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THE FATTY ACID 8,11,14-EICOSATRIENOIC ACID INDUCES SPAWNING IN THE MALE LUGWORM *ARENICOLA MARINA*

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Summary

Spawning of the lugworm *Arenicola marina* (L.) (Annelida: Polychaeta) occurs in both sexes following the release of a sex-specific maturation hormone from the prostomium. In males this has been termed sperm maturation factor (SMF). Chromatographic purification of SMF, using an *in vitro* bioassay, has led to it being putatively identified as the 20-carbon fatty acid 8,11,14-eicosatrienoic acid and this paper describes the induction of spawning *in vivo* by injection of 8,11,14-eicosatrienoic acid into sexually mature individuals.

It is shown that spawning in male *A. marina* occurs following the injection of prostomial homogenate or 8,11,14-eicosatrienoic acid into the coelomic cavity of sexually mature specimens. The injection of 8,11,14-eicosatrienoic acid into the coelomic cavity of females does not cause spawning although it can be induced by a hormone present in the prostomium. There are, therefore, clear sex-specific differences in the chemical signal that causes spawning in this species. Sex-specific differences in the behaviour of spawning animals are also described.

The study identifies a role for the fatty acid 8,11,14-eicosatrienoic acid in the spawning of male *A. marina*. This is the first description of a hormone in the annelids, for which both the chemical structure and the endocrine role are known.

Introduction

Spawning, in species that are broadcast spawners and which have external fertilisation, is frequently the most dramatic event in their life cycle. Often, the spawning of individuals is synchronised so that the chances of gamete interaction are maximised; it is thought that successful fertilisation may pose a significant problem during the life history of broadcast spawners (Pennington, 1985; Denny and Shibata, 1989). Synchronous spawning between neighbouring individuals has been well documented and is known to occur in species from a number of phyla. Perhaps the most spectacular is that occurring on the Great Barrier Reef in Australia, when over 100 species spawn together on only a

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few nights each year (Babcock *et al.* 1986). In such circumstances, possible environmental and/or physiological cues which could control synchronous spawning events have often been sought.

In the polychaete *Arenicola marina*, at some localities, spawning between neighbouring individuals is highly synchronised. It is easily identified by the appearance, during periods of low tide, of sperm puddles which have been ejected from the burrows of spawning males onto the surface of the beach sediment. Such a phenomenon has attracted the attention of a number of workers and Pirlot (1933), Newell (1948), Howie (1959) and Duncan (1960) have described synchronised (epidemic) spawning at numerous locations around the coasts of northern Europe. Several other authors have described spawning at a further twenty sites where it is not well synchronised and the patterns of natural spawning of *A. marina* have been discussed more recently by Howie (1984).

In an attempt to investigate the possibility that spawning in *Arenicola marina* is regulated, at least in part, by an internal (endocrine) stimulus, Howie investigated the spawning mechanism experimentally. In a series of papers he noted that spawning can be induced by an endocrine substance (Howie, 1961*a*) present in the prostomium (Howie, 1963, 1966) and he suggested that its mode of action is directly on the gametes (Howie, 1961*b*,*c*, 1962). Gametogenesis in *A. marina* is of particular interest because in both sexes it occurs almost entirely in the coelomic cavity (see Olive, 1983*a*,*b*, for a review); in females, oogenesis results in the production of oocytes that are arrested in the prophase of their first meiotic division (Howie, 1961*b*; Meijer, 1979*a*) and, in males, spermatogenesis results in the formation of sperm morulae in which several hundred spermatozoa are cytoplasmically connected to form a syncitium (Meijer, 1979*b*; Bentley and Pacey, 1989; Pacey and Bentley, 1992). Howie suggested that the mode of action of the endocrine substance, which stimulates spawning in ripe individuals, is by inducing gamete maturation. In females the maturation of the oocytes (i.e. the movement from prophase to metaphase of the first meiotic division and the breakdown of the germinal vesicle) results in a change in oocyte shape (Howie, 1961*b*; Meijer, 1979*a*) whereas during gamete maturation in males, the structure of the sperm morula breaks down and individual spermatozoa become free (Howie, 1961*a*; Meijer, 1979*b*). Howie suggested that it is this morphological change in the nature of the gametes that is fundamental to the spawning mechanism of *A. marina*. He suggested that once this has taken place the gametes are automatically accepted by the ciliated funnels of the nephromixia and are spawned. Unripe gametes are rejected by the nephromixia and are, therefore, retained within the body cavity and are not spawned (Howie, 1961*b*,*c*, 1962). For this reason, the endocrine factor which induces spawning in male and females has become known as a 'maturation' hormone rather than a 'spawning' hormone.

Some progress in the chemical identification of the maturation hormones of *Arenicola marina* was made by Howie, and he noted that the factor stimulating spawning in males is heat-stable and can be extracted from whole-body homogenates by benzene and ether (Howie, 1961*a*,*b*). Further investigation demonstrated that the active substance can be isolated from the saponifiable lipids (which contain fatty acids) and, of this fraction, the saturated fatty acids appear not to induce spawning (Howie, 1961*a*). This suggests that the maturation factor in males (now termed sperm maturation factor, after Bentley, 1985)

is a lipid. Fractionation of prostomial homogenates confirms this conclusion, although the fact that Howie was able to isolate active factors from both the lipid and non-lipid components during extraction with ether (Howie, 1963; Meijer, 1979*b*) suggests that there may be more than one active substance present within the prostomium. However, as Bentley (1985) points out, this result can equally be obtained by the incomplete separation of the lipid in the solvent system used.

Howie noted that the active substance can be extracted from prostomia taken from both sexes (Howie, 1966) but that the lipid substance inducing spawning in males cannot induce spawning in females. From this he concluded that the female maturation hormone has a different chemical structure to that of males, but no further progress has been made in identifying its chemical nature. More recently, oocyte maturation by the prostomial hormone was investigated by Meijer and although this work gave an insight into the mode of action of the hormone (Meijer and Durchon, 1977; Meijer, 1979*a*,*b*, 1980) it shed no new information on its chemical structure.

Progress in the chemical identification of sperm maturation factor (SMF) was made by Bentley (1985), who developed an *in vitro* bioassay for SMF. Using this bioassay, he was able to demonstrate that the presence of SMF in the prostomia of *A. marina* is not constant throughout the year, but that it is present only during the breeding season; following spawning, the levels of SMF quickly become undetectable. Bentley also reconfirmed the lipid nature of SMF and demonstrated that it has very similar thin layer chromatographic characteristics to non-steroid pharmacologically active lipids such as the unsaturated fatty acid arachidonic acid. More recently, and using a variety of chromatographic and enzymic techniques, work in this laboratory putatively identified SMF as the 20-carbon free fatty acid 8,11,14-eicosatrienoic acid (Bentley *et al.* 1990).

It is clear from the preliminary purification of SMF that the chemical and biological properties of both SMF and 8,11,14-eicosatrienoic acid, so far investigated, are identical (Bentley *et al.* 1990). In addition, the results from a number of other *in vitro* investigations, which will be reported separately, also support this conclusion. Clearly, the real test of the hypothesis that 8,11,14-eicosatrienoic acid and SMF are the same substance is to confirm the chemical nature of SMF by a full structural analysis of a purified sample. In the absence of such information, however, a major step forward would be to demonstrate a true role for 8,11,14-eicosatrienoic acid in the natural spawning process. This paper, therefore, investigates the ability of 8,11,14-eicosatrienoic acid to induce spawning in sexually mature specimens of *Arenicola marina*.

Materials and methods

Collection and maintenance of animals

Sexually mature specimens of *Arenicola marina*, for use in spawning experiments, were collected from populations at St Andrews (SE Scotland) and at Budle Bay (NE England) during the few weeks immediately prior to the date of natural spawning. In the case of individuals obtained from St Andrews, this was during the early part of October (spawning usually occurs during late October or early November) and for those at Budle Bay collections were made during early December (spawning at this locality occurs

during December). Animals were transported back to the laboratory, sexed, and placed individually in plastic containers filled with sea water. The animals were maintained at a constant 10 °C under constant illumination and were allowed to empty the gut before spawning experiments were initiated. Spawning experiments were carried out within 5 days of collection of the animals.

Animals were sexed by the direct observation of gametes present in the coelomic cavity. This was carried out in one of two ways: either the gametes present in the coelomic cavity were observed *in situ* through the thinner areas of the body wall such as at the position around the ventral nerve cord or, when this was not possible or proved inconclusive, a small drop of coelomic fluid was removed from the animal using a disposable syringe and needle, and was observed using light microscopy. Observation of the gametes through the body wall is usually possible during the period immediately prior to spawning because the coelomic cavity at this time becomes filled with gametes. A high density of sperm morulae in the coelomic cavity forms a milky white suspension whereas oocytes give the coelomic fluid a granular orange appearance in which individual oocytes (diameter approximately 180 μm) can usually be observed.

Experimental design

Animals were placed into individual pre-cleaned plastic containers containing a standard volume of 180 ml of sea water. A total volume of 200 μl of prostomial homogenate, 8,11,14-eicosatrienoic acid or 11,14,17-eicosatrienoic acid, from an appropriate stock solution, was then injected into the coelomic cavity of individuals to give the desired final concentration for each treatment group. In the control group, individuals were injected with 200 μl of twice-filtered sea water (TFSW.) Following injection, the animals were left undisturbed for 24 h, at 10 °C, after which the number of gametes released by each individual was determined.

The number of spawned oocytes was estimated by counting the total number of oocytes (using a 10× objective of a compound microscope) present in three replicate subsamples, each of 1 ml, taken from the standard volume after thorough mixing. Sperm numbers were determined in a similar way but, in instances where high densities of sperm were released, the subsamples were usually diluted between 1:1000 to 1:5000 (v/v) before counting in a Neubauer haemocytometer. Owing to the highly motile nature of the spermatozoa released during spawning, estimates of sperm numbers were carried out after sperm motility had ceased (usually within 48 h of spawning).

Spawning experiments

Three spawning experiments were carried out. In the first, 24 male and 24 female worms were divided into three treatment groups, each of eight animals. In one treatment group for each sex, individuals were injected with 200 μl of either crude prostomial homogenate or 8,11,14-eicosatrienoic acid to give a final concentration of 0.1 prostomium g^{-1} body mass, or 13 $\mu g\, g^{-1}$ body mass respectively. Animals in the third treatment group were injected with 200 μl of TFSW.

In the second experiment, the above procedure was repeated with the addition of a fourth treatment group, in which 200 μl of the fatty acid 11,14,17-eicosatrienoic acid was

injected to give the same concentration as that used in the first experiment (i.e. 13 $\mu g\,g^{-1}$ body mass). For analysis, the data from these two experiments were combined to give a total of 16 replicates for injection with prostomial homogenate and 8,11,14-eicosatrienoic acid, and eight replicates for the 11,14,17-eicosatrienoic acid treatment group, for each sex. The doses used in each experiment were chosen on the basis of *in vitro* dose–response experiments with these substances and were typical mid-range concentrations capable of stimulating sperm morula breakdown *in vitro* (see Bentley *et al.* 1990).

A third experiment was carried out in which the effect of dose was investigated. For each dose–response analysis, 48 animals in eight treatment groups were injected with prostomial SMF, 8,11,14- or 11,14,17-eicosatrienoic acid, or TFSW. The experimental protocol was unchanged from that described in the previous two experiments; however, each treatment group consisted of six rather than eight individuals. Injections were carried out to give a final concentration of 0.25, 0.125, 0.0625 or 0.0125 prostomia g^{-1} body mass and then tenfold dilutions down to a concentration of 1.25×10^{-6} prostomia g^{-1} body mass for prostomial SMF, or 13, 2.6, 1.3 or 0.26 $\mu g\,g^{-1}$ body mass and then tenfold dilutions down to a concentration of 2.6×10^{-5} $\mu g\,g^{-1}$ body mass for the fatty acids.

Results

The pooled results of both the first and the second experiments demonstrate that spawning in sexually mature male *Arenicola marina* can be induced by a coelomic injection of either prostomial homogenate or 8,11,14-eicosatrienoic acid (Fig. 1). In both of these treatment groups a similar number of spermatozoa ($2.53\times10^{8}\pm0.58\times10^{8}$ and $1.51\times10^{8}\pm0.22\times10^{8}$ sperm ml^{-1}, respectively) were released during spawning and all animals that were injected underwent a spawning response (100 % response). No spawning was observed following the injection of either 11,14,17-eicosatrienoic acid or TFSW (0 % response).

Spawning in female *Arenicola marina* was stimulated only following an injection of prostomial homogenate (Fig. 2) and the mean spawning response was $1.17\times10^{5}\pm0.259\times10^{5}$ oocytes (mean ± S.E.M.) released per individual. All individuals injected with prostomial homogenate at this concentration underwent a spawning response (100 % response). Microscopic examination of spawned oocytes indicated that they had undergone germinal vesicle breakdown (GVBD) and consequently maturation of the oocytes had occurred. Oocyte release was observed in one female injected with 8,11,14-eicosatrienoic acid.

The effect of dose on the spawning response of both male and female individuals was investigated in the third experiment and these results are shown in Figs 3 and 4. Spawning in male *Arenicola marina* was stimulated above concentrations of 1.25×10^{-2} μg prostomia g^{-1} body mass of prostomial extract (Fig. 3A) and 1.3 $\mu g\,g^{-1}$ body mass of 8,11,14-eicosatrienoic acid (Fig. 3B). At these concentrations, however, only two out of six animals spawned in response to the fatty acid (33.33 % response) and only one out of six animals spawned in response to prostomial SMF (16.67 % response).

No spawning was observed at any dose of 11,14,17-eicosatrienoic acid (Fig. 3C), confirming the observations made in the previous experiment that this eicosatrienoic isomer does not stimulate spawning. Above the threshold concentrations of prostomial SMF, Fig. 3A shows clearly that spawning is an all or nothing response with a similar number of sperm released over a range of concentrations. Slightly more variation is observed, however, at above threshold concentrations of 8,11,14-eicosatrienoic acid

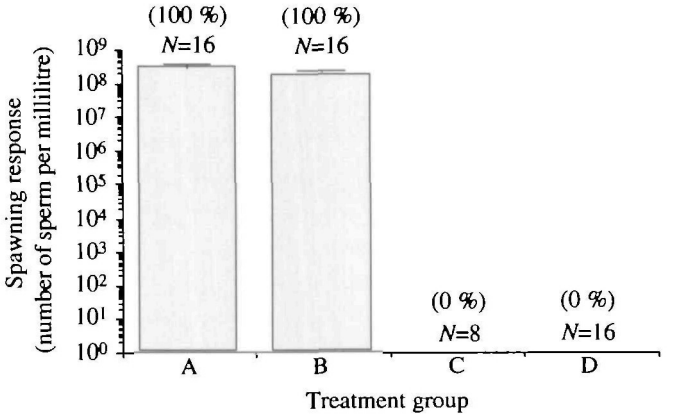

Fig. 1. Spawning response of sexually mature male *Arenicola marina* to the injection of (A) prostomial homogenate, (B) 8,11,14-eicosatrienoic acid, (C) 11,14,17-eicosatrienoic acid or (D) twice-filtered sea water. The spawning response is indicated as the number of sperm per millilitre released into 180 ml of sea water. Figures in parentheses indicate the percentage of individuals in the treatment group which underwent a spawning response. Data shown are the mean spawning response + S.E.M.; *N* is the number of individuals per treatment group.

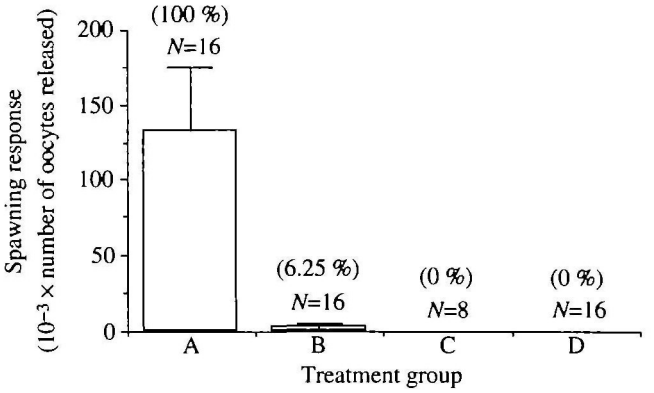

Fig. 2. Spawning response of sexually mature female *Arenicola marina* to the injection of (A) prostomial homogenate, (B) 8,11,14-eicosatrienoic acid, (C) 11,14,17-eicosatrienoic acid or (D) twice-filtered sea water as in Fig. 1. The spawning response is indicated as the total number of oocytes spawned (mean + S.E.M.). Figures in parentheses indicate the percentage of animals in the treatment group which underwent a spawning response; *N* is the number of individuals per treatment group.

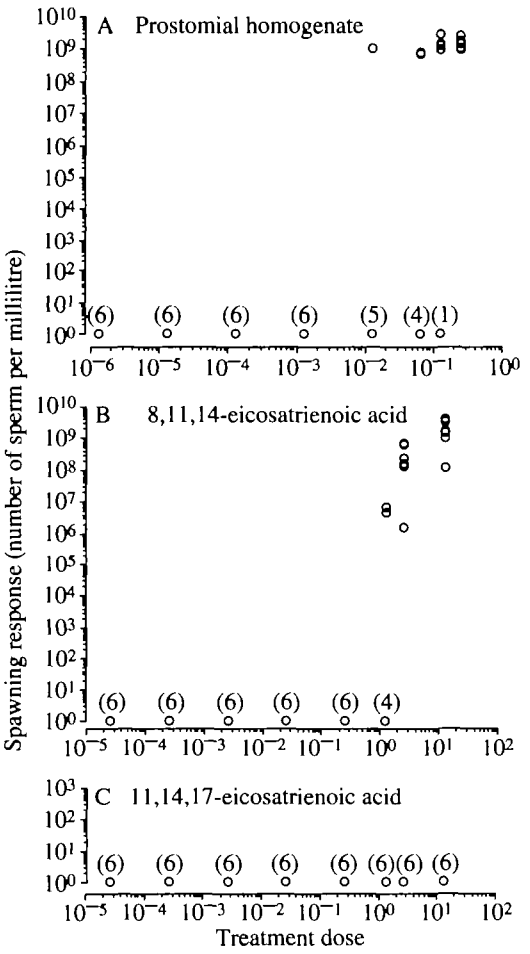

Fig. 3. Spawning response of male *Arenicola marina* to a variation in dose of (A) prostomial homogenate, (B) 8,11,14- and (C) 11,14,17-eicosatrienoic acid. Dose is expressed as the number of prostomia g^{-1} body mass for prostomial homogenate and $\mu g\, g^{-1}$ body mass for the two fatty acids. The spawning response is expressed as the number of sperm per millilitre released into 180 ml of sea water. The mean spawning response of each individual in the treatment group is shown and figures in parentheses show the number of individuals that failed to release spermatozoa after 24 h.

(Fig. 3B), in terms both of the number of animals responding and the number of sperm released, although this response can be still regarded as all or nothing.

The responses of females to comparable doses also confirms the results obtained in the first two experiments. Spawning is not stimulated by either of the fatty acid isomers, but only occurs in response to prostomial homogenate (Fig. 4). Spawning was stimulated above a concentration of 6.25×10^{-2} μg prostomia g^{-1} body mass (Fig. 4A) although, at this concentration, only one animal out of six underwent spawning (16.67 % response), and at the maximum concentration tested (0.25 μg prostomia g^{-1} body mass) only an 83.33 % response was obtained. In comparison to the spawning response observed in

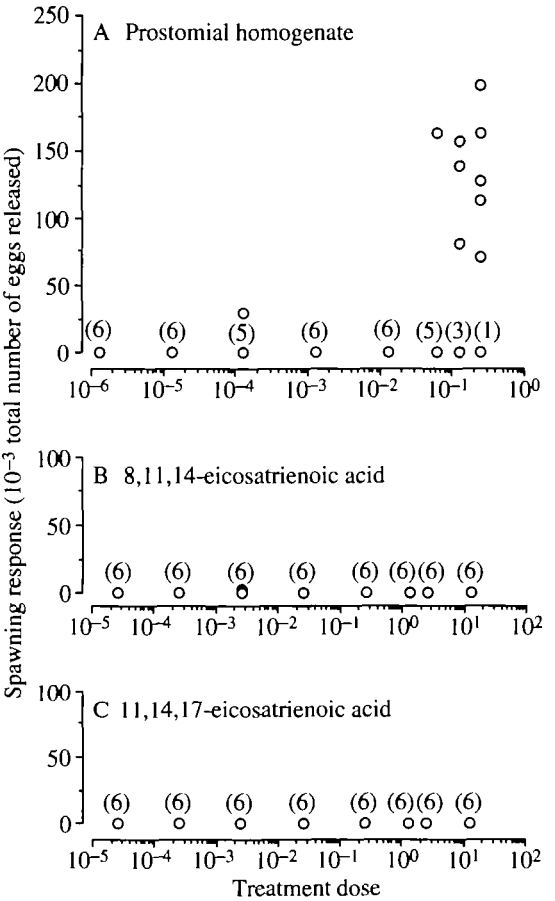

Fig. 4. Spawning response of female *Arenicola marina* to a variation in dose of (A) prostomial homogenate, (B) 8,11,14- and (C) 11,14,17-eicosatrienoic acid. Treatment doses are expressed as the number of prostomia g^{-1} body mass for prostomial homogenate and $\mu g\, g^{-1}$ body mass for the two fatty acids. The spawning response is expressed as the total number of eggs released per animal. The mean spawning response of each individual in the treatment group is shown and figures in parentheses show the number of individuals that had not released eggs at a given concentration after 24 h.

males, these data suggest that there is a greater variation in the number of gametes released during the female spawning response. During the dose–response experiment to 8,11,14-eicosatrienoic acid (Fig. 4B) one female released approximately 4000 oocytes, but these had not undergone GVBD and, since oocytes at this stage cannot be fertilised, this did not constitute a true spawning response, but was probably the result of leakage from the body cavity.

The experiments described above demonstrate clearly the specificity of 8,11,14-eicosatrienoic acid in the induction of spawning in males and indicate that there is a difference in the chemical nature of the endocrine signal between males and females. In addition to these observations, however, sex-specific behavioural modification was also noted in spawning animals.

Spawning in males was accompanied by intermittent muscular contractions of the body wall, which caused ejaculation through six pairs of ducts (nephromixia) associated with the nephridia in the trunk region. Powerful ejaculations usually resulted in simultaneous sperm release from all these gonopores and ejaculatory bursts were often separated by periods of quiescence that lasted for up to several minutes (Fig. 5A–F). During spawning, stimulated both by the injection of prostomial homogenate and of 8,11,14-eicosatrienoic acid, ejaculations began and spawning commenced about 50–60 min after injection and ejaculatory bursts often continued for more than 1 h or until the animal was spent. Animals that had been injected with either the fatty acid isomer 11,14,17-eicosatrienoic acid or TFSW, neither of which induced spawning, did not exhibit similar rhythmic muscular contractions.

Spawning in females differs in several respects from that observed in males: the time scale over which spawning occurred was different, with females not commencing spawning until 5 h following injection. Individuals in which spawning had been induced continued to spawn for up to 19 h. Spawning in females was a much less active process and was not accompanied by any noticeable muscular contractions.

Discussion

The experiments described here suggest that there is a true hormonal role for 8,11,14-eicosatrienoic acid in the induction of spawning in male *Arenicola marina*. In addition, the study also highlights several important aspects of the spawning mechanism in this species.

First, the observation that 8,11,14-eicosatrienoic acid is able to stimulate spawning in males, but not in females, indicates that there are sex-specific differences in the chemical signal that induces spawning in *A. marina*. This has been demonstrated previously and, in his studies into the nature of the maturation hormones of *A. marina*, Howie (1961*a*) noted that whilst the saponifiable lipids of whole-body homogenates are capable of inducing spawning in males, they did not do so in females. The saponifiable lipids contain the fatty acid fraction and these observations, therefore, concur with the results described here. Clearly, spawning in females can be induced by a maturation hormone present in the prostomium, but this is not 8,11,14-eicosatrienoic acid. However, little information exists on the chemical nature of the maturation hormone in females although the results of Howie suggest that it is not a lipid.

Second, the observation that the fatty acid 11,14,17-eicosatrienoic acid (an isomer of the active 8,11,14-eicosatrienoic acid) does not stimulate spawning in males indicates that the reception of the chemical signal is quite specific. Spawning induced by 8,11,14-eicosatrienoic acid may result from the fact that the 8,11,14-isomer can be metabolised by a cascade of enzymes in a similar way to arachidonic acid whereas the 11,14,17-isomer cannot (this difference being related to the differences in the positioning of the double bonds in the two isomers).

Finally, the study identifies subtle sex-specific differences with regard to the spawning behaviour in this species that had earlier been reported by Howie during his investigations into the nature of the maturation hormones (Howie, 1961*a*,*b*). Spawning in males takes

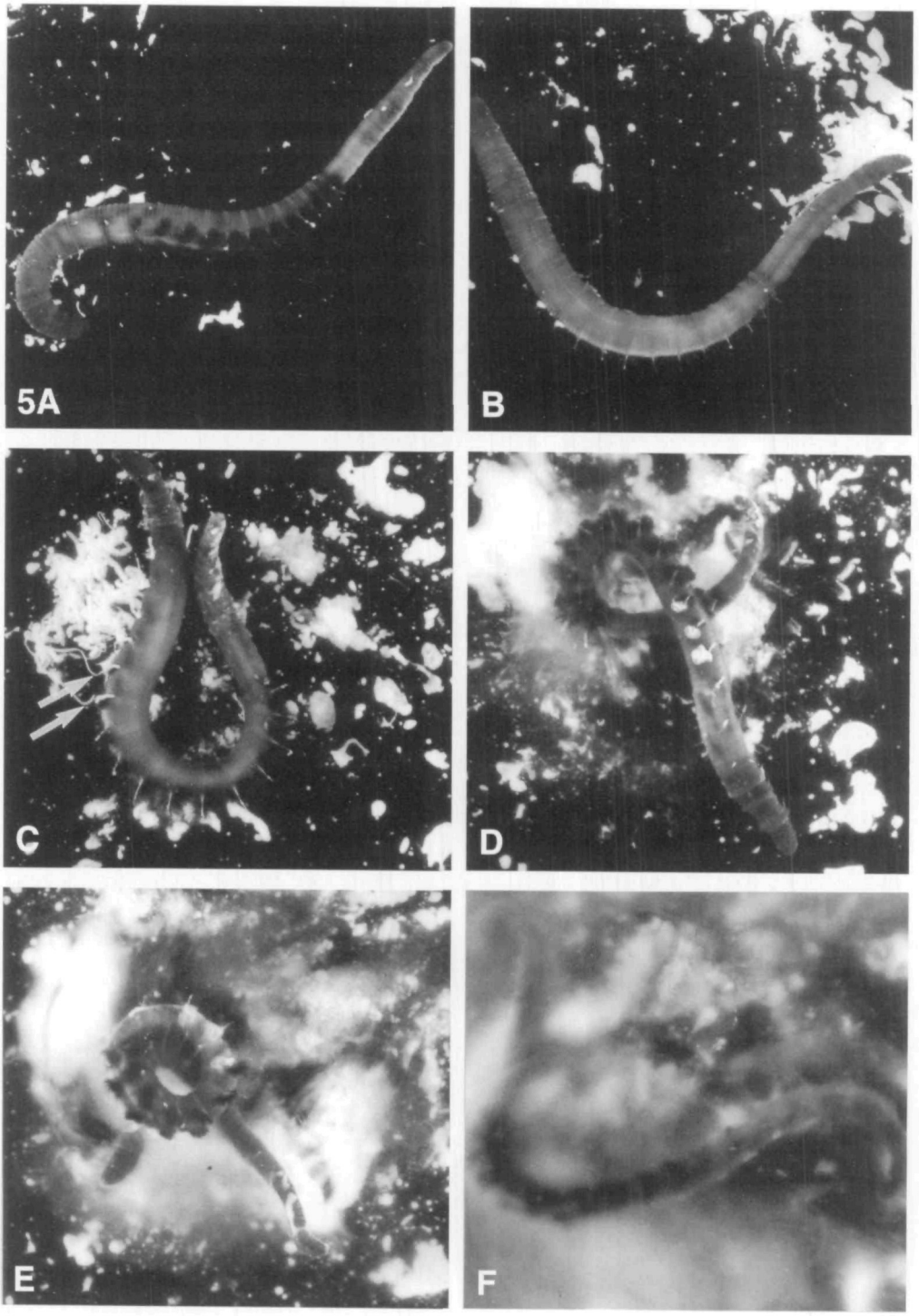

Fig. 5

Fig. 5. Spawning of male *Arenicola marina* following injection of 8,11,14-eicosatrienoic acid. The sequence of photographs (A–F) illustrates the progressive release of sperm from the coelom *via* the nephridia. (A) The animal following an initial burst of spawning activity 50 min after a coelomic injection of fatty acid (final concentration 13 $\mu g\,g^{-1}$ body mass). During the spawning process, periods of relative quiescence are followed by bursts of ejaculatory activity (B,C) during which sperm suspensions are clearly visible as they are released to the exterior (arrowed in C). (C) A powerful ejaculation and the simultaneous release of sperm through all six nephridia along one side of the animal. Spawning continues for a variable period, usually more than 1 h (D), at which point the worm begins to be obscured by the cloud of spermatozoa in the surrounding sea water (E,F).

place usually within 1 h and is characterised by strong muscular contractions resulting in the ejection of spermatozoa from the gonopores. In comparison, spawning in females involves no visible muscular contractions and usually commences several hours after injection. In addition, it is interesting to note that the spawning response of females is much more variable than that of males, in terms both of the number of animals that respond and also in the number of gametes released. The study clearly demonstrates that spawning in males (whether stimulated by SMF present in prostomial homogenates or by 8,11,14-eicosatrienoic acid) is an all or nothing response. The variability in the extent of gamete release that has been observed in other polychaetes (Olive *et al.* 1981*a*,*b*, 1985) does not seem to occur in this species.

In addition to observations on the maturation hormones, Howie (1961*a*) also proposed the existence of a separate muscle stimulant in tissue extracts. He noted that individual worms reacted violently to an injection of emulsified whole-body homogenates and suggested that the muscle stimulant and the spawning stimulant (SMF) were not the same because they were obtained from the aqueous fraction and the organic fraction of homogenates, respectively (Howie, 1961*a*,*b*). During the course of the investigations outlined in this paper, worms did not react violently to the injection of the fatty acid or crude homogenates of the prostomia (although they did undergo muscular contractions during the spawning process) and, therefore, this study also supports the conclusion that the activity of the muscle stimulant is not associated with the activity of SMF. The active ejection of gametes in *Arenicola marina* contradicts one of the generally accepted views of the endocrine control of spawning in this species, which had suggested that it is different from the mechanism described in some other polychaetes (see Franke and Pfannenstiel, 1984, for a review). Previously it was seen as a passive process in which gamete release resulted from the change in nature of the gametes occurring after maturation instead of relying upon muscular contractions to eject gametes from the body cavity. Howie (1961*b*,*c*) concluded from his early observations that, although the emission of gametes involves primarily a change in the state of the gametes, there may also be some active uptake (and shedding) of gametes (see also Howie, 1984). The results of the present investigation have led us to propose that SMF in addition to its action on spermatozoa should also be regarded as a true spawning hormone (see Bentley and Pacey, 1992, for a review).

Spawning hormones, both in polychaetes and in other marine invertebrates, have often been shown to have a multiplicity of roles during the spawning process (see Schroeder,

1984; Giese and Kanatani, 1987). As a spawning hormone, SMF may be no exception. It clearly acts upon the gametes directly and can bring about their maturation (Bentley, 1985; Bentley and Pacey, 1989; Bentley *et al.* 1990; Pacey and Bentley, 1992) but it also induces spawning, either as a direct response to SMF or possibly secondarily *via* factors released from other endocrine organs or from sperm. It may be significant that a behaviour pattern similar to that described here in spawning individuals can be induced in non-gravid individuals by an injection of 8,11,14-eicosatrienoic acid into the coelomic cavity (M. G. Bentley, unpublished observations). This suggests that the induction of spawning is not dependent on the presence of gametes. Furthermore, Howie (1961*c*) showed that injection of mature spermatozoa into the coelomic cavity resulted in their shedding from the nephromixia but this shedding was passive and was not accompanied by the muscular contractions typical of spawning animals. It would seem, therefore, that the presence of active spermatozoa in the coelom does not induce spawning behaviour.

Complex endocrine systems, in which hormones act at a number of target sites, or when one hormone stimulates or inhibits the secretion of another, were once thought to be absent in relatively simple invertebrates. However, their existence is now more widely realised as the endocrine systems of a number of species become better understood. For example, it has been shown that, in the induction of spawning in starfish, 1-methyl adenine has a number of roles *in vivo* (Shirai *et al.* 1986). Similarly, the interaction of two distinct hormonal substances from two endocrine glands has been demonstrated to be involved in the control of stolonisation in *Typosyllis prolifera* (see Franke and Pfannenstiel, 1984). The action of SMF *in vivo* is a subject that requires more detailed investigation.

In addition to the complexity of invertebrate endocrine systems, it is becoming clear that the nature of the endocrine signalling molecules are equally diverse. Fatty acids do not fit into the conventional categories of hormonal substances; by far the majority of hormones that have been positively identified are either peptides or steroids. However, the role of other classes of molecule as hormones is becoming more widely realised and has now been demonstrated in a number of marine invertebrates. For example, a trihydroxy metabolite of 5,8,11,14-eicosatetraenoic acid has recently been identified as the barnacle hatching factor (Holland *et al.* 1985) and prostaglandins have been implicated in the spawning of the molluscs *Haliotis rufescens* and *Mytilus californianus* (Morse *et al.* 1977; Fitt and Trench, 1981). Fatty acids have, in addition, been demonstrated to have signalling roles in a number of other systems: they are known to modulate adenylate cyclase (Baba *et al.* 1984) and protein kinase activity (Speizer *et al.* 1991), to activate guanylate cyclase (Wallach and Pastan, 1976) and potassium channels (Ordway *et al.* 1989) and to inhibit Na^+/K^+-ATPase activity (Chan *et al.* 1983); they also have other non-specific effects upon membranes (Baumgold, 1980). A review of the role of lipids as hormones or second messengers in biological systems has recently been published (Merrill and Liotta, 1991).

It has been noted previously (Bentley *et al.* 1990) that 8,11,14-eicosatrienoic acid is a particularly interesting molecule because its metabolism gives rise to a number of pharmacologically active products (including eicosanoids) whereas that of 11,14,17-eicosatrienoic acid does not (see Stanley-Samuelson, 1987, for a review). There remains,

therefore, the possibility that eicosanoids may be involved in the spawning mechanism and this would account for the failure of the 11,14,17-isomer to induce spawning. During the investigations on SMF-induced breakdown of sperm morulae *in vitro,* there was no evidence to suggest that any eicosanoid metabolism occurred (Bentley *et al.* 1990), but that does not rule out the possibility that eicosanoids are involved during spawning. Equally, however, there is no reason to suggest that 8,11,14-eicosatrienoic acid alone is not responsible.

Sufficient amounts of SMF from *A. marina* to enable gas chromatographic–mass spectrometric analysis to confirm the results of partial purification (see Bentley *et al.* 1990) have not yet been obtained. We have, however, outlined a number of *in vitro* investigations (Bentley *et al.* 1990) that strongly support the hypothesis that SMF is 8,11,14-eicosatrienoic acid. The experiments described in this paper represent a clear step forward in the understanding of endocrine-controlled spawning in *A. marina.* They demonstrate that there is a precise and sex-specific endocrine mechanism that induces spawning and this is particularly interesting in the light of the chemical nature of 8,11,14-eicosatrienoic acid and the obvious relevance of this to eicosanoid biochemistry. In addition, this is the first description of a hormone in the annelids for which both the chemical structure and the functional role are known.

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References

BABA, A., TATSUNO, T. AND IWATA, H. (1984). Modulation by unsaturated fatty acids of norepinehrine- and adenosine-induced formation of cyclic AMP in brain slices. *J. Neurochem.* **42**, 192–197.

BABCOCK, R. C., BULL, G. D., HARRISON, P. L., HEYWARD, A. J., OLIVER, J. K., WALLACE, C. C. AND WILLIS, B. L. (1986). Synchronous spawning of 105 scleractinian coral species on the Great Barrier Reef. *Mar. Biol.* **90**, 379–394.

BAUMGOLD, J. (1980). ^{3}H-Saxitoxin binding to nerve membranes: inhibition by phospholipase A_2 and by unsaturated fatty acids. *J. Neurochem.* **34**, 327–334.

BENTLEY, M. G. (1985). Sperm maturation response in *Arenicola marina* L.: An *in vitro* assay for sperm maturation factor and its partial purification. *Invertebr. Reprod. Dev.* **8**, 139–148.

BENTLEY, M. G., CLARK, S. AND PACEY, A. A. (1990). The role of arachidonic acid and eicosatrienoic acids in the activation of spermatozoa in *Arenicola marina* L. (Annelida: Polychaeta). *Biol. Bull. mar. biol. Lab., Woods Hole* **178**, 1–9.

BENTLEY, M. G. AND PACEY, A. A. (1989). A scanning electron microscopical study of sperm development and activation in *Arenicola marina* L. *(Annelida: Polychaeta). Invertebr. Reprod. Dev.* **15**, 211–219.

BENTLEY, M. G. AND PACEY, A. A. (1992). Physiological and environmental control of reproduction in polychaetes. In *Oceanography and Marine Biology: An Annual Review*, vol. **30** (ed. M. Barnes and R. N. Gibson), pp. 443–481. London: University College London Press.

CHAN, P. H., FISHMAN, R. A., CHEN, S. AND CHEW, S. (1983). Effects of temperature on arachidonic acid-induced cellular edema and membrane perturbation in rat brain cortical slices. *J. Neurochem.* **41**, 1550–1557.

DENNY, M. W. AND SHIBATA, M. F. (1989). Consequences of surf-zone turbulence for settlement and external fertilization. *Am. Nat.* **134**, 859–889.

DUNCAN, A. (1960). The spawning of *Arenicola marina* (L.) in the British Isles. *Proc. zool. Soc., Lond.* **134**, 137–156.

FITT, W. K. AND TRENCH, R. K. (1981). Spawning, development and acquisition of zooxanthellae by *Triidacna squamosa* (Mollusca, Bivalvia). *Biol. Bull. mar. biol. Lab., Woods Hole* **61**, 213–235.

FRANKE, H.-D. AND PFANNENSTEIL, H.-D. (1984). Endocrine control of reproduction. In *Polychaete Reproduction. Fortschritte der Zoologie*, Band 29 (ed. A. Fischer and H.-D. Pfannenstiel), pp. 53–72. Stuttgart, New York: Gustav Fischer-Verlag.

GIESE, A. C. AND KANATANI, K. (1987). Maturation and Spawning. In *Reproduction of Marine Invertebrates*, vol. IX. *General Aspects: Seeking Unity in Diversity* (ed. A. C. Giese, J. S. Pearse and V. B. Pearse), pp. 251–329. Palo Alto, California: Blackwell Scientific Publications and Pacific Grove, California: The Boxwood Press.

HOLLAND, D. L., EAST, J., GIBSON, K. H., CLAYTON, E. AND OLDFIELD, A. (1985). Identification of the hatching factor of the barnacle *Balanus balanoides* as the novel eicosanoid 10,11,12-trihydroxy 5,8,14,17-eicosatrienoic acid. *Prostaglandins* **29**, 819–830.

HOWIE, D. I. D. (1959). The spawning of *Arenicola marina* (L.). I. The breeding season. *J. mar. biol. Ass. U.K.* **38**, 395–406.

HOWIE, D. I. D. (1961*a*). The spawning of *Arenicola marina* (L.) II. Spawning under experimental conditions. *J. mar. biol. Ass. U.K.* **41**, 127–144.

HOWIE, D. I. D. (1961*b*). The spawning of *Arenicola marina* (L.) III. Maturation and shedding of the ova. *J. mar. biol. Ass. U.K.* **41**, 771–783.

HOWIE, D. I. D. (1961*c*). Spawning mechanism in the male lugworm. *Nature* **192**, 1100–1101.

HOWIE, D. I. D. (1962). Neurosecretion in relation to spawning and maturation of the gametes in *Arenicola marina* L. *Gen. comp. Endocr.* **2**, 32.

HOWIE, D. I. D. (1963). Experimental evidence for the humoral stimulation and ripening of the gametes and spawning in the polychaete *Arenicola marina* (L). *Gen. comp. Endocr.* **3**, 660–668.

HOWIE, D. I. D. (1966). Further data relating to the maturation hormone and its site of secretion in *Arenicola marina* Linnaeus. *Gen. comp. Endocr.* **6**, 347–361.

HOWIE, D. I. D. (1984). The reproductive biology of the lugworm, *Arenicola marina* L. In *Polychaete Reproduction. Fortschritte der Zoologie*, Band 29 (ed. A. Fischer and H.-D. Pfannenstiel), pp. 247–263. Stuttgart, New York: Gustav Fischer-Verlag.

MEIJER, L. (1979*a*). Hormonal control of oocyte maturation in *Arenicola marina* L. (Annelida, Polychaeta). I. Morphological study of oocyte maturation. *Dev. Growth Differ.* **21**, 303–314.

MEIJER, L. (1979*b*). Hormonal control of oocyte maturation in *Arenicola marina* L. (Annelida, polychaeta). II. Maturation and fertilization. *Dev. Growth Differ.* **21**, 315–329.

MEIJER, L. (1980). Hormonal control of oocyte maturation in *Arenicola marina* L.: (Annelida, Polychaeta). III. Involvement of Ca^{2+} and –SH groups in meiosis reinitiation. *Dev. Growth Differ.* **22**, 33–38.

MEIJER, L. AND DURCHON, M. (1977). Contrôle neurohormonal de la maturation ovocytaire chez *Arenicola marina* (Annélide Polychète). Etude *in vitro*. *C. R. hebd. Séanc. Acad. Sci. Paris* **285**, 377–380.

MERRILL, A. M. AND LIOTTA, D. C. (1991). Lipids as hormones and second messengers. *Curr. Opin. struct. Biol.* **1**, 516–521.

MORSE, D. E., DUNCAN, H., HOOKER, N. AND MORSE, A. (1977). H_2O_2 induces spawning in mollusks with activation of prostaglandin endoperoxide synthetase. *Science* **196**, 298–300.

NEWELL, G. E. (1948). A contribution to our knowledge of the life history of *Arenicola marina* (L.). *J. mar. biol. Ass. U.K.* **27**, 554–580.

OLIVE, P. J. W. (1983*a*). Annelida–Polychaeta. In *Reproductive Biology of Invertebrates*, vol. I, *Oogenesis, Oviposition and Oosorption* (ed. K. G. Adiyodi and R. G. Adiyodi), pp. 357–422. New York: John Wiley and Sons Ltd.

OLIVE, P. J. W. (1983*b*). Annelida–Polychaeta. In *Reproductive Biology of Invertebrates*, vol. II, *Spermatogenesis and Sperm Function* (ed. K. G. Adiyodi and R. G. Adiyodi), pp. 321–341. New York: John Wiley and Sons Ltd.

OLIVE, P. J. W., BENTLEY, M. G., WRIGHT, N. H. AND MORGAN, P. J. (1985). Reproductive energetics, endocrinology and population dynamics of *Nephtys caeca* and *N. hombergi*. *Mar. Biol.* **88**, 235–246.

OLIVE, P. J. W., GARWOOD, P. R. AND BENTLEY, M. G. (1981*a*). Oosorption and reproductive failure in Polychaeta in relation to their reproductive strategy. *Bull. Soc. Zool. France.* **106**, 263–268.

OLIVE, P. J. W., GARWOOD, P. R., BENTLEY, M. G. AND WRIGHT, N. H. (1981*b*). Reproductive success, relative abundance and population structure of two species of *Nephtys* in an estuarine beach. *Mar. Biol.* **63**, 189–196.

ORDWAY, R. W., WALSH, R. V. AND SINGER, J. J. (1989). Arachidonic acid and other fatty acids directly activate potassium channels in smooth muscle cells. *Science* **244**, 1176–1178.

PACEY, A. A. AND BENTLEY, M. G. (1992). An ultrastructural study of spermatogenesis and sperm morula breakdown in *Arenicola marina* (L.) (Annelida: Polychaeta). Helgoländer. Meersunters. **46**, 185–199.

PENNINGTON, J. S. (1985). The ecology of fertilization of echinoid eggs: the consequence of sperm dilution, adult aggregation and synchronous spawning. *Biol. Bull. mar. Lab., Woods Hole* **169**, 417–430.

PIRLOT, J. M. (1933). La ponte de l'*Arenicola marina* Linné à la Côte Belge. *Bull. mus. roy. Hist. nat. Belg.* **9**, 1–7.

SCHROEDER, P. C. (1984). A summary of the endocrine control of gametogenesis in invertebrates other than insects. In *Advances in Invertebrate Reproduction,* vol. 3. (ed. W. Engels), pp. 203–211. New York: Elsevier Science Publishers.

SHIRAI, H., BULET, P., KONDO, N., ISOBE, M., IMAI, K., GOTO, T. AND KUBOTA, I. (1986). Endocrine control of oocyte maturation and spawning in starfish. In *Advances in Invertebrate Reproduction*, vol. 4 (ed. M. Porchet, J.-C. Andries and A. Dhainaut), pp. 249–256. Amsterdam: Elsevier Science Publishers.

SPEIZER, L. A., WATSON, M. J. AND BRUNTON, L. L. (1991). Differential effects of omega-3 fish oils on protein kinase activities *in vitro*. *Am. J. Physiol.* **261**, E109–E115.

STANLEY-SAMUELSON, D. W. (1987). Physiological roles of prostaglandins and other eicosanoids in invertebrates. *Biol. Bull. mar. Lab., Woods Hole* **173**, 92–109.

WALLACH, D. AND PASTAN, I. (1976). Stimulation of guanylate cyclase of fibroblasts by free fatty acids. *J. biol. Chem.* **251**, 5802–5809.