Reef Research

Determining the reproductive cycle of *Eunicella verrucosa*

A project supported by Species Challenge Grant No SC7473

FINAL REPORT
July 2004

A REPORT TO CCW

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Report by:

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1. INTRODUCTION

Gorgonian dominated reefs are recognised as 'a habitat whose conservation requires the designation of Special Areas of Conservation' under Annex 1 of the EC Habitats Directive (UK Marine SAC's Project, 2003). The temperate gorgonian *Eunicella verrucosa* is listed as 'vulnerable' within the IUCN Red list of Threatened species (IUCN, 2002). Within UK waters it is classed as a 'nationally scarce species', it is protected under the UK Wildlife and Countryside Act 1981 (Sanderson, 1996) & is the subject of national and regional species action plans (JNCC, 1999)

Over the past 10 years *Reef Research* have been researching the ecology of *E. verrucosa*, in collaboration with the Countryside Council for Wales and English Nature. Since 1998 detailed time-series data relating to recruitment, mortality, colony growth and water temperature has been collected at a permanent seabed monitoring station at East Tennants Reef, Lyme Bay (Munro, 2002a). We have studied the population dynamics and genetic variability of populations at the extreme northern limits of the species range (Munro, 2001, Munro 2002a, Munro & Munro, 2004). In addition we have conducted a detailed two-year study of *E.verrucosa's* reproductive cycle, sampling seafan populations at two sites: the East Tennants Reef (ETR), SW England, and around Skomer Marine Nature Reserve (MNR), SW Wales (Munro & Munro, 2002b, 2003a, 2003b & 2003c).

Prior to *Reef Research's* study of *Eunicella* verrucosa's reproductive cycle there was no data on the species' reproductive ecology. Research of other gorgonian species (Brazeau & Lasker, 1989; Coma *et al.*, 1995; Grigg, 1977; Lasker, 1990 & Weinberg, 1979), indicates that there is considerable variation in reproductive ecology between different gorgonian species in terms of;

- 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 of *E.verrucosa's* reproductive cycle study aimed to determine

- the duration and pattern of *E.* verrucosa's reproductive cycle
- what environmental cues (if any) influence the synchronisation of spawning
- the fecundity of individuals and populations, and the levels of larval survivorship

- the age / size at which colonies reach reproductive maturity and
- sex ratios (ie proportion of male to female colonies) among UK seafan populations

Our research into the reproductive cycle of *Eunicella verrucosa* has been enabled through the financial assistance provided by the Countryside Council for Wales' Species Challenge Fund. This final report details the methodology, results and conclusions of our work. Interim reports for the project have been compiled by Munro & Munro (2002b), Munro & Munro (2003a) and Munro & Munro (2003b). Details of this project and associated research can be found at the Reef Research website www.reef-research.org. A DVD describing the *E. verrucosa* reproduction study is available from *Reef Research*.

2. Methodology

Eunicella verrucosa colonies within populations at Skomer MNR and East Tennants Reef were sampled between July 2002 and September 2003. Skomer lies on the northern edge of *E. verrucosa* distribution in British waters, while East Tennants Reef lies close to the species' eastern distribution limit. The seafans around Skomer occur in far lower densities than those on the ETR, and summer water temperatures in this area are significantly lower those recorded within Lyme Bay. Samples from Skomer Marine Nature Reserve were taken from two sites; Bull Hole (51^{o} 44.462' N 05^{o} 18.654'W) where depths range from 14 – 18m below chart datum (bcd) and Bernies Rock (51^{o}44.654'N 05^{o}17.653'W) where depths ranged from 14 to16m bcd. Samples from East Tennants Reef (50^{o}39.175'N 02^{o}52.509'W) were collected from 10 colonies close to our permanent monitoring station at a depth of 23m below chart datum.

2.1 Sampling

All sampling was conducted using SCUBA. Samples consisted of apical fragments one to three centimetres in length cut from the upper, side and lower branches of individual colonies. At each sampling, between four and six branch fragments were cut from each colony, placed in labelled bags and then transferred to seawater tanks, where they were maintained for up to 14 days prior to analysis. In the laboratory polyps were dissected under a low power microscope. On each clipping five polyps were chosen arbitrarily for dissection. For each polyp we recorded the number of oocytes or spermaries found, gonadal diameter and a description of the reproductive structures observed. Where possible photo and video microscopy was used to provide a visual record of our observations.

2.2 Yearly reproductive cycle

To follow the yearly reproductive cycle, six colonies at East Tennants Reef were tagged using fluorescent ribbon. Samples were collected from each of these colonies in July, August, & September 2002, and February, March, May, July, August & September 2003. All sampling at East Tennants Reef was conducted by Reef Research Staff and volunteers.

At Skomer MNR, the marine team selected large seafans for sampling at Bernies Rock and Bull Hole sites. In total twelve colonies were sampled (four from Bernies Rock and eight from Bull Hole). From this pool of twelve seafans, a maximum of seven colonies were sampled each month, to minimise the effects of repeated sampling. The Skomer marine team carried out all sample collection at SKOMER MNR

2.3 Time of spawning

To determine the timing of spawning we conducted repeated sampling at East Tennants Reef in late August 2003. Among the six tagged colonies, pairs of branch clippings were collected every 2-3 days between 13/08/03 and 22/08/03. In September 2003 a final sample was collected from three of the largest colonies to confirm the endpoint of spawning.

One branch from each pair collected was dissected within 24 hours of collection. In female colonies eggs were counted according to two size categories; less than 0.3mm (typically light orange in colour) and greater than 0.3mm (typically red in colour). An overall count of individual spermaries (incorporating all size classes) was made in male colonies. The remaining branch from each collected pair was placed in a tank of aerated seawater & checked regularly for signs of egg / spermary release.

3. RESULTS

3.1. Gonad development

Oocytes

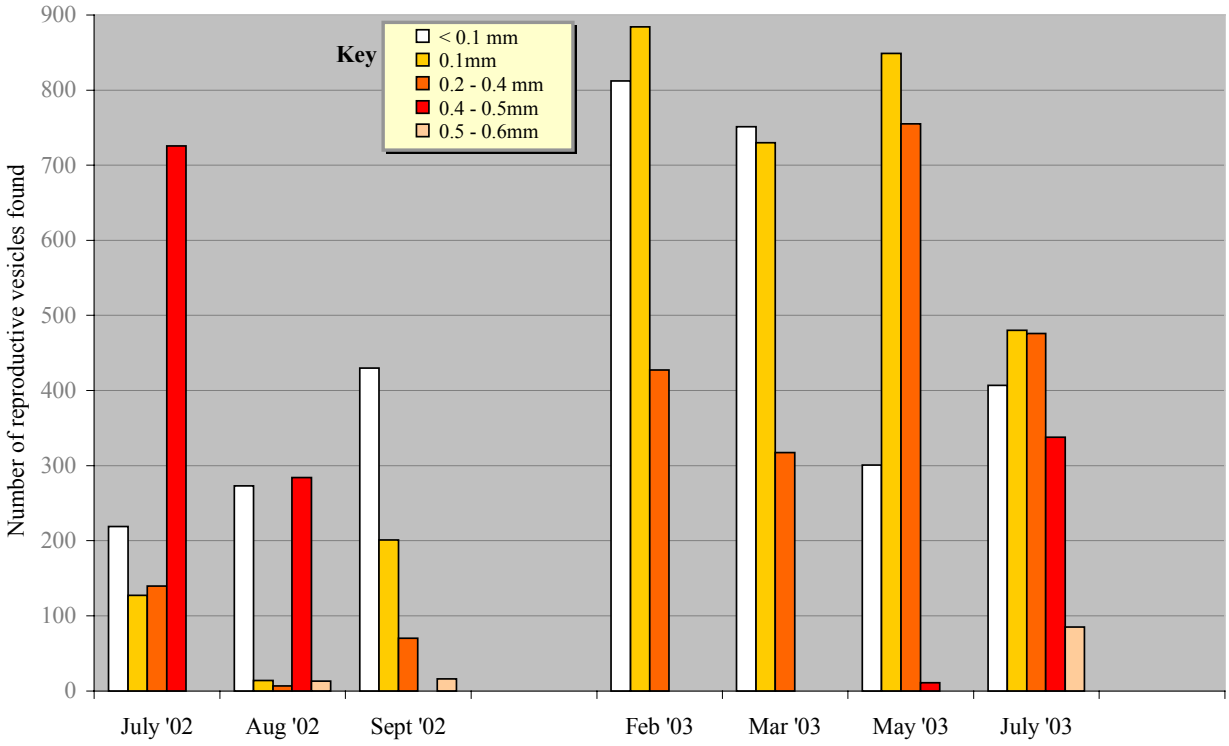
Throughout the entire 14-month study newly formed oocytes were observed within the polyps of female colonies. These new oocytes, which can be recognised by their small size (<0.1mm) and colourless, translucent appearance, appear to originate from immature gonad coils in a process which is continuous throughout the year. As the oocytes ripen their mean diameter increases and they appear coloured, first orange (up to a diameter of around 0.3mm) and then deepening red (as they mature to a maximum diameter of 0.6mm). Detailed description of the oocyte development though the full reproductive cycle is given by Munro & Munro (2003b).

Figures 1 & 2 illustrate the pattern of oocyte development. The number of new oocytes increased steadily from May through to February. A pre-spawning maturation phase was observed between March and July 2003. During this period there was a clear “upward shift” in the size distribution of eggs. Between March and May an increased frequency of oocytes in the 0.2 – 0.4mm size class was accompanied by a decreased frequency of eggs in the <0.1mm size class. Similarly between May and July an increase in the 0.4 - 0.5mm and 0.5 – 0.6mm size classes was accompanied by a decrease in the 0.1 – 0.2mm size class. Over this period the total counts of eggs remained similar between samples as shown in Table 1. By September nearly all the large oocytes had disappeared, but many smaller sized eggs (<0.2mm diameter) were still present in the polyps.

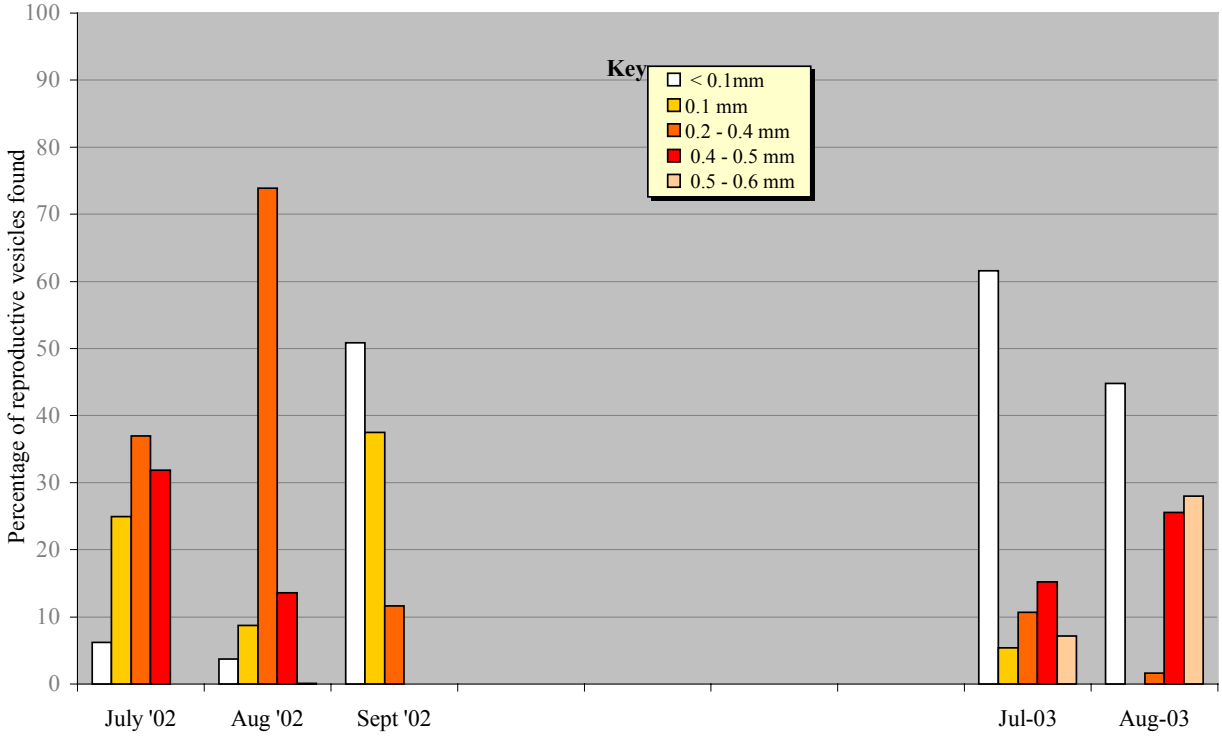
Figure 1. Graphs showing the size distribution of reproductive vesicles found in female colonies collected at Lyme Bay (graph i) and Skomer (graph ii) between July 2002 and August 2003.

***NB** Skomer sample size varied considerably between 2002 and 2003 therefore Skomer data has been presented using a percentage scale to indicate frequency. Lyme Bay data shows frequency by total number of vesicles found in each size class.*

Graph i



Graph ii



	July '02	***Aug'02***	***Sept '02***	***Feb '03***	***Mar '03***	***May '03***	***Jul '03***
No Oocytes	1212	591	717	2123	1798	1916	1786
No. polyps	91	90	120	120	120	115	125

Table 1 *showing the total number of oocytes found and number of polyps dissected in samples from Lyme Bay (East Tennants Reef) July 2002 – July 2003.*

This pattern suggests a cycle of oocyte development lasting up to 18 months, beginning between February and May and culminating in spawning during the late summer (August / September) of the following year. Since spawning occurs each year, two overlapping cohorts of oocytes are present in the polyps from May (or possibly earlier) till spawning in August / September. Mature oocytes seem to develop from a pool of young oocytes present in the polyps throughout the year. In the early stages oocyte development is not synchronous between the polyps, but increased synchrony of development occurs during the final maturation phase. As shown in Figure 1, a similar pattern of reproductive development was observed for both populations considered in this research.

Spermaries

Newly developed spermaries (<0.1mm) were present within the polyps of male colonies for all samples except September 2003, as shown in Figure 3. These newly developed spermaries were colourless and translucent in appearance and could not be distinguished from young oocytes. Between May and July there was an increase in the number of spermaries measuring 0.1 – 0.2mm, followed in July to August, by an increase in the number of spermaries with a diameter of 0.2 – 0.4mm. As spermatogenesis progressed the spermaries could be distinguished from the oocytes by their milky appearance and delicacy (spermaries were quite easily damaged during dissection in contrast to the relatively robust oocytes). By mid September no gonads were present in any male polyps. Thus in male colonies gametes appear to mature over a shorter period, growing from the early reproductive phase to maturity in around 5 – 6 months between March and August, and being released as a single cohort.

3.2 Sexuality and sex ratio

In the eighteen colonies included in this study, none had polyps containing both spermaries and oocytes, suggesting the sexes are separate. No change in sex was recorded for any of the colonies during the 14-month year study period. Ten of the colonies were female and five male, with a sex ratio of 2:1 being maintained in both Skomer and Lyme Bay populations (Lyme Bay 4 female : 2 male; Skomer 6 female, 3 male). It was not possible to determine the sex of three Skomer seafans (BHO17, BHO1, and BRK 2).

3.3 Fecundity of individuals and populations

Across each sample there was considerable variation in the distribution of spermaries and oocytes between polyps. Amongst female colonies the maximum number of oocytes removed from a single polyp was 73 (ETR SF10, Top branch 1, Feb 2003).

Figure 2 Showing mean number of oocytes /polyp in samples collected from Lyme Bay July '02 – Sept '03 against seabed (23m bcd) water temperature measured at the sampling site, Sept 02 – Sept 03. Three size classes of oocyte are shown, (<0.2mm, >0.2<0.4mm & >0.4mm)

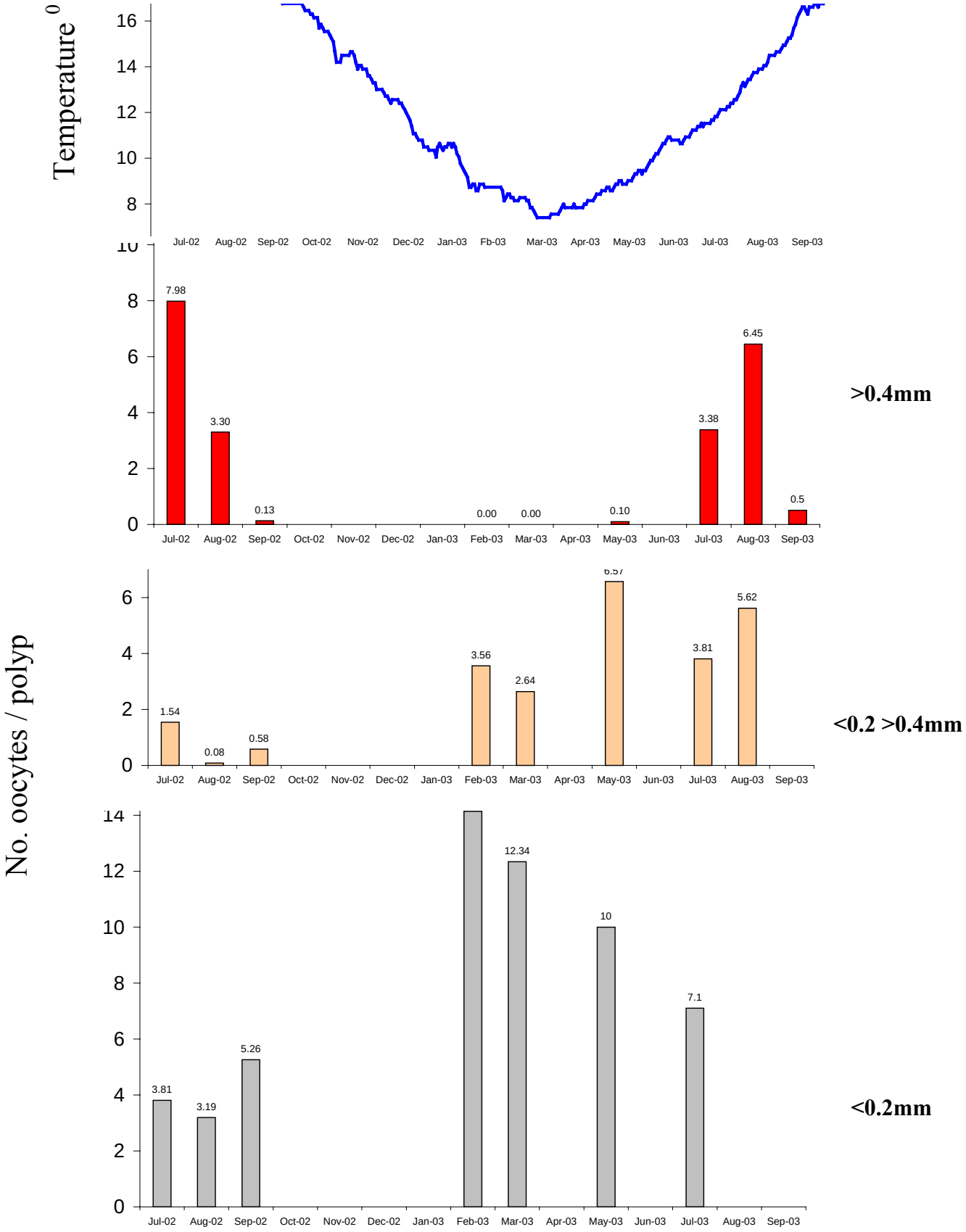
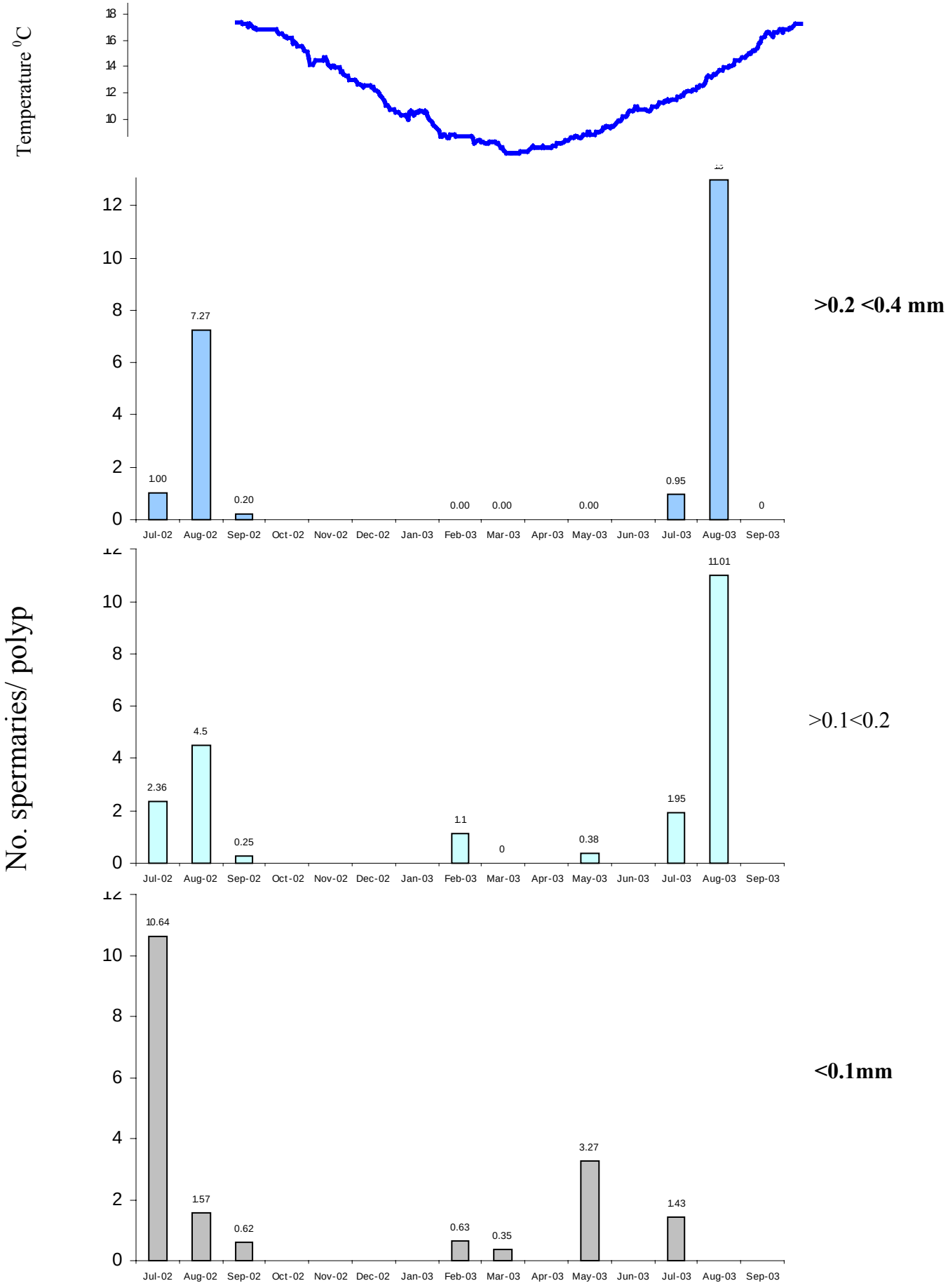


Figure 3 Showing mean number of spermaries /polyp in samples collected from Lyme Bay July '02 – Sept '03 against seabed (23m bcd) water temperature measured at the sampling site, Sept 02 – Sept 03. Three size classes of spermary are shown, (<0.1mm, >0.1 <0.2mm & >0.2 <0.4mm)



Amongst male colonies the maximum number of spermaries found was 40 (ETR SF3, Top branch 2). In most samples some polyps contained no gonads. Table 2 provides summary information on the densities of mature oocytes and spermaries within both Skomer and East Tennants Reef populations. The mean number of oocytes per polyp were calculated from samples collected in July, since both cohorts of eggs were present during this month, and maturing oocytes reached their annual maxima in terms of size (mean diameter) and number. The mean number of spermaries / polyp were calculated from August sample data, when spermaries were at their maximum size and number.

	Site	% polyps with gonads present	Max N^{o} gonads w/i single polyp	Mean N^{o} gonads/ polyp *(SD)*
Spermaries >0.2mm	Skomer	100	14	10.30 *(2.87)*
	ETR	25	18	7.27 *(5.23)*
Ooctyes >0.4mm	Skomer	55	14	3.21 *(3.60)*
	ETR	66	17	3.38 *(4.24)*

Table 2 *showing the density of mature spermaries and oocytes in samples from Skomer (2002) and East Tennants Reef (2003). For each sample the percentage of polyps containing gonads is shown together with mean number of gonads per polyp for the sample and the maximum number of gonads found within a single polyp*

Data from repeated polyp counts conducted as part of the reproduction study indicate a mean value of 29 polyps / cm of branch, as described in Munro & Munro (2003b).

3.4 Spawning

The final stages of gonad ripening was synchronous in male and female colonies with maximum density of spermaries and oocytes occurring in August samples from both Skomer and East Tennants Reef (Figures 1, 2 and 3). In both years of the study, abundant mature oocytes and spermaries were found in samples collected in August, but had disappeared by September. This indicates a pattern of gamete release occurring in late August / early September co-incident with peak water temperature As shown in Figure 4, in 2002, spawning at ETR occurred as the moon waned (with egg release from lab-based samples occurring 7-10 days after the full moon). In 2003 release of oocytes and spermaries at ETR occurred in the waxing phase between new moon and full moon (Figure 5). Samples collected from Skomer in August 2002 indicated that colonies had not spawned before 30^{h} August (by which time ETR samples had begun to spawn). Thus spawning at Skomer may lag slightly behind that at ETR.

Following spawning there was no evidence of internal or external brooding of gametes.

Figure 4 Showing the time of sample collection and reproductive phase observed in samples from, Skomer and East Tennants Reef, summer 2002. Data is presented against local seabed water temperature (collected at East Tenants Reef and Skomer MNR) and lunar phases. The assumed time of spawning is shown for each population.

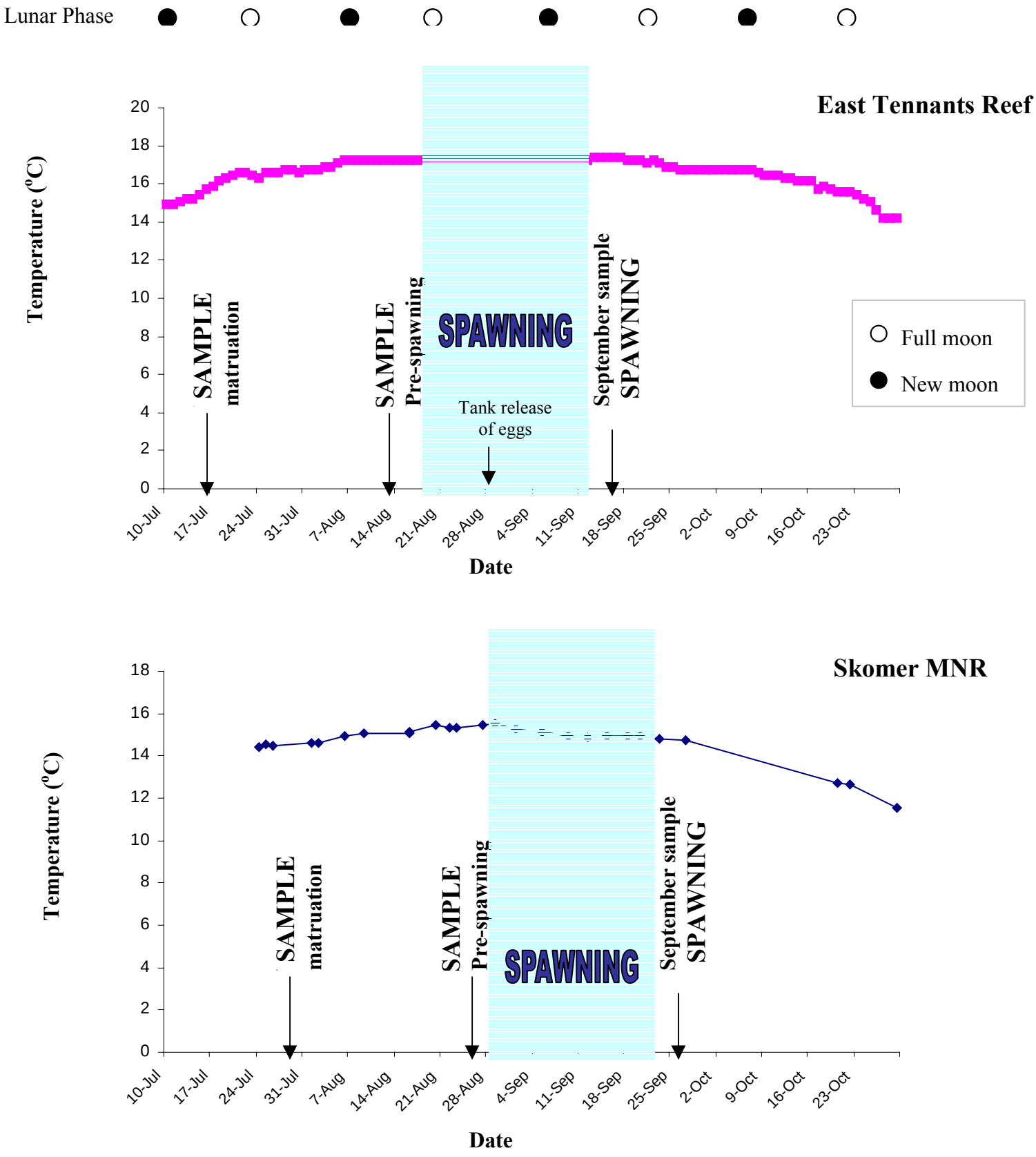
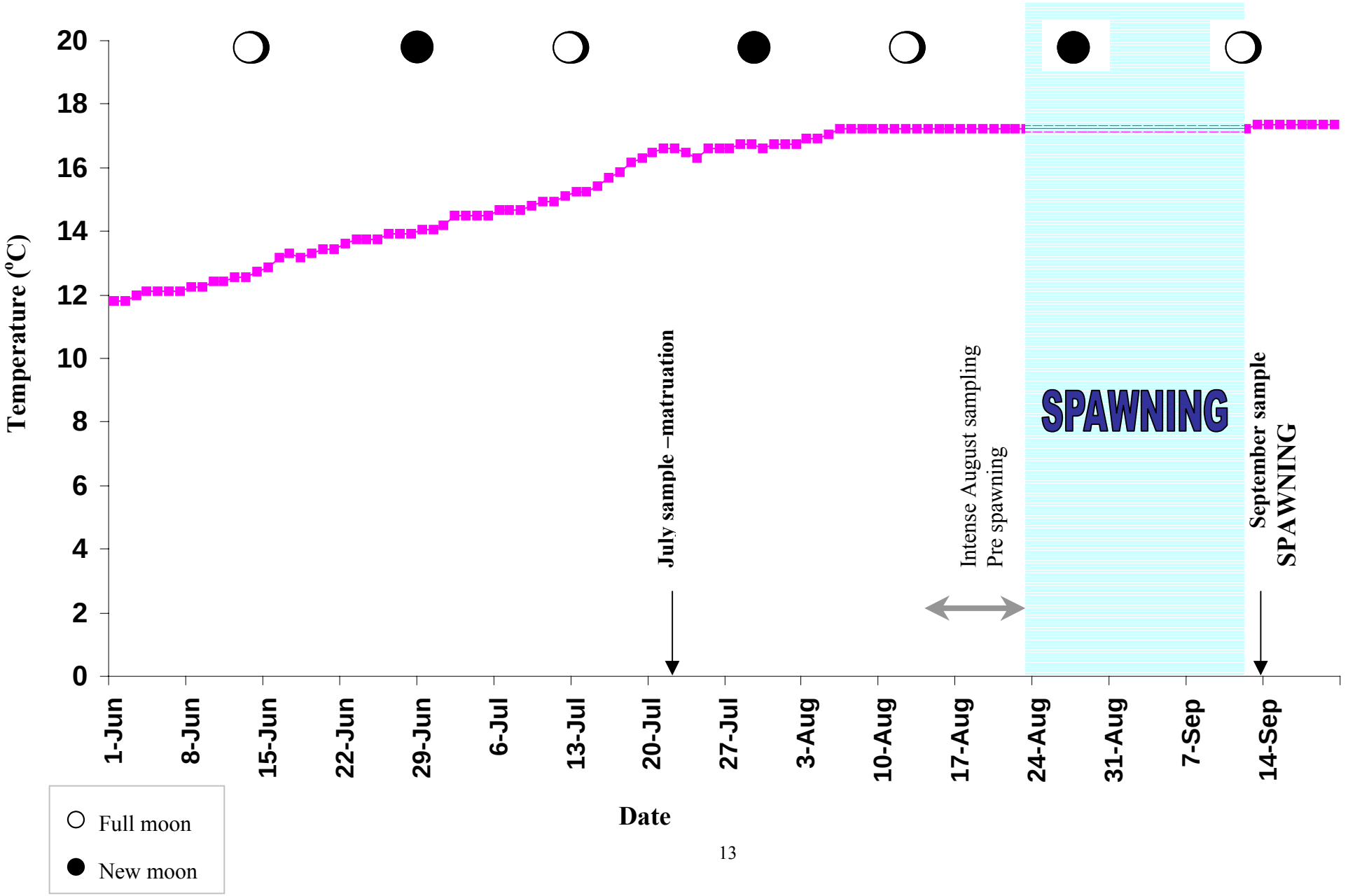


Figure 5 Showing the time of sample collection and reproductive phase observed in samples from East Tennants Reef, summer 2003. Data is presented against local seabed water temperature (collected at East Tenants Reef) and lunar phases. The assumed time of spawning is shown for each population.



4. DISCUSSION

Previous research has shown a broad diversity in the reproductive strategies gorgonian species (AIMS, 2003). This section discusses the reproductive biology of *Eunicella verrucosa* in relation to that described for other gorgonian species. We outline the relevance of this project in improving our understanding of seafan population ecology. In addition we discuss the implications of our research for the management of *Eunicella verrucosa* and the habitats in which it occurs.

4.1 Sexuality and sex ratio

Gonochorism (separation of the sexes) is common among most octocorals, and has been described for many gorgonians including the Mediterranean species *Eunicella singularis* (Weinberg & Weinberg 1979) & *Paramuricea clavata* (Coma et al, 1995). Gonochorism has also been found in gorgonian species from tropical (Beiring & Lasker, 2000; Coffroth & Lasker, 1998) and polar (Orejas et al, 2002) regions. Whilst a wider, population-level survey would be needed to confirm the absence of hermaphrodism in *E. verrucosa,* the apparent separation of sexes in all colonies included in this study provides good indication that the species is gonochoristic.

In this study we observed a sex ratio of 1 : 2 males:females at both sampling sites. Sex ratios of 1 : 1 have been commonly observed amongst other temperate gorgonian species including *Eunicella singularis* (Weinberg & Weinberg 1979), *Paramuricea clavata* (Coma et al, 1995), *Muricea californica & M. fruticosa* (Grigg, 1977).

Highly skewed sex ratios have been recorded in the Caribbean corals *Briareum asbestinum (*2.2 : 1 male:female, Brazeau & Lasker, 1990) and *Plexaura A,* where no male (or hermaphroditic) colonies were found in a survey of 265 colonies (Brazeau & Lasker, 1989). It is possible that these species do not have such a strict requirement for parity of sex distribution, since their primary means of reproduction is asexual stolonisation rather than sexual reproduction involving gamete release. However in ten years studying *Eunicella verrucosa* we have seen no indication of asexual reproduction by stolonisation. It is possible that the species is able to reproduce asexually by fragmentation. Coma *et al* (1995) describe the distinct appearance of *Paramuricea clavata* colonies that have originated from fragmentation (attached to substrate at several points with parallel branches growing up from a branch on the substrate), as opposed to those developing from larvae (single point of attachment with single branch growing from the base). Whilst we have frequently observed small sections of branch on the seabed at East Tennants Reef and other seafan dominated reefs in Lyme Bay, we have never seen any colonies which appear to have originated from these fragments.

In this study the observed sex ratio of 2 : 1 female : male colonies was derived from extremely small sample sizes at both Skomer and ETR sites. A population-level survey would be needed to confirm the distribution of the sexes in *Eunicella verrucosa,* and provide additional data on the species' potential for asexual reproduction.

4.2 Duration of oogenesis and spermatogenesis

Disparity in the development times for oocytes and spermaries is a common feature of alcyonaceans, octocorals and many scleractinians. Studies of other gorgonian species indicate that it is common for oogenesis to last longer than spermatogenesis. As an example, Coma *et al* (1995) found that in *Paramuricea clavata* oogenesis lasted between 13 and 18 months, while spermaries developed in 6-7 months. In species where there are disparities between the development times of male and female gonads, the onset of spermatogenesis is often delayed in order to achieve synchrony of gamete maturation and release (Coma *et al*, 1995). This study suggests that the oocytes of *Eunicella verrucosa* develop over a much longer period than spermaries (oogenesis lasts up to 18 months as opposed to spermatogenesis which takes a maximum of 6 months). A delay in the onset of spermatogenesis enables mature spermaries and oocytes to be released in a single, synchronous spawning event.

In *Eunicella verrucosa* the long oocyte development period, coupled with annual release of gametes, results in two cohorts of oocytes developing within the polyps of female colonies for at least part of the year. This pattern is similar to that observed for P*aramuricea clavata,* where two cohorts of ooctyes are present within the polyps between February and June / July (initial appearance of a second cohort of new oocytes in February alongside a cohort of maturing oocytes prior to spawning in June / July). However in *Eunicella verrucosa* very small "primary" oocytes were present within the female polyps in all months sampled. Brazeau and Lasker (1989) observed a similar pattern in the Caribbean gorgonian *Plexaura A*, where a "pool" of primary oocytes (from which the mature oocytes develop) was present throughout the year. In their 1989 paper Brazeau & Lasker indicate that the continual maintenance of an oocyte pool within female polyps is "unknown for any other coral species".

4.3 Fecundity

As shown in Table 3, the size of mature oocytes and spermaries in *Eunicella verrucosa* falls within the upper range of maximum gonad diameters recorded for gorgonian species. In *Eunicella verrucosa* the mean number of eggs / polyp is relatively high, and the maximum numbers of oocytes / spermaries removed from single polyps are far higher than has been recorded for other species. The combination of large oocytes / spermaries with high densities of gonads per polyp indicates a high expenditure on sexual reproduction.

In this study the mean number of mature spermaries per polyp was more than 2.1 times greater than the mean number of mature oocytes / polyp. It is possible that the values for mean gonad / polyp were skewed by small sample size. It is also possible that within *E. verrucosa* populations, the skewed sex ratio of colonies described earlier in this report, (2:1 females to males at both Skomer and ETR) is offset by higher productivity among male colonies.

Our observations on the density of eggs / spermaries provides a valuable starting point for estimating the fecundity of *Eunicella* verrucosa populations. A very crude estimate of egg density per cm branch can be obtained by combining values for the mean number mature gonads per polyp (ETR = ♀ 3.38, ♂ 7.27) with values for mean number polyps per cm branch (ETR = 29 polyps cm^{-1}). Thus for colonies at East

Tennants Reef we might expect each centimetre of distal branch to produce up to 98 mature oocytes and 210 mature spermaries.

Species	***Maximum oocyte diameter (μm)***	***Mean N° mature eggs/ polyp***	***Maximum spermary diameter (μm)***	***Source***
Eunicella verrucosa	*600*	*3.38*	*500*	*This study*
Paramuricea clavata	*400-500*	*13*	*500-700*	*Coma et al (1995)*
Plexaura A	*500 – 600*	*1.9*		*Brazeau & Lasker (1989)*
Muricea californica	*600*	*1.6*		*Grigg (1977)*
Muricea fruticosa	*700*	*3.8*		*Grigg (1977)*
Ainigmaptilon antarcticum	*900*	*–*		*Orejas et al (2002)*

Table 3 *Showing the maximum diameter of oocytes and spermaries and mean number of eggs per polyp recorded for five gorgonian species, including* Eunicella verrucosa*.*

Studies of other gorgonian species have found significant variability in reproductive effort dependent on polyp position within the colony. As an example Coma et al (1995) found decreasing fecundity across five orders of branch in the Mediterranean gorgonian *Paramuricea clavata,* with distal branches having a higher percentage of fertile polyps and more gonads per polyp than inner branches. Since all gonad density and polyp count data for this study is based on samples collected from distal branch fragments, extrapolations of fecundity at whole colony level are, at best, extremely crude, and **must be viewed with extreme caution**.

As an example, Figure 6 shows a medium sized seafan (height = 18 cm) from East Tennants Reef. Results from our photo monitoring study show the total branch length for this seafan is 123.4 cm. Therefore the **maximum** fecundity estimate for this seafan would be 12093.2 oocytes (98 x 123.4) or 25914 spermaries (210 x 123.4) per year (with actual fecundity likely to be a much lower value)

In order to make accurate estimates of fecundity in *Eunicella verrucosa* populations it will be necessary to determine

- The extent to which reproductive effort varies with a) colony size and b) polyp position within the colony.
- The age / size at which colonies first become reproductively viable, and
- The age / size structure of the population in question

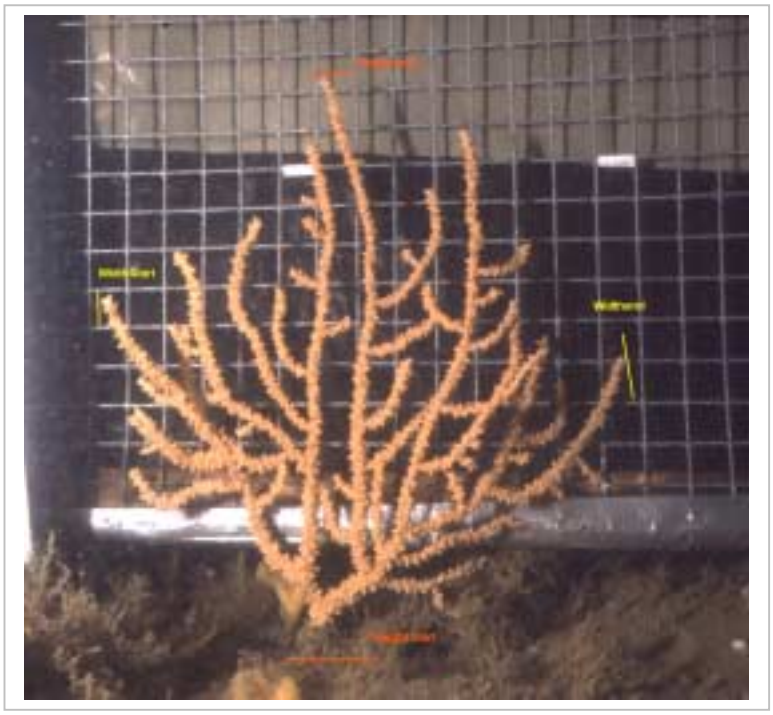


Figure 6 showing seafan 35 from our permanent monitoring station. The seafan is 184mm in height and 166 mm across its widest point. Total branch length is 1234mm. The seafan is classed as "medium" sized & its age is unknown.

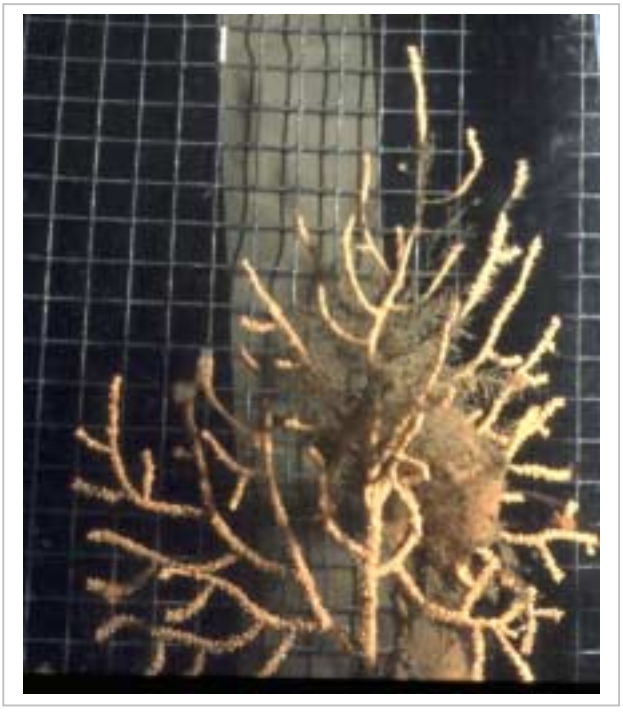


Figure 7 Showing a seafan entangled in fishing line. Loss of living tissue (top branches of the colony) and some overgrowth (centre of the colony) is apparent.

4.4 Broadcast spawning

In this study we found no evidence of internal fertilisation or brooding of gametes (either internally or externally). Unusually for a gorgonian species, *E. verrucosa* appears to release large numbers of unfertilised eggs and spermaries which we presume are subsequently fertilised within the water column. The species' broadcast spawning strategy may be advantageous for the colonisation of remote locations and for the maintenance of high genetic diversity, but is unlikely to be favourable for maintaining existing population stability (AIMS, 2003). A detailed study of reproductive success in the broadcast spawning gorgonian *Plexaura kuna* was made by Lasker et al (1996). In that study very low rates of reproductive success were recorded, despite production and release of prodigious gametes. Failures in fertilisation, low levels of larval recruitment and high post settlement mortality were identified as the main factors contributing to the species' low success rates for sexual reproduction.

In all other gorgonian species where sexual reproduction is the primary reproductive strategy, gametes are internally fertilised and subsequently brooded either internally (eg *Eunicella singularis)* or externally in mucus coating (eg *Paramuricea clavata)* (Theodor, 1967; Coma et al 1995). In *E. verrucosa*, it seems unusual that the species' high investment in egg / spermary production is coupled with an extremely uncertain strategy for fertilisation and subsequent larval settlement. However, long term monitoring of the *Eunicella verrucosa* population at East Tennants Reef provides evidence that UK populations do recruit successfully. Over a five-year study period, *Reef Research* has found between 7 and 18 newly recruited seafans per year within a study area $4m^2$. The average survival rate for newly recruited fans is around 50% (Munro & Munro, 2003d).

Within the Skomer population very few small, newly recruited seafans are present, with none found in the three years preceding this study (Skomer, pers. comm.). This study has shown that the Skomer colonies are reproductively viable. The absence of small seafans from this population may, in part, be due to the species' use of broadcast spawning as its primary means of reproduction. Lasker & Kim (1996) calculate that for broadcast spawning species, planulae may travel tens of kilometers even if they spend as little as 3-8 days in the water column. The Skomer population of *Eunicella verrucosa* represents an isolated population at the Northern limit of the species range. It is possible that gametes released at spawning are carried away from the MNR and that the lack of adjoining *E. verrucosa* populations results in poor larval supply and depressed recruitment within the Skomer population. Within Skomer, limited and variable recruitment success may contribute to the maintenance of a skewed age-frequency distribution. In a long-term study of recruitment and gorgonian community structure in Puerto Rico, Yoshioka (1996) found highly variable recruitment over an 8-year study period and reported a major, long-term effect from this on colony densities and age-frequency distributions.

4.5 Environmental cues for gamete release

There is growing evidence that several gorgonian require a peak summer temperature above a certain threshold as a cue for spawning (Coma, 1995). In two other studies of temperate gorgonian species Weinberg & Weinberg (1979) and Grigg (1977)

observed staggered spawning between populations occupying habitats with dissimilar temperature regimes. In both studies gamete release appeared to be triggered by a "threshold" temperature, since spawning in deeper (colder) water populations lagged behind that of populations in warmer shallow waters. In this study, we observed strong synchrony of spawning between Skomer and East Tennant Reef populations, despite the dissimilar temperature regimes for the two sites. Spawning of both populations occurred in late August / early September. At the time of spawning the water temperature at Skomer had already started to decline from its annual maximum of 15.5^{o}C to around 15.2^{o}C. At East Tennants Reef the water temperature reaches 15^{o}C in early July. However spawning is delayed until late August, by which time water temperature has reached 17^{o}C (a peak temperature, which is maintained for 2-3 weeks after spawning). We suspect that changes in water temperature are important in synchronising the final stages of gonad development, and that spawning may be timed to coincide with peak water temperature. However our results do not indicate that the achievement of a threshold water temperature triggers spawning in *Eunicella verrucosa.*

Coma *et al* (1995) observed that the lunar cycle appears to be a primary influence in "..synchronising the discrete periods in which spawning actually takes place…" In this study spawning occurred during a different lunar phase in 2002 compared with 2003. In both years gamete release occurred outside the periods of full and new moons, and therefore coincided with neap rather than spring tides. At present we have no information of links between tidal phase and spawning synchrony in gorgonian species. Tidal strength will influence the larval dispersal range, and hence the reproductive success of broadcast spawning species. It therefore seems possible that gamete release in broadcast spawners is somehow cued to occur during the most favourable tides for successful larval dispersal.

4.6 Management implications of this study

This study provides preliminary data on the reproductive biology of *Eunicella verrucosa.* Whilst our discussion is based around *Eunicella verrucosa*, we believe that many of our recommendations are relevant to other reef species whose ecology and reproductive biology is less well understood. The findings of our study have important implications for the management of UK gorgonian populations and the reef habitats on which they occur. In this section we consider these management implications and make recommendations for future population monitoring.

The UK Biodiversity Group's Species Action Plan (SAP) outlines current factors thought to cause loss or decline to *Eunicella verrucosa* populations and lists a series of objectives and targets for research and management of the species (UK Biodiversity Action Group, 1999). This document is currently used as the basis for planning and implementation of UK management strategies relating to pink seafan populations.

The Species Action Plan for *Eunicella verrucosa* states that "*.the effects that climate change may have on the current UK distribution of this species are not known. Natural environmental factors affecting pink sea-fan populations globally need to be identified in order to differentiate them from local, anthropogenic impacts*".

The action plan outlines the requirement to " *Ensure that the pink sea-fan maintains its current geographical distribution from the 1998 baseline*".

For many gorgonian species, populations will remain viable only within a narrow range of temperatures, since a requirement for a peak water temperature to trigger spawning is often coupled with an intolerance of extreme high summer temperature (Munro & Munro, 2003e). Our research indicates that peak water temperature is an important factor in synchronising reproductive development (and possibly spawning) in *E. verrucosa,* and thus the distribution of populations in UK waters is at least partially dependent on the local water temperature regime. Since *Eunicella verrucosa* is a broadcast spawner with gametes dispersed in the water column, its distribution is also likely to be influenced by the existing current regime.

In the UK a range of marine programmes have been implemented to monitor the biota of benthic communities. Examples include monitoring within Special Areas of Conservation (SAC's), no-take-zones, marine nature reserves and in response to developments which may impact on the marine environment. One of the primary reasons for monitoring biotic communities is to detect cycles, shifts in equilibrium, or trends which may be linked to other physical or biotic changes. This can give warning of adverse human impacts or facilitate detection of natural cycles or long-term changes (such as those highlighted in individual species and biotope action plans). However, correlations between physical and biotic events can only be drawn with confidence if the physical conditions likely to influence community structure have also been monitored.

It is likely that as our climate changes, increased UK water temperatures and changes in existing current systems will result in changes to the pink seafans' current UK distribution. Similarly, annual fluctuations in water temperature and natural changes in current speed and direction could result in natural year on year variation in the species' reproductive success. Continued annual monitoring of oocyte and spermary production within *E. verrucosa* populations may help confirm natural variability in the species reproductive capacity. **Any such monitoring should be conducted in conjunction with physical data collection** in order to assess accurately the species response to changes in the physical environment.

The action plan targets also include a need to " *Ensure that the pink sea-fan maintains its current abundance from the 1998 baseline*". (UK Biodiversity Action Group,1999)

Baseline data on pink seafan abundance and distribution has been provided by a broadscale survey of seafan populations from South Wales to the Channel Islands (MCS, 2004). This survey collected data on seafan size (height and width measurements) and condition.

As discussed earlier in this report, information on the size of colonies and age structure of *E. verrucosa* populations is particularly important in defining the reproducing population. Abundance monitoring should take account of the amount of living tissue within seafan populations, not just the number of individual colonies present. Changes in overall colony size and development of immature seafans to reproductive maturity represent important shifts in a population's ecology which

cannot be detected in monitoring programmes using counts of individuals to measure abundance.

The majority of monitoring programmes within the UK rely on abundance measurements based on counts of individuals or estimates of cover to describe and monitor the marine biota. The MNCR's SACFOR scale (JNCC, 2004) is one of the most commonly used abundance scales and is used as standard in both commercial and statutory marine monitoring programmes.

Using the SACFOR scale a population of small, reproductively immature seafans occurring at densities of one colony / m^2 would be ascribed the same abundance as a population of large, reproductively mature seafans occurring at densities of nine colonies / m^2. In ecological terms the abundance of two such populations would be very different; a shift from one to the other would represent an important environmental change which monitoring using the SACFOR scale would not even detect. In order to effectively monitor the abundance of UK seafan populations it is considered essential that future monitoring programmes move away from basic counts of individual colonies to measure the species' abundance.

Even if seafan abundance can be more accurately monitored, it is difficult to envisage how future management strategies can meet the BAP target to "*Ensure that the pink sea-fan maintains its current abundance from the 1998 baseline" (*UK Biodiversity Action Group, 1999). The abundance of *Eunicella verrucosa* is largely dependent of the species reproductive success. Results from this study indicate that reproductive success in *Eunicella verrucosa* is dependent on a range of factors including water temperature, lunar phase, spawning synchrony, habitat availability, and pre and post settlement mortality. The majority of these factors cannot be controlled by human intervention.

Since the species reproduces by broadcast spawning, it is likely that interbreeding populations extend over many kilometres and that stands of seafans found in discrete reef patches (such as those in Lyme Bay) are reproductively interdependent. Thus any activity which results in the isolation of seafan stands (for example habitat destruction and /or the loss of adjacent seafan stands) will impact on the reproductive success of remaining seafans. It is now accepted that activities such as scallop dredging can lead to destruction of the sublittoral reefs on which *Eunicella verrucosa* commonly occurs.

It is important that management strategies aim to minimise habitat degradation in order to ensure

a) there are suitable habitats for new seafans to colonise, and
b) maintenance of populations over sufficiently large areas to allow interbreeding / recruitment from adjacent populations

The protection of marine habitats is currently implemented through the award of conservation status (eg designation as Marine Nature Reserve (MNR), Special Areas of Conservation (SAC) or No Take Zone (NTZ)) to discrete geographic areas, often for a finite time period. In order for such areas to function effectively, the spatial and temporal limits and management plans of such protected areas should be based on the

life histories of the species / communities they contain. Thus management aimed at protecting seafan populations and their associated reef habitat should consider

- the range of larval supply
- the time from recruitment to reproductive maturity
- the species' densities required to maintain reproductively viable populations (ie fecundity and rates of reproductive success)

At present these parameters are unknown for *Eunicella verrucosa* and many of its associated reef species. Research into these areas is urgently required, and will be fundamental in deciding the most appropriate spatial and temporal extents for habitat protection.

In relation to species management and protection the SAP proposes action to "*Investigate causes of decline and take the appropriate management response where human activities are implicated*." (UK Biodiversity Action Group, 1999)

As outlined above, it is believed that habitat destruction by intense benthic fishing activities poses a threat to UK seafan populations. Results of this study help clarify the importance of fishing line damage as a potential threat to *Eunicella verrucosa.*

As noted by Garrabou et al (2001), the "..reproductive capacity in clonal organisms is positively related to the amount of living tissue.." This study indicates that *Eunicella verrucosa* does not commonly reproduce asexually. Thus the only way for pink seafan populations to recover from a disturbance is through sexual recruitment. Thus any activity which causes damage to the living tissue of *E. verrucosa* colonies will result in reduced reproductive capacity, and will consequently reduce the capacity for short term recovery.

Long term monitoring of *Eunicella verrucosa* populations in Lyme Bay indicates that tissue damage most commonly occurs when colonies become snagged in fishing line. Initial damage occurs when coenchyme tissue is worn away (presumably by abrasion from the fishing line). The removal of coenchyme leaves exposed patches of gorgonin axis, which are readily colonised by epibionts, leading to secondary damage, colony overgrowth and further loss of living (reproductive) tissue. Figure 7 shows the type of colony overgrowth commonly associated with entanglement in fishing line. In a broadscale survey of UK *Eunicella verrucosa* populations very few colonies (7/1000) were found with fishing line attached (MCS, 2004). This finding contrasts with out own observations of *E. verrucosa* populations in Lyme Bay, where around 25% of colonies have fishing line attached.

In relation to future research and monitoring the Species Action Plan for E. verrucosa highlights the need to "*Continue to monitor the abundance and condition of sea-fans as a part of established monitoring work".*

The MCS's 2001-2002 baseline survey of UK seafan populations included condition assessment of 1000 individual colonies. A 0-5 scale was used to describe overall colony condition in relation to tissue damage and fouling by epibionts and human debris (MCS 2004).

We recommend that future condition monitoring consider the reproductive status of individual colonies and or populations. Results from two years' monitoring at two different sites indicate that mature gonads are present within polyps during July and August, and are sufficiently large to be visible using a hand lens. Simple field-based observations of seafan reproductive status are therefore possible with very little expenditure of time or effort. If included in future condition monitoring work, such observations would provide valuable ecological data to inform our understanding of the reproductive viability of seafan populations and appropriate strategies for their management.

Also included in the research and monitoring recommendations for *E. verrucosa* is the need to "*Research the factors which affect recruitment and survival of pink sea-fan."* (UK Biodiversity Action Group, 1999)

As noted by Lasker *et al* (1998) "..recruitment is not a single event but a series of independent processes.." including gamete production and release, fertilisation, the dispersal and settlement of larvae and the early survival of newly settled individuals. This study has provided baseline data on gamete production and release in *Eunicella verrucosa.* Our work provides initial indication as to the levels of fecundity, mode of fertilisation and dispersal of larvae in seafan populations. However, hard scientific data on the many aspects of the species' reproductive ecology is still lacking. Areas recommended for additional research include

- The extent to which reproductive effort varies with a) colony size and b) polyp position within the colony.
- The age / size at which colonies first become reproductively viable, and
- The age / size structure of the UK seafan populations
- The length of time larvae spend in the water column prior to settlement
- The extent to which seafan populations on adjacent reefs are reproductively interdependent.

4.7 Final conclusions

Our study has generated data to show that *Eunicella verrucosa*

- Is a gonochoristic species
- Produces large oocytes and spermaries
- Has a relatively high reproductive capacity (in comparison with other gorgonian species)
- Has a fecundity of around 98 oocytes cm^{-1} and 210 spermaries cm^{-1} in distal branch segments
- Releases mature oocytes and gametes annually in spawning events which are synchronous over time and space

- Is probably reliant on temperature and lunar cues to synchronise spawning
- Does not brood its larvae either internally or externally
- Possibly occurs in sex ratios of 2 : 1 females : males, but produces gametes at a ratio of 1 : 2 oocytes : spermaries (further research is required to support this finding).

Our work indicates that *E. verrucosa's reproductive strategy is* unusual in combining a high expenditure on gamete production with broadcast spawning and external fertilisation / development of gametes. It is our expectation that the larvae of *Eunicella verrucosa* have a high dispersal capacity. We anticipate that seafan populations occurring on patch reef systems (eg within Lyme Bay) are interdependent for larval supply. We predict that activities causing loss or damage to seafans at one site will have secondary impacts on seafan recruitment at sites remote from the initial impact, due to reduced larval supply. We expect larval supply, recruitment and overall population structure to be highly variable amongst populations at the extremes of the species range (eg Skomer), since larval flow into these sites is "one-way". We expect that, over time, the geographic limits of *Eunicella verrucosa's* UK distribution will shift in response to a combination of natural and anthropogenic factors. We hope that continued monitoring and research of *Eunicella verrucosa* populations will extend the existing scientific platform for future marine management within the UK.

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