

# Control of oocyte maturation, sperm activation and spawning in two lugworm species: *Arenicola marina* and *A. defodiens*

G. J. Watson<sup>1,\*</sup>, P. S. Cadman<sup>2</sup>, L. A. Paterson<sup>1</sup>, M. G. Bentley<sup>1</sup>, M. F. Auckland<sup>1</sup>

<sup>1</sup>Gatty Marine Laboratory, School of Environmental and Evolutionary Biology, University of St Andrews, St Andrews, Fife KY16 8LB, Scotland, UK

<sup>2</sup>Faculty of Agriculture and Biological Sciences, Department of Marine Sciences and Coastal Management, Ridley Building, University of Newcastle, Newcastle-upon-Tyne NE1 7RU, England, UK

**ABSTRACT:** In addition to the morphological differences between *Arenicola defodiens* (Cadman and Nelson-Smith, 1993) and *Arenicola marina* (L.) this study demonstrates clear differences in the reproductive biology, timing of reproduction at a population level, and endocrine control of reproduction in females. Fecundity is significantly higher in females of *A. marina* and the mean diameter of their fully grown oocytes is also significantly larger than in *A. defodiens*. The timing of reproduction at a population level is also different with *A. defodiens* spawning approximately 1 to 2 mo later than sympatric *A. marina*. *In vitro* and *in vivo* experiments have established fundamental differences as well as similarities in the hormonal control of reproduction of both sexes between the 2 species. Male *A. defodiens* can be induced to spawn by the injection of 8,11,14-eicosatrienoic acid into the coelomic cavity, putatively shown to be the natural sperm maturation factor and spawning hormone in *A. marina*. In females, injection of female prostomial homogenate from either species into the coelomic cavity induces oocyte maturation and subsequent spawning in both species. However, oocytes of *A. marina*, whilst maturing in the presence of CMF (Coelomic Maturation Factor) *in vitro*, do not mature in the presence of prostomial homogenate of either species, whereas *A. defodiens* oocytes do. In *A. marina*, oocyte maturation and subsequent spawning is under the control of 2 hormones: (1) a substance from the prostomium, Prostomial Maturation Hormone (PMH), which induces the production of (2) CMF which acts on the oocyte. In *A. defodiens*, we show that only PMH is required for oocyte maturation and spawning. The *in vitro* induction of oocyte maturation in *A. marina*, by a variety of chemicals and treatments that have already been shown to induce maturation in *A. defodiens* and species from other phyla, was investigated. None of the chemicals and treatments tested induces maturation in *A. marina* or *A. defodiens* oocytes.

**KEY WORDS:** *Arenicola marina* · *A. defodiens* · CMF · Hormone · Oocyte maturation · Polychaeta Spawning · Sperm activation

## INTRODUCTION

Gamble & Ashworth (1898) were the first to distinguish 2 'varieties' of *Arenicola marina* which they termed the 'littoral' and 'laminarian' varieties, but this distinction was subsequently lost from the literature. The recent work of Cadman & Nelson-Smith (1990,

1993) and Cadman (1997) has shown, through the examination of a number of morphological, ecological and genetic characteristics, that this distinction was valid, and consequently the 'laminarian' variety has been classed as a separate species, *Arenicola defodiens* (Cadman & Nelson-Smith).

*Arenicola marina* and *A. defodiens* are dioecious, annually iteroparous species. *A. marina* has a wide distribution, being found in sandy beaches and estuaries across Northern Europe (Wells 1963, Beukema & de

\*E-mail: gjw@st-andrews.ac.uk

Vlas 1979, Farke & Berghuis 1979, Farke et al. 1979). The distribution of *A. defodiens* is, however, largely unknown, with only the original work of Gamble & Ashworth (1898) and a recent study by Cadman (1997) having examined its distribution and abundance. In both South Wales (UK) and several sites on the Northumberland coast (UK), locations where *A. defodiens* only are present are relatively common, but there are also a number of sites where both species exist sympatrically. The sites investigated where only *A. defodiens* are present were more likely to be of an exposed nature than sites where only *A. marina* occur. *A. defodiens* was also absent from all the estuaries investigated, in contrast to *A. marina*, densities of which are often at their highest in estuaries. Where both species coexist, *A. defodiens* are usually found at a lower position on the shore (in relation to tidal height) than sympatric *A. marina* (Cadman 1997).

The hormonal control of reproduction and spawning in *Arenicola marina* is now one of the most comprehensively studied systems in polychaetes. In males, the fatty acid 8,11,14-eicosatrienoic acid has been putatively identified as the Sperm Maturation Factor (SMF) and spawning hormone found in the prostomium (Bentley 1985, Bentley et al. 1990, Pacey & Bentley 1992a, b, Bentley & Hardege 1996). In females, oocyte maturation involves the progression from late prophase of the first meiosis to metaphase following germinal vesicle breakdown (GVBD) (Howie 1961). Oocyte maturation and subsequent spawning are induced by a hormone from the prostomium (Howie 1961, 1963, 1966, Pacey & Bentley 1992a). Recent work has shown that oocyte maturation and subsequent spawning requires 2 steps: production of the substance from the prostomium, termed the Prostomium Maturation Hormone (PMH), which induces the production of a second substance, the Coelomic Maturation Factor (CMF), which is detectable in the coelomic fluid prior to spawning (Watson & Bentley 1997, 1998a, b). Oocytes can only be matured *in vitro* in the presence of CMF and will not mature when incubated with PMH (Watson & Bentley 1997).

The work by Meijer & Durchon (1977) and Meijer (1979a, b, 1980) on what was originally identified as *Arenicola marina*, but is now considered to be *A. defodiens* (*A. marina* [sic] = *A. defodiens*) and so for ease of writing will be referred to in this paper as *A. defodiens*, are the only investigations into gamete maturation and spawning in this species. In females, spawning can be induced by the injection of female prostomial homogenate; however, oocytes will undergo maturation *in vitro* when incubated in only prostomial homogenate, suggesting a single step mechanism. Meijer (1980) also showed that calcium plays an important role in oocyte maturation as a

number of chemicals affecting calcium induced maturation *in vitro*. In males, induction of spawning and the activation of sperm *in vitro* were induced with male prostomial homogenate, suggesting similarities with *A. marina*.

The collection of both species from a number of populations around the UK that spawn at different times enabled the reproductive biology of both species to be investigated simultaneously. This paper examines differences and similarities between the 2 species in their control of reproduction and investigates the differences in reproductive morphology and timing of reproduction at a number of sites where both species are found sympatrically. Experimentally, we have investigated the role of 8,11,14-eicosatrienoic acid in the control of spawning in male *Arenicola defodiens*. In females, reciprocal experiments examine the role of PMH found within each species' prostomial homogenate in inducing spawning in the other. Focusing on the oocytes of both species, the role of CMF in inducing maturation *in vitro* in comparison to prostomial homogenate from both species was also assessed. The chemical pathways involved in oocyte maturation within oocytes were also examined through a number of different treatments, and compared and contrasted to previous results for *A. defodiens* (Meijer 1980) and other systems from other phyla.

## MATERIALS AND METHODS

**Collection of mature specimens.** The majority of sexually mature *Arenicola marina* were collected as described previously (Watson & Bentley 1997). The remainder of the *A. marina* and all the *A. defodiens* were also collected as described previously, but at varying times prior to the spawning of the population and were then maintained in culture facilities at Seabait Ltd (Ashington, Northumberland, UK) under ambient conditions until required. In all experiments, only specimens with a homogenous population of fully developed gametes were used.

**Observations and investigations of oogenesis, female fecundity and spawning in the field.** Oocyte size frequency measurements were taken from mature females of 7 *Arenicola marina* and 5 *A. defodiens* collected from Hauxley Haven (Northumberland, UK) where both species exist sympatrically. Data for *A. marina* and *A. defodiens* were from specimens collected on 25 October and 20 December 1995 respectively. Both dates were just prior to the spawning of each population (see 'Results'). For the oocyte size frequency data, samples of coelomic fluid were removed from each specimen and the diameters of approximately 50 oocytes were measured. Fecundity of

females of both species taken from ongoing cultures at Seabait Ltd was measured by taking replicate subsamples of spawned oocytes and recording the wet weight of each specimen. Observations on the spawning of populations in the field were obtained whilst collecting specimens for experiments and inferred from the spontaneous spawning of specimens within the laboratory.

**Induction of spawning.** Three sets of spawning experiments were performed. All injection techniques, production of prostomial homogenate and the experimental design were as described previously (Pacey & Bentley 1992a, Watson & Bentley 1997). All specimens injected with prostomial homogenate were injected to give a final concentration equivalent to 1 prostomium per specimen.

For the first experiment (performed in 1997), 18 male *Arenicola defodiens* and 40 male *A. marina* were divided into 5 treatment groups. Six *A. defodiens* and 20 *A. marina* (positive controls) were each injected with 8, 11, 14-icosatrienoic acid to give a final concentration of  $13 \mu\text{g g}^{-1}$  body mass. As a further positive control, 6 *A. defodiens* were injected with male *A. defodiens* prostomial homogenate. As a negative control, 6 *A. defodiens* and 20 *A. marina* were each injected with 200  $\mu\text{l}$  twice filtered seawater (TFSW). All specimens were left for 12 h at  $10^\circ\text{C}$  before being assessed for spawning.

The second experiment was performed in 1993 with 24 female *Arenicola marina* divided equally into 3 treatment groups. Individuals in the first group were each injected with female *A. marina* prostomial homogenate, and those in the second group with female *A. defodiens* prostomial homogenate. Individuals in the final treatment group were each injected with 200  $\mu\text{l}$  TFSW as a negative control. All specimens were left for 12 h at  $10^\circ\text{C}$  before being assessed for the presence of spawned oocytes.

For the third experiment (performed in 1997) 15 female *Arenicola defodiens* and 17 female *A. marina* were divided into 3 treatment groups. In the first treatment group, 5 *A. defodiens* and 6 *A. marina* were each injected with female *A. marina* prostomial homogenate. In the second group, 5 *A. defodiens* and 5 *A. marina* were each injected with female *A. defodiens* prostomial homogenate. In the final treatment group 5 *A. defodiens* and 6 *A. marina* were each injected with 200  $\mu\text{l}$  TFSW as a negative control. All specimens were left for 12 h at  $10^\circ\text{C}$  before being assessed for the presence of spawned oocytes.

**In vitro maturation of oocytes.** Oocytes of both species were incubated in coelomic fluid containing CMF from 5 females and prostomial homogenate of *Arenicola marina* and *A. defodiens* at respective concentrations of 0.2, 1, 10 and 0.2, 1 and 5 prostomial  $\text{ml}^{-1}$ .

Oocytes of both species were also incubated in TFSW as a negative control. Prostomial homogenate for both species was produced using the same technique as described previously for *A. marina*, as was the induction of CMF production in the *A. marina* females (Watson & Bentley 1997). In all experiments, 5  $\mu\text{l}$  of oocytes from each of 2 females of each species was incubated in 50  $\mu\text{l}$  of the test solution and incubated for 3 h at  $10^\circ\text{C}$  before assaying for maturation. Oocyte maturation in both species was assessed as described previously (Watson & Bentley 1997).

**Chemical induction of oocyte maturation in *Arenicola marina*.** Five  $\mu\text{l}$  of pooled oocytes (from a number of oocyte donors) was added to 1 ml of each treatment at each concentration and to the controls. All incubations were performed at  $10^\circ\text{C}$  in the dark. Oocytes were sampled after 2 and 4 h and then assessed for maturation as described above. Artificial seawater (ASW), made up according to Baloun & Morse (1984), was used for all chemical dilutions. All chemicals were dissolved directly in ASW except for ionophore A23187 and arachidonic acid which were first dissolved in ethanol and then subsequently diluted in ASW to give a final ethanol concentration of 1%.

**Chemicals and treatments tested for maturation inducing activity:** Oocytes were incubated in coelomic fluid containing CMF as positive controls. To control for spontaneous maturation and the possible effects of ethanol in the arachidonic acid and ionophore treatments, oocytes were also incubated in ASW and 1% ethanol in ASW. To assess the effect of pH on maturation, ASW with a pH of 5.6, 7, 8, 9, 10, 11 and 12 (adjusted with either 1 M HCl or 1 M NaOH) were all tested for maturation inducing activity. All chemicals, at the highest concentrations used, had a pH of between 7.0 and 7.6, except lanthanum chloride (pH of 5.6). ASW was determined to have a pH of 7.8. Tetra-caine, procaine, propranolol, oxprenolol, lanthanum chloride, ionophore A23187, DL-dithiothreitol, arachidonic acid and ammonium ions (ammonium sulphate) were also all tested for their ability to induce maturation. The concentrations of all the chemicals used were  $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$  M, except for ionophore A23187 ( $10^{-5}$ ,  $10^{-6}$  and  $10^{-7}$  M), arachidonic acid ( $10^{-4}$ ,  $10^{-5}$  and  $10^{-6}$  M) and ammonium sulphate ( $10^{-2}$  and  $5 \times 10^{-2}$  M). ASW with elevated KCl at concentrations of  $2.6 \times 10^{-2}$ ,  $5.4 \times 10^{-2}$  and  $1.04 \times 10^{-1}$  M and elevated calcium at concentrations of  $6.8 \times 10^{-2}$  and  $1.36 \times 10^{-1}$  M was also tested for maturation inducing activity as was calcium-free ASW made up according to Kuriyama et al. (1986). The maturation inducing ability of trypsin solution (1 and 3% Type III bovine pancreas) was also tested. All chemicals were purchased from Sigma Chemical Co. except for propranolol which was purchased from ICN Biomedicals, Inc.

**Chemical induction of oocyte maturation in *Arenicola defodiens*.** Oocytes from 2 donors were incubated in arachidonic acid and ionophore A23187 at concentrations of  $10^{-5}$  and  $10^{-6}$  M for both chemicals and also  $10^{-7}$  M for arachidonic acid. As positive and negative controls, oocytes were incubated in female *A. defodiens* prostomial homogenate at a concentration of  $0.5$  prostomia  $\text{ml}^{-1}$  and ASW only respectively.

**Statistical analysis.** A Kolmogorov-Smirnov 2-sample test was used to analyse the data on oocyte size frequencies (Fig. 1). To test for a significant difference in female fecundity between species (Fig. 2) a Student *t*-test was used to compare the regression coefficients for each regression line. For the results presented in Figs. 3, 4 & 5, differences between all treatments were analysed using an  $R \times C$  test of independence using a G-test. Pairwise comparisons were then performed using Tukey-type multiple comparison tests for proportions.

## RESULTS

### Observations on gametogenesis

Frequency polygons of oocyte diameters for mature *Arenicola defodiens* and *A. marina* collected from Hauxley Haven are shown in Fig. 1. Oocytes of *A. defodiens* are fully grown but immature at a diameter of  $159 \pm 0.46 \mu\text{m}$  (mean  $\pm$  SEM) and are a dull brown in colour as opposed to *A. marina* which are fully grown

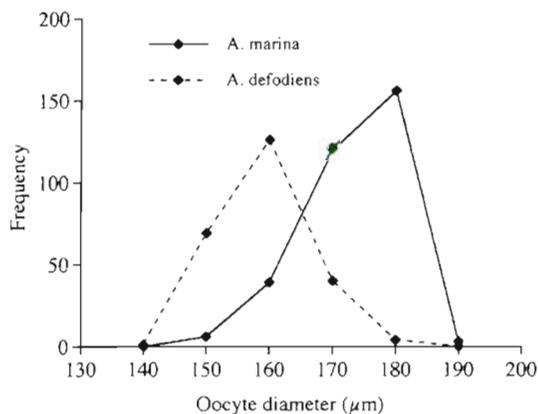


Fig. 1. Frequency polygon of mean oocyte diameters from 7 *Arenicola marina* and 5 *A. defodiens* females (approximately 50 oocytes counted per female). All specimens were collected from sympatric populations at Hauxley Haven, Northumberland, UK. Data for *Arenicola marina* and *A. defodiens* were from specimens collected on the 25 October and 20 December 1995, respectively. Both dates were just prior to the natural spawning of each population

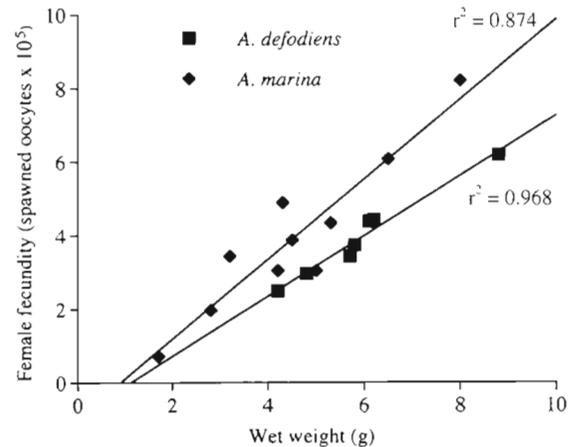


Fig. 2. Correlation between fecundity (total number of oocytes spawned  $\times 10^5$  per female) and wet weight (g) of females for *Arenicola marina* ( $n = 10$ ) and *A. defodiens* ( $n = 7$ ). ( $r^2 = 0.968$  for *A. defodiens* and  $r^2 = 0.874$  for *A. marina*)

at  $173 \pm 0.39 \mu\text{m}$  and are bright orange. Statistical analysis shows that the distribution of fully grown oocytes of *A. defodiens* is significantly different from *A. marina* ( $D = 0.6778$ ,  $p < 0.001$ ).

The correlations between fecundity (number of eggs spawned  $\times 10^5$ ) and wet weight (g) for females of each species is shown in Fig. 2. Results presented here show that as the wet weight of specimens of both species increases there is an increase in the total number of oocytes spawned ( $r^2 = 0.968$  and  $0.874$  for *Arenicola defodiens* and *A. marina*, respectively). However, the increase in fecundity, corrected for body weight, for *A. defodiens* is significantly lower when compared to *A. marina* ( $t = 3.482$ ,  $p = 0.05$ ).

### Observations on the spawning of natural populations

At sites where both species exist sympatrically, for example at West Sands, St Andrews (E. Scotland), *Arenicola marina* spawn in late October or early November at spring tides (the peak of spawning in 1997 was from the 30 October to the 2 November). *A. defodiens* from that site, however, were still mature approximately 1 mo later after the *A. marina* population had spawned, although the exact date of spawning was not recorded. At Hauxley Haven in 1995, *A. marina* spawned on the spring tides of the week beginning 30 October with sympatric *A. defodiens* spawning on the week beginning 15 January 1996. Other populations of *A. defodiens* also spawn much later in the year, with a population from Sand Head on the West Coast of Scotland spawning around the 1 February 1996.

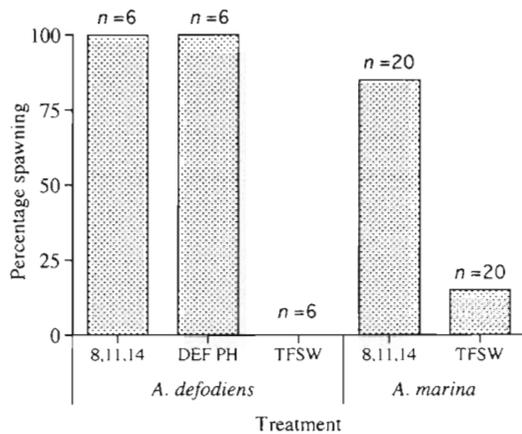


Fig. 3. Percentage spawning response of male *Arenicola marina* and *A. defodiens* to various treatments. 8,11,14: injection of 8,11,14-eicosatrienoic acid to give a final concentration of  $13 \mu\text{g g}^{-1}$  body mass; DEF PH: injection with male *A. defodiens* prostomial homogenate to give a final concentration of 1 prostomium equivalent per male; TFSW: injection of 200  $\mu\text{l}$  twice filtered seawater. All specimens were left for 12 h at  $10^\circ\text{C}$  before being assessed for spawning.  $n$  is the number of individuals in each treatment group

#### Induction of spawning of males with 8,11,14-eicosatrienoic acid

The induction of spawning in male *Arenicola defodiens* and *A. marina* with 8,11,14-eicosatrienoic acid is shown in Fig. 3. Statistical analysis using an  $R \times C$  test of independence using a  $G$ -test shows that highly significant differences ( $\chi^2 = 42.57$ ,  $p < 0.001$ ) exist between all treatments. The injection of 8,11,14-eicosatrienoic acid induced 100% of the *A. defodiens* and 85% of the *A. marina* to spawn. Statistical analysis of pairwise comparisons using a modified Tukey test shows that the injection of 8,11,14-eicosatrienoic acid induces highly significant levels of spawning in both species ( $q = 6.032$ ,  $p < 0.001$  for *A. defodiens* and  $q = 6.01$ ,  $p < 0.001$  for *A. marina*) when compared to their respective controls. The injection of male *A. defodiens* prostomial homogenate also induced highly significant levels ( $q = 6.032$ ,  $p < 0.001$ ) of spawning in *A. defodiens* (100%). No significant differences exist when these 3 treatments are compared ( $q = 3.858$ ,  $p > 0.05$ ). None of the seawater injected *A. defodiens* and only 15% of *A. marina* spawned spontaneously.

#### Reciprocal induction of spawning in females

The spawning in females of both species can be induced by either *Arenicola marina* or *A. defodiens* female prostomial homogenate (Fig. 4). In 1997, the injection of *A. defodiens* with either species' pro-

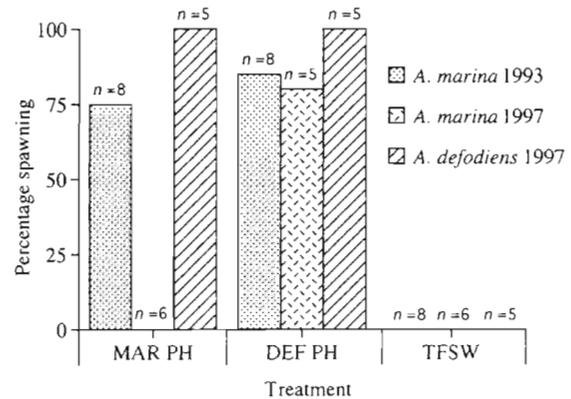


Fig. 4. Percentage spawning response of female *Arenicola marina* in 1993 and 1997 and *A. defodiens* 1997 after injection with various treatments. DEF PH: injection with female *A. defodiens* prostomial homogenate to give a final concentration of 1 prostomium equivalent per female; MAR PH: injection with female *A. defodiens* prostomial homogenate to give a final concentration of 1 prostomium equivalent per female; TFSW: injection of 200  $\mu\text{l}$  twice filtered seawater. All specimens were left for 12 h at  $10^\circ\text{C}$  before being assessed for spawning.  $n$  is the number of individuals in each treatment group

mial homogenate induced 100% of each treatment group to spawn. Of the *A. marina* injected with *A. defodiens* prostomial homogenate, spawning was induced in 85 (1993) and 80% (1997) of the specimens. In 1993, 75% of the *A. marina* injected with *A. marina* prostomial homogenate spawned; however, in 1997 the repeat experiment resulted in none of the *A. marina* spawning. No females of either species injected with TFSW spawned.

Statistical analysis of the data, as performed previously, shows that highly significant differences are present between all treatments ( $\chi^2 = 26.124$ ,  $p < 0.001$ ). Pairwise comparisons of these differences confirm that a significant level of spawning is induced by all treatments, when compared to their respective controls (TFSW injected), except for *A. marina* injected with *A. marina* prostomial homogenate in 1997 ( $q = 0.11$ ,  $p > 0.05$ ). No significant differences ( $p > 0.05$ ) are present between pairwise comparisons of the treatments that induce significant spawning.

#### In vitro induction of oocyte maturation

The *in vitro* induction of oocyte maturation in both species by incubation in prostomial homogenate and CMF is shown in Fig. 5. Statistical analysis using an  $R \times C$  test of independence using a  $G$ -test shows that highly significant differences exist between all treatments ( $\chi^2 = 2509.2$ ,  $p < 0.001$ ). Subsequent pairwise comparisons using the modified Tukey test show that

significant maturation of *Arenicola marina* oocytes, when compared with controls and other treatments, occurred only when they were incubated in coelomic fluid containing CMF. The levels of maturation achieved in the different females' coelomic fluid were not significantly different from each other except for coelomic fluid Sample 1, which was significantly higher than all other coelomic fluid samples. Incubation of *A. marina* oocytes in prostomial homogenate of either species up to a concentration of 10 prostomia ml<sup>-1</sup> for *A. marina* and 5 prostomia ml<sup>-1</sup> for *A. defodiens* failed to induce any significant maturation when compared to the control. In contrast, pairwise comparisons show that oocytes of *A. defodiens* underwent very significant levels of maturation (mean value of 90%) when incubated in a range of concentrations of prostomial homogenate of either species or in the coelomic fluid containing CMF. Pairwise comparisons also show that there is no significant difference in the number of *A. defodiens* oocytes maturing when incubated in either species' prostomial homogenate. There is no significant maturation of oocytes from either species when incubated in the seawater control.

#### Chemical induction of oocyte maturation

Maturation of each species' oocytes only occurred when they were incubated in their respective positive controls. For *Arenicola marina* 79% of the oocytes matured in coelomic fluid with CMF activity, while 100% of *A. defodiens*' oocytes matured when incubated in female *A. defodiens* prostomial homogenate at a concentration of 0.5 prostomia ml<sup>-1</sup>. All the following treatments, at all concentrations tested and incubated for either 2 or 4 h, failed to induce any maturation of oocytes of *A. marina*: tetracaine, procaine, propranolol, oxprenolol, lanthanum chloride and DL-dithiothreitol (10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> M), ionophore A23187 (10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup> M), arachidonic acid (10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> M), ammonium sulphate (10<sup>-2</sup> and 5 × 10<sup>-2</sup> M), KCl enriched ASW (2.6 × 10<sup>-2</sup>, 5.4 × 10<sup>-2</sup> and 1.04 × 10<sup>-1</sup> M), calcium enriched ASW (6.8 × 10<sup>-2</sup> and 1.36 × 10<sup>-1</sup> M), trypsin (1 and 3% solutions of Type III bovine pancreas), ASW (pH adjusted to 5.6, 7, 8, 9, 10, 11 and 12), calcium free ASW, ASW only and ASW with 1% ethanol. The following treatments also failed to induce any maturation in *A. defodiens* oocytes: ionophore A23187 (10<sup>-5</sup> and 10<sup>-6</sup> M), arachidonic acid (10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup> M) and ASW only.

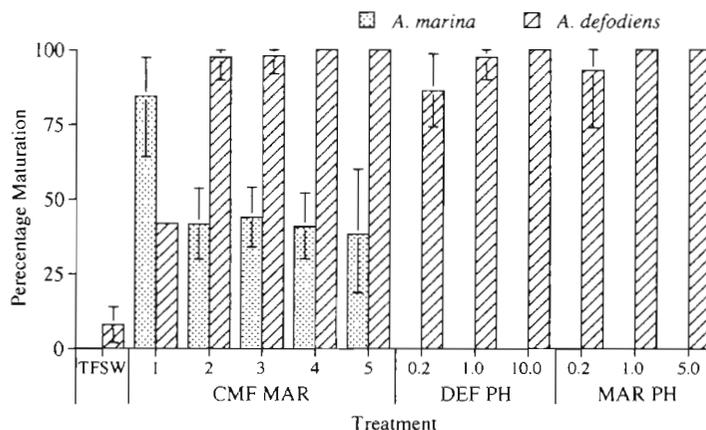


Fig. 5. Maturation response of oocytes of *Arenicola marina* and *A. defodiens* incubated in various treatments. TFSW: TFSW only; CMF MAR 1, 2, 3, 4, 5: coelomic fluids from 5 female *A. marina* containing CMF; MAR PH: prostomial homogenate of *A. marina* at concentrations of 0.2, 1 and 10 prostomial ml<sup>-1</sup>; DEF PH: prostomial homogenate of *A. defodiens* at concentrations of 0.2, 1 and 5 prostomial ml<sup>-1</sup>. Response expressed as arcsine back-transformed mean percentage ( $\pm$ SEM) of oocytes matured

#### DISCUSSION

*Arenicola marina* and *A. defodiens* can be separated by a variety of ecological, morphological and genetic criteria (Cadman & Nelson-Smith 1990, 1993, Cadman 1997). However, until now, no direct comparisons or experiments have been undertaken to examine whether these differences extend to the reproductive biology of the 2 species. Observations described here have confirmed that the reproductive biology of these 2 species, at least in females, is different. Vitellogenesis in *A. marina* begins in mid-June and results in the production of fully-grown (approximately 175  $\mu$ m diameter) but immature oocytes (Howie 1961). In contrast oocytes of *A. defodiens* are smaller (160  $\mu$ m diameter) and a different colour (dull brown as opposed to bright orange). Fecundity of females of *A. defodiens* is also lower when compared to similarly sized *A. marina*. Results presented in Fig. 2 show that as females increase in size, fecundity of *A. defodiens* increases at a slower rate than that of *A. marina*. It is likely that a similar situation exists in males, although these data were not recorded. The lower levels of fecundity in *A. defodiens* compared to *A. marina* may be related to the reduced available coelomic fluid volume for developing oocytes and sperm. This lower available volume of the coelomic cavity for gamete development is probably due to the thicker body wall of *A. defodiens* (authors' pers. obs.).

Reproductive differences between the species have also been shown to extend to the population level. Most UK populations of *Arenicola marina* spawn in autumn or early winter (Howie 1959, Duncan 1960,

Williams et al. 1997). Observations from sites around the UK indicate that the timing of spawning of *A. defodiens* is approximately 1 to 2 mo later than sympatric *A. marina*. The observed spawning of *A. marina* at sites such as West Sands and Hauxley Haven is highly synchronised and is restricted to 2 or 3 days in the year (Bentley & Pacey 1992, Williams et al 1997, G. J. Watson pers. obs.); however, there have been no observations of spawning in the field of *A. defodiens*. It is unknown, therefore, whether *A. defodiens* exhibits the same level of within-population synchronisation as *A. marina*. Similarly, the spawning behaviour of *A. defodiens* in the field has not been observed. Although personal observations of males in the laboratory suggest that they spawn with less muscular contractions than *A. marina*, the method of spawning has not been recorded. What is clear is that the reproductive seasons are significantly separated in time to prevent cross fertilization (artificial cross fertilizations have so far not produced viable larvae; P. S. Cadman pers. obs.)

*Arenicola marina* and *A. defodiens* are clearly different in their reproductive biology. In particular there are subtle differences in the hormonal control of spawning. Results presented above have shown that both sexes from both species share certain common elements of control as well as exhibiting fundamental differences. Males of both species can be induced to spawn with the injection into the coelomic cavity of 8,11,14-eicosatrienoic acid, which has already been putatively identified as the spawning inducer and SMF for *A. marina* (Bentley et al. 1990, Pacey & Bentley 1992a). To conclude, however, that in males, the system of sperm maturation and spawning is identical in both species would be premature. Results obtained with females (see below for discussion) show that 2 systems may contain similar or identical components, but, upon further examination, are different. Clearly, 8,11,14-eicosatrienoic acid plays an important role in sperm activation and spawning in *A. defodiens*, although its exact role is unknown.

The injection of female *Arenicola marina* prostomial homogenate into the coelomic cavity of female *A. marina* is now a reliable endocrine technique to induce spawning of ripe females (Howie 1961, Pacey & Bentley 1992a, Watson & Bentley 1997, 1998b). The results shown in Fig. 4, however, show that, although 75% of the 1993 females spawned, all the 1997 *A. marina* failed to spawn after injection. This was unexpected, but one possible explanation for the failure to spawn relates to the titres of PMH present within the *A. marina* prostomial homogenate and the threshold concentration of PMH required to induce spawning in females. Previous work (Watson 1996) has shown that in *A. marina* the nearer to the natural spawning time of a population you inject females with prostomial

homogenate, the greater the number of females will spawn. This could be because as the natural spawning approaches the levels of PMH within the prostomium increases concurrently with the receptivity of females to spawn (a possible decrease in threshold level required for PMH). The *A. defodiens* used in the 1997 experiments may have been closer to spawning spontaneously than the *A. marina* and therefore higher levels of PMH would have been present in their homogenate and they would also have been more receptive to injection (had lower PMH thresholds) compared to the *A. marina*. Consequently, when the *A. marina* were injected with both species' homogenates the *A. defodiens* homogenate induced them to spawn due to the higher levels of PMH in the homogenate exceeding the threshold levels of the *A. marina*. The lower levels of PMH in the *A. marina* homogenate would have failed to reach the higher threshold level required by the *A. marina*, resulting in a failure to spawn. However, because there would be enough PMH present in the *A. marina* homogenate to exceed the lower threshold level of PMH required in the *A. defodiens*, all of the *A. defodiens* injected with *A. marina* prostomial homogenate were induced to spawn.

The results of the *in vitro* experiments (Fig. 5), confirming our earlier work (Watson & Bentley 1997), show that *Arenicola marina* oocytes will undergo maturation only in coelomic fluid containing CMF. The presence of this substance is essential for oocytes of *A. marina* to mature, but oocytes of *A. defodiens* require only the presence of PMH (from either species). In *A. defodiens*, CMF is not produced, and oocyte maturation only requires the presence of PMH. In *A. marina*, PMH is also essential but is the first of 2 steps, with CMF as a second substance which then acts on the oocyte to induce maturation.

A comparison of the control of reproduction and oocyte maturation in these 2 species with other systems, for example the asteroid echinoderms, demonstrates a unique level of interspecific control of oocyte maturation. In all of the asteroids so far studied, oocyte maturation and spawning is under the control of a 2-step hormonal system. This involves the production of a Gonad Stimulating Substance (GSS) which in turn induces the production of the meiosis inducing substance (MIS), 1-methyladenine, from the follicle cells which induces spawning and oocyte maturation (for review, see Meijer & Mordet 1994). This system has therefore been conserved across all species of asteroids so far investigated. In molluscs there is also evidence, from a number of species of bivalves, that neurotransmitters, in particular serotonin, are involved in the control of reproduction (Matsutani & Nomura 1982, 1986, Gibbons & Castagna 1984, Hirai et al. 1988, Ram

et al. 1993). In polychaetes, it seems that many more systems have evolved in the control of reproduction (see for example the diversity of pheromones involved in nereid spawning [Zeeck et al. 1988, 1990, 1991, Hardege et al. 1991]) with the different systems of oocyte maturation in *Arenicola marina* and *A. defodiens* being an extreme example.

At the level of the oocyte, the system of maturation also appears different. Meijer (1980) suggested that, because maturation in *Arenicola defodiens* could be induced by chemicals affecting intracellular calcium levels (tetracaine, procaine, propranolol, oxprenolol, verapamil and lanthanum chloride), calcium plays a key role in the maturation process in this species. The results presented here show that in *A. marina*, elevation of intracellular calcium, specifically caused by these chemicals, does not alone induce maturation. The failure of any of these chemicals to induce maturation is in contrast to results for *A. defodiens* (Meijer 1980). However, the results presented here are consistent with some work on starfish: increases in intracellular calcium do occur during maturation but they are not a requirement for maturation, or sufficient to induce maturation themselves (Kikuyama & Hiramoto 1991). Further work to examine the exact role of calcium and whether it is essential for oocyte maturation in starfish has provided contradictory results. An increase in calcium in the nucleus, although not in the cytoplasm, is essential for maturation in starfish (Santella & Kozzuka 1994). Other conflicting results on the role of calcium are the lack of maturation in oocytes of starfish and *A. defodiens* when exposed to ionophore A23187, whereas the oocytes of other species such as *Spisula solidissima* and *Pectinaria gouldii* mature in its presence (Schuetz 1975, Anstrom & Summers 1981). It has been suggested that in starfish the surge of calcium induced by the ionophore A23187 is too large (20 times greater than the calcium increase due to 1-methyladenine) and outside the limits required to induce maturation (Moreau et al. 1978).

The failure of oocytes to mature when incubated with DTT DL-dithiothreitol suggests that the reduction of disulphide bonds also does not play a significant

part in the process of maturation in *Arenicola marina*. These findings contrast with the maturation process of both *A. defodiens* and starfish, in which DTT and another disulphide reducing agent (2,3-dimercaptoethanol) induce significant maturation (Kishimoto & Kanatani 1973, Meijer 1980).

The complete failure of *Arenicola marina* oocytes to mature in all of the test chemicals is in contrast to the situation found not only in *A. defodiens* but also starfish and many other species. An increase in pH of seawater has been shown to induce maturation in the molluscs *Patella vulgata* (Guerrier et al. 1986) and *Cel-lana nigrolineata* (Catalan & Yamamoto 1993), suggesting that internal pH changes may play a role in the maturation of oocytes of these species. In *Nereis virens* and *Spisula* spp., oocytes undergo maturation when external KCl and calcium are elevated, supporting a role for the opening of calcium channels in the oocyte membrane (Heilbrunn & Wilbur 1937, Allen 1953). In starfish, Meijer et al. (1984), showed that arachidonic acid and related compounds can induce oocyte maturation, suggesting that these may also play a role within the oocyte. In the hydrozoan, *Phialidium gregarium*, oocyte maturation can be induced by calcium ionophore A23187 and ammonia, also suggesting a role of calcium and intracellular pH changes in oocyte maturation in the cnidarians (Freeman & Ridgway 1993).

The results presented here demonstrate that in *Arenicola marina* the mechanism of oocyte maturation, specifically the transduction of the CMF signal to the activation of MPF, is different to that of *A. defodiens*, and may also be different to the other more extensively studied systems of other species described already. The chemicals and treatments tested here fail to induce maturation because (1) they have no essential role in oocyte maturation so that changes induced are irrelevant; (2) they induce too little or too much change in the oocyte to induce maturation; and (3) they may be acting, in this case, in a different way to their reported pharmacological action. Further studies on *A. marina* investigating the internal changes in pathways such as calcium and pH with microinjection and other tech-

Table 1. Summary of the endocrine control of spawning and oocyte maturation in females of *Arenicola marina* and *A. defodiens*. PMH: Prostomial Maturation Hormone; CMF: Coelomic Maturation Factor

Species	Hormonal step 1	Hormonal step 2	Effect on oocyte	Requirement for Ca <sup>2+</sup>	Requirement for disulphide bond reduction
<i>Arenicola marina</i>	PMH ( <i>A. marina</i> or <i>A. defodiens</i> )	CMF	Maturation from prophase to metaphase of meiosis I	No	No
<i>Arenicola defodiens</i>	PMH ( <i>A. marina</i> or <i>A. defodiens</i> )	-	Maturation from prophase to metaphase of meiosis I	Yes	Yes

niques are required to ascertain the role of these chemical pathways in the maturation of the oocytes.

The results presented in this paper show for the first time that differences between *Arenicola marina* and *A. defodiens* also extend to the reproductive biology, timing of spawning and specific elements of the endocrine control of oocyte maturation. A summary diagram of the endocrine control of reproduction in females of both species is shown in Table 1. Clearly such fundamental differences between closely related species have evolutionary implications in relation to the ecological niche occupied by each species. However, it is clear from Table 1 that all aspects of both species' reproductive biology warrant further investigation.

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