INTRODUCTION: The beneficial role of diatoms in marine ecosystems is currently under review. Studies using planktonic copepods have shown that diatom feeding can inhibit larval development and hatching success [1]. Wound-activated diatom-derived short chain aldehydes (SCAs) and oxo-acids are embryotoxic and teratogenic. Here we investigate the effects of the bioactive SCA 2E,4E-decadienal (DD) on hatching and fertilization success in broadcast spawning benthic macroinvertebrates. We aim to highlight the potential of diatom-derived toxins to limit secondary productivity in soft sediment communities by interference with reproductive processes.

METHODOLOGY: Details of in vitro hatching and fertilization assays can be found in Caldwell et al. (2002) [2]. Sub-lethal exposure trials were conducted in sterile glass crystallizing dishes. Details are given elsewhere [3]. Sperm motility was monitored using a capillarity motility assay [4] whereby sperm front migration rates were recorded over time in tubes loaded with increasing DD concentrations. The effects of DD during oocyte maturation in Asterias rubens was conducted as follows: prophase oocytes were dissected from ovaries in Ca\(^{2+}\) free seawater and were stocked in test solutions. 1-methyladenine was added at a concentration of 1 mM to initiate maturation. After 1 hour gametes were assessed for necrotic effects.

Diatom extracts were analysed by GC-MS. 25 ml of dense culture was sonicated 3 times on ice for 30 s. The headspace was extracted using Solid Phase Microextraction for 30 min with a 100 µm poly(dimethylsiloxane) fibre (Supelco) at 40°C.

RESULTS AND DISCUSSION: Both hatching and fertilization success were inhibited in a dose-dependent manner, with hatching the more sensitive of the assays. Hatching was completely blocked at conc. greater than 0.25 µg ml\(^{-1}\) (well within theoretical environmental limits of SCAs). DD has since been shown to block oocyte fertilization and plasma membrane voltage gated Ca\(^{2+}\) currents at a conc. of 2.2 µg ml\(^{-1}\) [5]. DD retards development in a dose-dependent manner. Embryos abort at conc. of only 0.05 µg ml\(^{-1}\), following exposure sperm remained alive as evidenced by continued oscillation of the sperm head. However, flagellar beating was completely inhibited. This is the first evidence of sperm motility inhibition by an unsaturated SCA. It has been suggested that DD is interfering with Ca\(^{2+}\) signal transduction [4] which is vital for the maintenance and control of sperm swimming behaviour. It is evident that diatom-derived aldehydes contribute to fertilization failure by acting against both male and female gametes [4, 5].

REFERENCES:

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Fig. 1. Effect of DD on A) fertilization success and B) hatching success in Nereis virens. Mean ± SE, n = 4.

Fig. 2. Larval development of N. virens at different DD concentrations. A) 1 µg ml\(^{-1}\); B) 0.05 µg ml\(^{-1}\); C) 0.01 µg ml\(^{-1}\); D) 0 µg ml\(^{-1}\). DD retards development in a dose-dependent manner. Embryos abort at cleavage stages at conc. of 1 µg ml\(^{-1}\) (A). Sub-lethal exposure produces phenodeviants (B) or slows developmental rate (C compared with D).

Fig. 3. Effects of DD on invertebrate sperm motility.

DD was found to inhibit sperm motility in a time- and dose-dependent manner. Inhibitory effects were determined at a conc. of only 0.05 µg ml\(^{-1}\). Following exposure sperm remained alive as evidenced by continued oscillation of the sperm head. However, flagellar beating was completely inhibited. This is the first evidence of sperm motility inhibition by an unsaturated SCA. It has been suggested that DD is interfering with Ca\(^{2+}\) signal transduction [4] which is vital for the maintenance and control of sperm swimming behaviour. It is evident that diatom-derived aldehydes contribute to fertilization failure by acting against both male and female gametes [4, 5].

Fig. 4. DD induces oocyte necrosis during in vitro maturation.

At maturation, oocytes of Asterias rubens enter metaphase from prophase arrest. We have shown that exposure to DD during maturation triggers necrosis. Neither filtered seawater (FSW), the fatty acid EPA or the saturated aldehydes decanal or undecalanal had any effect. The response to DD was dose-dependent. Fig 4 shows oocytes exposed to 1.5 µg ml\(^{-1}\) DD (A = normal, B = necrotic).

Fig. 5. Identification of SCAs from Nitzschia commutata.

Two stereoisomers of DD were identified from N. commutata extracts together with some non-toxic saturated SCAs. Previously, the benthic diatom Phaeodactylum tricornutum was identified as a producer of oxo-acids [6]. We have shown that N. commutata also synthesises toxic compounds in response to wounding. Toxin production has been identified in a number of planktonic species [7, 8]. Planktonic and benthic diatoms represent an important food source for benthic invertebrates. As such the potential exists to limit secondary benthic productivity. We also suggest that the presence of diatom toxins is an important selective pressure in the evolution of reproductive seasonality in the benthos.