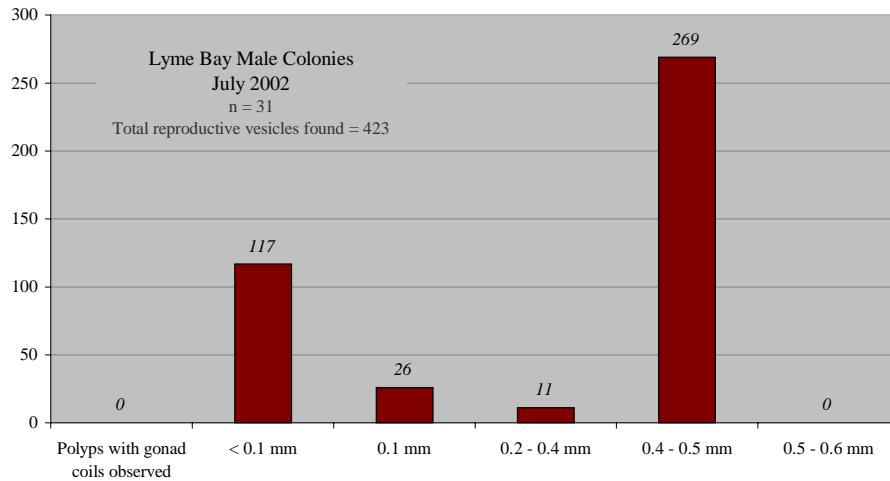
ii) Graph showing distribution of reproductive vesicles by size class for samples collected August 2002



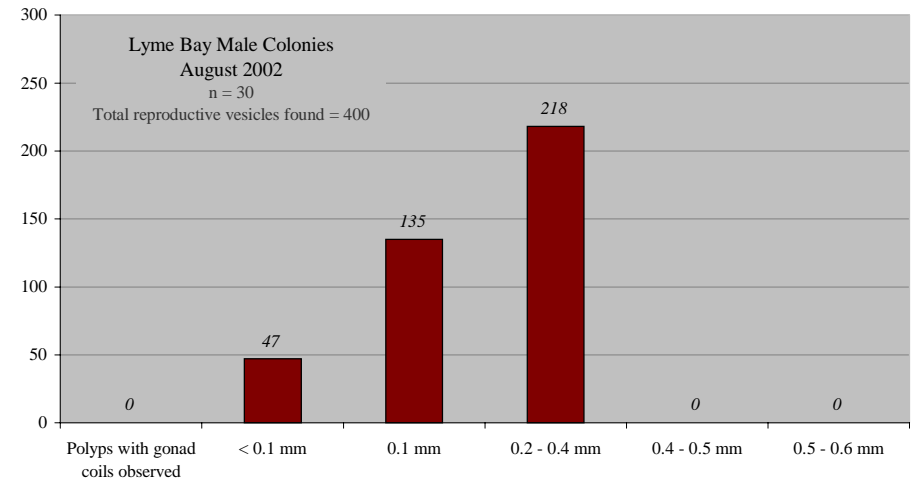
iii) Graph showing distribution of reproductive vesicles by size class for samples collected September 2002

iv) Graph showing distribution of reproductive vesicles by size class for samples collected February 2003

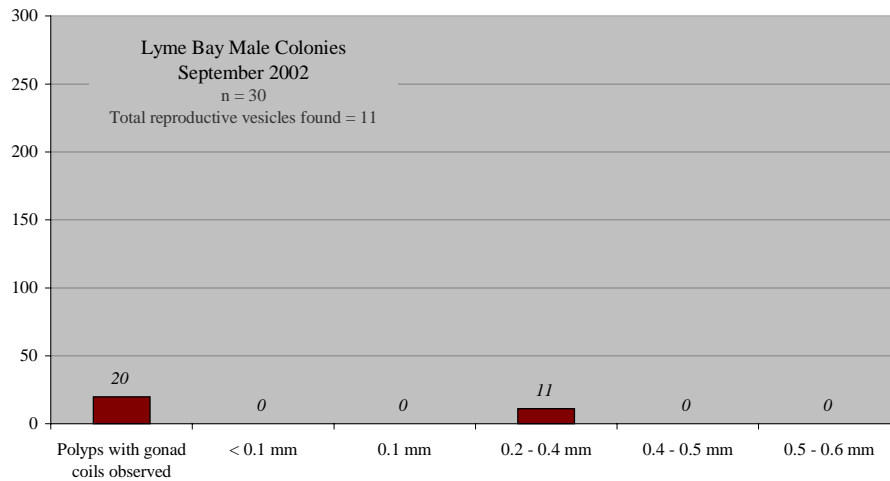
Figure 9. Graphs showing male reproductive vesicles found in *Eunicella verrucosa* samples taken from the East Tennants population of seafans in July, August and September 2002 and February 2003.



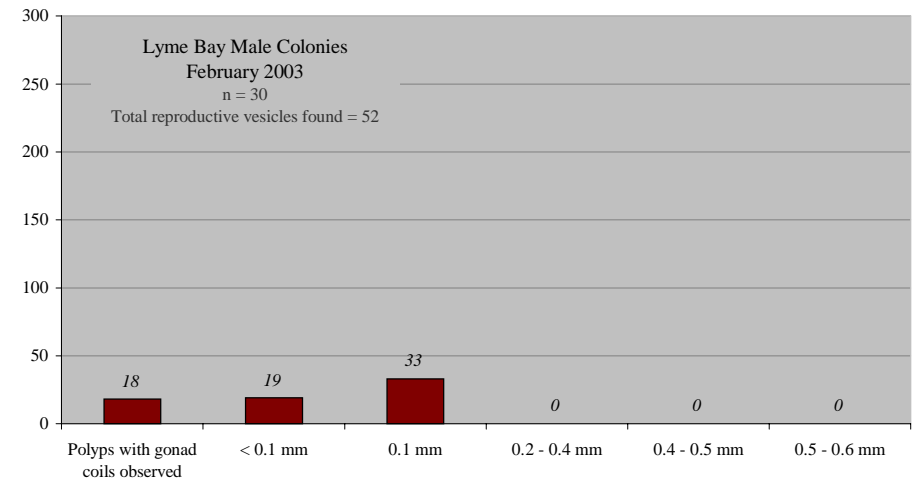
i) Graph showing distribution of reproductive vesicles by size class for samples collected July 2002



ii) Graph showing distribution of reproductive vesicles by size class for samples collected August 2002



iii) Graph showing distribution of reproductive vesicles by size class for samples collected September 2002



iv) Graph showing distribution of reproductive vesicles by size class for samples collected February 2003

5. DISCUSSION

Using data obtained in the first six months of the reproduction study we are able to make a preliminary interpretation of the development pattern for *Eunicella verrucosa*'s reproductive structures.

NB At this stage any interpretation of results must be undertaken with caution because

- 1) we remain uncertain of the sex of all colonies and
- 2) some reproductive structures are hard to accurately quantify because of their small size and / or because they are easily destroyed or damaged during dissection.

For the first three months of the study the broad pattern of reproductive development appears synchronous for Skomer and Lyme Bay populations; a high proportion of medium and large reproductive bodies (>0.2mm diameter) were observed in samples collected in July and August 2002. During the same months gonad coils were observed in very few polyps across all populations. In September 2002 the size distribution and type of structures found within dissected polyps appeared rather different to observations for July & August 2002. The majority of reproductive structures observed were small (<0.2mm diameter). A few, very large (0.5 – 0.6mm) reproductive vesicles were found in female colonies from East Tennants Reef. Some medium sized vesicles (0.2 – 0.4mm) were found in all populations. Very small reproductive vesicles were not apparent in male colonies from Lyme Bay. Gonad coils were found in a high proportion of polyps from all populations sampled in September.

We believe that spherical reproductive bodies within the size range 0.4-0.5 mm are “mature” egg and sperm vesicles, which will be found in highest densities just prior to spawning. Reproductive bodies in the size class 0.2 – 0.4mm are thought to be maturing egg and sperm vesicles (the minimum size for a viable reproductive body is as yet unknown). It is thought likely that medium sized reproductive bodies will be apparent within the polyps for a period prior to spawning, during which they will continue to mature and increase in size. The exact duration of the pre-spawning, maturation phase is currently unknown. It is thought likely that the presence of gonad coils and very small reproductive bodies within the polyps is indicative of an early developmental stage in the species reproductive cycle.

Following these proposed reproductive stages, the observed monthly reproductive status of Skomer and Lyme Bay populations are summarised in Figure 10. At present we cautiously describe the observed reproductive cycle as follows;

July & early August 2002 –	Maturation
Late August – early September 2002 –	Maturity and Spawning
Late September 2002	Early development of next year's gametes

Our observations of spawning in tank held clippings of *E. verrucosa* (from Lyme Bay) on 28th & 30th August & 18th / 19th Sept may provide further evidence of a spawning period in late August, early September, however tank spawning events do not necessarily coincide precisely with the spawning of field populations. Spawning in other species of temperate gorgonians is believed to be triggered by a combination of environmental cues such as water temperature (Grigg, R. W., 1977) the lunar cycle (Coma, R. *et al.*, 1995a). In our tank held populations it is possible that spawning may have been induced prematurely by raised water temperature (during August and September tank temperatures rose to 20°C, whereas the seawater temperature at East Tennants Reef peaks at 18°C).

Our field observations show that in all populations high numbers of larger reproductive bodies, which were visible in August, were no longer present by September. This suggests spawning in both populations occurred between their August and September samplings i.e. for

Skomer population between 28th - 30th Aug and 24th – 30th September 2002 and for Lyme Bay populations between 13th Aug & 13th September 2002. Continued research & sampling in 2003 should help us to determine the periodicity of spawning in *Eunicella verrucosa*, and the likely cues which trigger release of mature egg and sperm vesicles.

Results from the East Tennants Reef population indicate a significant increase in the density of small and medium sized female reproductive bodies between September and February. In February many of the smaller reproductive bodies (0.1mm and less) appeared to be contiguous with the gonad coils within dissected polyps. It is thought likely that by February the reproductive cycle of female colonies is in a definite 'growth phase', in which gonad coils ripen to produce reproductive bodies, & small sized female reproductive bodies begin to increase in diameter (see Figure 10).

In male colonies there also appears to be some growth of the reproductive bodies by February, with very small vesicles (0.1mm and less) apparent in a small proportion of the sampled polyps. Sampling over the next few months should help determine whether, as with the Mediterranean gorgonian *Paramuricea clavata* (Coma, R. *et al.*, 1995a), the growth and maturation phase of male colonies is shorter than that of female colonies for *Eunicella verrucosa*.

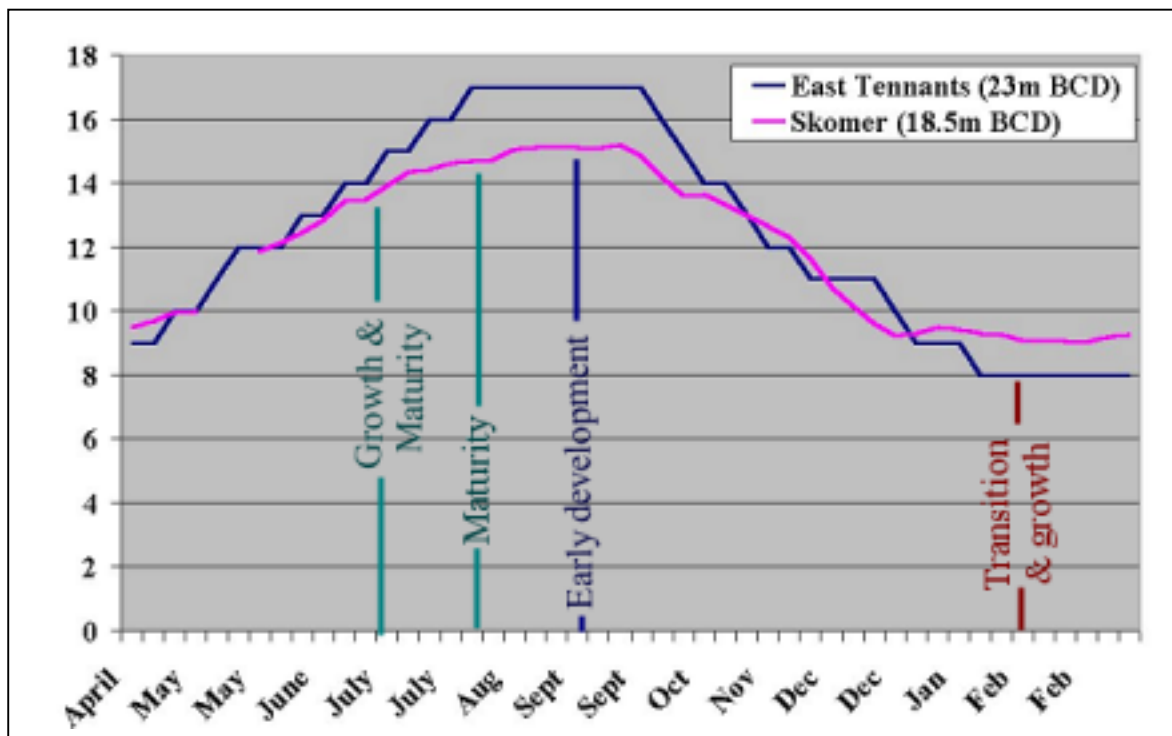


Figure 10 Showing a summary of the phases of reproductive maturity observed by dissection of polyps from Skomer and East Tennants Reef samples July 2002 – February 2003. The reproductive phases are presented against seabed temperature data (°C) collected at Skomer MNR (data supplied by CCW, Skomer) and East Tennants Reef.

6. SUMMARY OF OUTPUTS TO DATE

- 8 person-days fieldwork completed.
- 45 person-days lab/prep time completed
- 3 pages added to the Reef Research website plus one detailing our sponsorship
- 2 volunteers engaged on an occasional basis to assist with diving/lab work
- Presentation on the seafan project given to 200 delegates at the Reef Conservation UK conference held December 2002 at Royal Zoological Gardens, London.
- Presentation planned for Porcupine Natural History Society (March 2003)
- One equivalent full-time post created (i.e. two pers. inputting working 0.5 time each) since project commenced.

7. FORTHCOMING WORK

On March 14th 2003 we will deliver a talk at the annual meeting of the Porcupine Natural History Society detailing our work on *Eunicella verrucosa*.

Continued monthly sampling at both Skomer and East Tennants Reef will enable us to monitor the next phase of the reproductive cycle, and determine whether reproductive development remains synchronous between the two populations.

We hope to continue developing links with Dr Rafel Coma & Dr. Marta Ribes at CSIC who are conducting a parallel study of reproduction in the Mediterranean gorgonian *Eunicella singularis*.

8. REFERENCES

Brazeau, D. A. & Lasker, H. R. (1989). The reproductive cycle and spawning in a Caribbean gorgonian, *Biological Bulletin* **176**, 1 - 7.

Coma, R., Ribes, M., Zabala, M. & Gili, J.-M. (1995a). Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*, *Marine Ecology Progress Series* **117**, 173-183.

Coma, R., Zabala, M. & Gili, J.-M. (1995b). Sexual reproductive effort in the Mediterranean gorgonian *Paramuricea clavata*, *Marine Ecology Progress Series* **117**, 185-192.

Grigg, R. W. (1977). Population dynamics of two gorgonian corals, *Ecology* **58**, 278 - 290.

Lasker, H. R. (1990). Clonal propagation and population dynamics of a gorgonian coral, *Ecology* **71**, 1578-1589.

Weinberg, S. (1979). The light-dependent behaviour of planula larvae of *Eunicella singularis* and *Corallium rubrum* and its implication for octocorallian ecology, *Bijdragen tot de dierkunde* **49**, 16-30.

APPENDIX 1 ACTIVITY LOG

- | Date | Activity description |
|----------|--|
| 12.09.02 | Preparation for diving. Sample bags labelled and grouped ready for sample collection. Tanks cleaned out ready for new samples. Water containers and all diving gear checked & packed. Cylinders filled, slates prepared & temperature logger set for one year's recording. Emma Sheenan (English Nature) & Ben Rhodes (graduate volunteer) briefed on the project |
| 13.09.02 | Diving conducted at East Tennants Reef. Branch clippings collected from 4 large colonies which had been tagged in July and sampled July & August. Two additional large seafan colonies were tagged & branch clippings collected from them. Total of 6 clippings (2 x top branches, 2 x side branches, 2 x low branches) collected from each of 6 fans = 36 branch clippings. Photographed all large and medium colonies which have been sampled during the reproduction study. Re-attached <i>Minilog</i> temperature logger to the grid. Photographs of several fans within the grid collected as part of our ongoing time series size data. Checks made for newly recruited seafan colonies. Macro photographs of smallest seafan colonies obtained.

Two 10 gallon containers of seawater were collected.

Six dives completed.
Personnel; John Walker (skipper) Colin Munro (Diving Contractor), Lexie Munro (Dive supervisor) Kirsten Ramsay & Rohan Holt (CCW), Emma Sheenan (EN), John Bleach & Ben Rhodes (Graduate volunteers). |
| 14.09.02 | Maintenance & examination of Lyme Bay samples
Set up seawater tanks with branch clippings collected 13/09/02. Ten polyps were examined for egg & sperm vesicles. Several small orange spheres (<0.2mm) and a few very large, red spheres (0.5 – 0.6mm) were found in polyps from colony L2 (female ?). In several polyps from colony L3 (male ?) coiled structures (thought to be developing gonads) were visible within the gastrovascular cavity. Coils varied in colour from bright orange, to pale/ creamy with black "speckling". |
| 18.09.02 | 35mm film shot during survey work on 13/09/02 was developed using E6 processing. Examined polyps from a total of 12 branches (60 polyps in total). Found many small, orange, spherical bodies (all less than 0.3mm) and observed gonad coils within the gastrovascular cavity of the majority of polyps. |
| 19.09.02 | Examined polyps from 8 further branches (total of 40 polyps). Again found many small, orange, spherical bodies (all less than 0.3mm) and observed gonad coils within the gastrovascular cavity of the majority of polyps. |
| 20.09.02 | Examined polyps from 11 branches (total 55 polyps). Similar reproductive structures found to those of 18 & 19.09.02 |
| 21.09.02 | Examined the remaining 3 branches for reproductive structures. |
| 08.10.02 | Maintenance & Examination of Skomer samples
Receive Skomer samples & set up branch clippings in aerated seawater tank |
| 09.10.02 | Examined polyps from a total of 10 branches (50 polyps in total). No spherical reproductive bodies were observed in samples from Bull Rock, but the majority of polyps contained gonad coils within the gastrovascular cavity. Coils described variously as "loose pale / creamy coils" and "speckled black / white on translucent" |
| 10.10.02 | Examined polyps from a total of 14 branches (70 polyps in total). A few red and orange spheres (all less than 0.3mm diameter) were found in samples from Bull Hole. Developing gonad coils were found within the gastrovascular cavity of the majority of polyps examined. |
| 18.10.03 | Web development
Began to overhaul the Reef Research web site planning new pages detailing the reproduction study & project sponsorship from CCW |

- 21.10.03** Compiled an electronic map of study sites and scanned / notated all images intended for use in the reproduction study pages of the Reef Research web site.
- 23.10.03** Compiled text for the 'Background' and 'Study methods' pages of the Reef Research web site.
- 24.10.03** Compiled text for the 'Findings to date' page of the Reef Research web site.
- 12.11.03** Compiled 'sponsors' page of Reef Research web site. Add a discussion page to the site.
- 13.11.03** Checked the correct functioning of all links on the new Reef Research website. Made alterations as necessary & published the site.
- 07.12.02** **Conference presentation**
Gave a presentation on the Seafan Study at the RCUK (Reef Conservation UK) annual conference. Our talk detailed the methodology behind our research. We reviewed some of the problems in conducting temperate reef monitoring programmes involving repeat sampling of a discrete population & discussed some of the techniques we have developed to improve fieldwork efficiency.
- 06.02.03** **Diving conducted at East Tennants Reef.** Branch clippings were collected from 5 large colonies. One of the colonies had not been included in previous reproduction study sampling. The remaining four colonies (SF1, SF4, SF10 and SF3) had been tagged & sampled on previous visits. A total of 6 clippings (2 x top branches, 2 x side branches, 2 x low branches) were collected from each of the 5 fans = 30 branch clippings.
- One 10 gallon containers of seawater were collected.
- Two dives completed
- 07.02.03** **Sample maintenance.** Set up seawater tanks with branch clippings collected 06/02/03.
- 12.02.03** **Microscopic examination of branch clippings.**
Six branches from the previous week's sampling were examined under the low power microscope with 5 polyps (chosen at random) examined on each branch. Sketches of observations were made and photomicroscopy used to record results. Counts of developing gonads made with classification according to size and colour. A high power microscope was used to determine the size of reproductive vesicles (to an accuracy of 0.1mm).
- 13.02.03** **Microscopic examination of branch clippings.**
Eight branches were examined under the low power microscope with 5 polyps (chosen at random) examined on each branch. Sketches of observations were made and photomicroscopy used to record results. Counts of developing gonads made with classification according to size and colour.
- 14.02.03** **Microscopic examination of branch clippings.**
Four branches were examined under the low power microscope with 5 polyps (chosen at random) examined on each branch. Sketches of observations were made and photomicroscopy used to record results. Counts of developing gonads made with classification according to size and colour.
- 24.02.03** **Microscopic examination of branch clippings.**
Six branches were examined under the low power microscope with 5 polyps (chosen at random) examined on each branch. Sketches of observations were made and photomicroscopy used to record results. Counts of developing gonads made with classification according to size and colour.
- 25.02.03** **Microscopic examination of branch clippings.**
Six branches were examined under the low power microscope with 5 polyps (chosen at random) examined on each branch. Sketches of observations were made and photomicroscopy used to record results. Counts of developing gonads made with classification according to size and colour.
- 27.02.03** Analysis of data collected for reproduction study to date
- 28.02.03** Analysis of data collected for reproduction study to date
- 03.03.03** Analysis of data collected for reproduction study to date
- 04.03.03** Analysis of data collected for reproduction study to date

