Aune Conservation Association

John Crawford Environmental Awards

Summary of project: **Endocrine Disruption in Avon clams**

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EXECUTIVE SUMMARY

Clams *Scrobicularia plana* collected from the Avon Estuary in the summers of 2008 and 2009 were subjected to histological analysis to look for indications of intersex/ovotestis (the simultaneous presence of both eggs and sperm in the male gonad). This phenomenon is potentially a result of endocrine disruption (ED) and is thought to indicate pollution-induced feminisation in males. Exact cause is not yet known, though agricultural origins seem plausible. Compared to previous years, levels of intersex in 2008 were extremely low, coinciding with attempts to reduce the influence of cattle in the catchment and a reduction in dairy holdings nationally. However, the trend of declining intersex levels appears to have been reversed, partially, in 2009.

BACKGROUND AND RELEVANCE

Endocrine Disruption (ED) in aquatic environments is a priority issue. Nevertheless, whilst ED has been shown to occur widely in UK rivers as a result of oestrogenic hormones and hormone mimics, the risk of ED in the marine environment, including estuaries, is not clear and has been largely overlooked in invertebrates. The question of whether anthropogenic forces influence the sexuality and reproduction of aquatic invertebrates is important, as invertebrates comprise 95% of all animal species and are central to ecosystem function. Marine bivalves are also a significant food resource (nb in the Avon) and their well-being is of concern, both economically and for human and marine food chains.

High on the list of priorities is the requirement for more information on the symptoms and likelihood of impact of ED on shellfish: this is particularly relevant to estuaries such as the Avon which house important commercial shellfisheries.

Our studies on clams *Scrobicularia plana* from the Avon Estuary have shown that disruption to 'normal' gonadal development of male *S. plana* (feminisation) has been a consistent feature since sampling was initiated in 2004[1]. In these initial surveys a significant proportion (~half) of males displayed ovotestis (an intersex condition typified by the simultaneous presence of both testicular and ovarian tissue in the male gonad- see figure 2).

In order to come to a better understanding of the long-term trends, sources and risks to the Avon from environmental oestrogens, and to demonstrate a practical model for screening and managing ED threats, we have undertaken additional sampling of Avon clams in 2008-9. This has been made possible, with help of the Aune Conservation Association, by a donation to the MBA through the John Crawford Environmental Awards Scheme.

Moreover, the study of intersex in Avon clams is relevant in a wider context, in terms of developing and validating monitoring schemes to measure the health of estuarine systems and assessing the possible repercussions for humans. Benefits of this research will thus include provision of a more balanced strategy for investigating causes of ED. In turn this will help separate the relative importance of natural vs. urban and agricultural sources, and focus remediation –if needed- where it would be most beneficial.

Advantages are therefore environmental (water quality, habitats, biodiversity), and also economic and social (water industries, shellfisheries, aquaculture, tourism).

Objectives

The aims of this project were:

- To survey the clam population at Milburn Orchard (MO) at various times over the annual gametogenic cycle throughout 2008 and 2009, and to examine their sexuality and gonadal condition.

- To compare levels of intersex frequency and severity in current samples with those in previous years (since 2004) in order to establish evidence of temporal change (improvement or deterioration) in 'endocrine disruption' and links with potential causes (e.g. agricultural practice; consented discharges from waste water treatment works - WwTW).

- To extend axial surveys of clams along the Avon Estuary to include their upstream limit of distribution, to try and establish spatial trends in intersex which might indicate sources of endocrine disrupting compounds.

- To compare data on levels of intersex in clams from the Avon Estuary with other populations in the South-West and further afield: How good, or bad, is the status of the Estuary with reference to pristine sites?

Methodology

The clam population at Milburn Orchard (SX683466, figure 1) was surveyed on four separate occasions throughout the gametogenic cycle, during the summers of 2008 and 2009. On each sampling occasion we have examined, histologically, the sex and reproductive condition of 30 'adult' clams, collected inter-tidally. This sample size is sufficiently representative of a larger number of clams[1] and avoids over-sampling. All collections were mixed-sex samples since there is no external distinction between male and female. Light microscopy was used in the determination of gonadal structure and appearance, oocyte sizes and sex ratios - including the incidence (frequency and severity) of intersex. We have developed a six-point scale to describe the severity of the intersex condition. Details of these methods are now published[1].

General condition of clams (body weight, shell length) was also recorded. All biometric data including oocyte size, gender, incidence of intersex and its severity, have been archived in spreadsheets. Statistics have been applied to establish the significance of any differences and trends.

In 2008, an additional sample of clams was taken - upstream of Milburn Orchard (at SX686469, figure 1 -midway between MO and the road bridge over the Avon). This represents the upstream limit of *S.plana* distribution in the estuary. The clams sampled here were also examined histologically, to establish if there were potentially important ED sources signified in an upstream direction. Methods used to determine reproductive condition were identical to those described above.

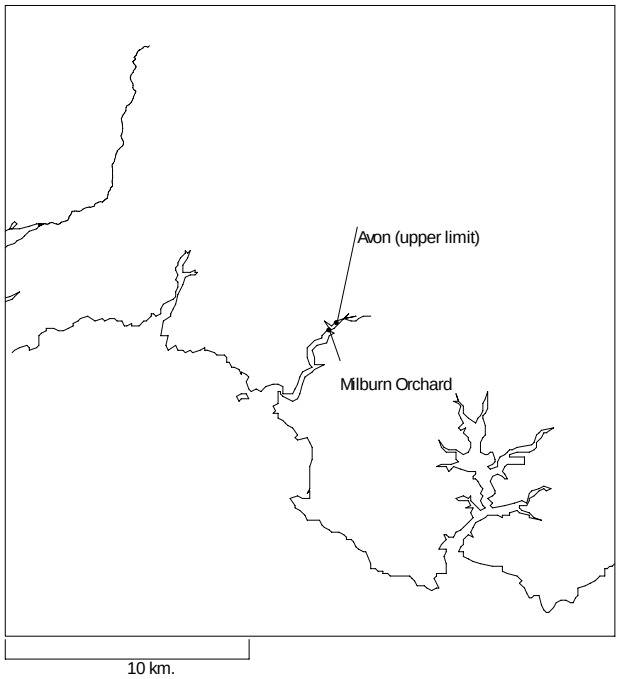

Figure 1 Map showing location of clam sampling sites, Avon Estuary- (1) Milburn Orchard and (2) upstream limit of distribution of Scrobicularia plana

Results and Discussion

Figure 2 shows the appearance of normal male (A) and female (B) gonad in the clam *Scrobicularia plana* together with an intersex male (C) in which five female oocytes are seen developing alongside male gametes.

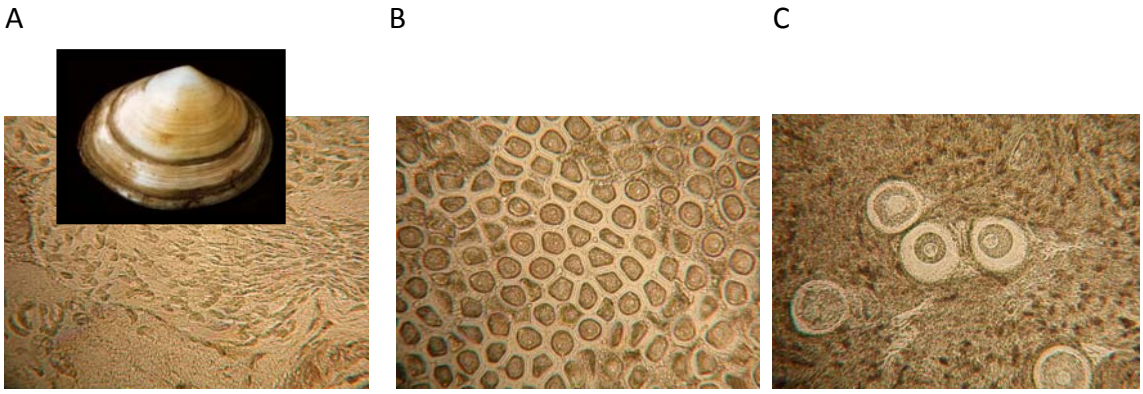

Figure 2. Scrobicularia plana. Gonad tissue of male (A), female (B) and intersex (C) clams

For the Milburn Orchard population, the results of intersex frequency and sex ratios in 2008 and 2009 are shown alongside earlier observations (Figure 3).

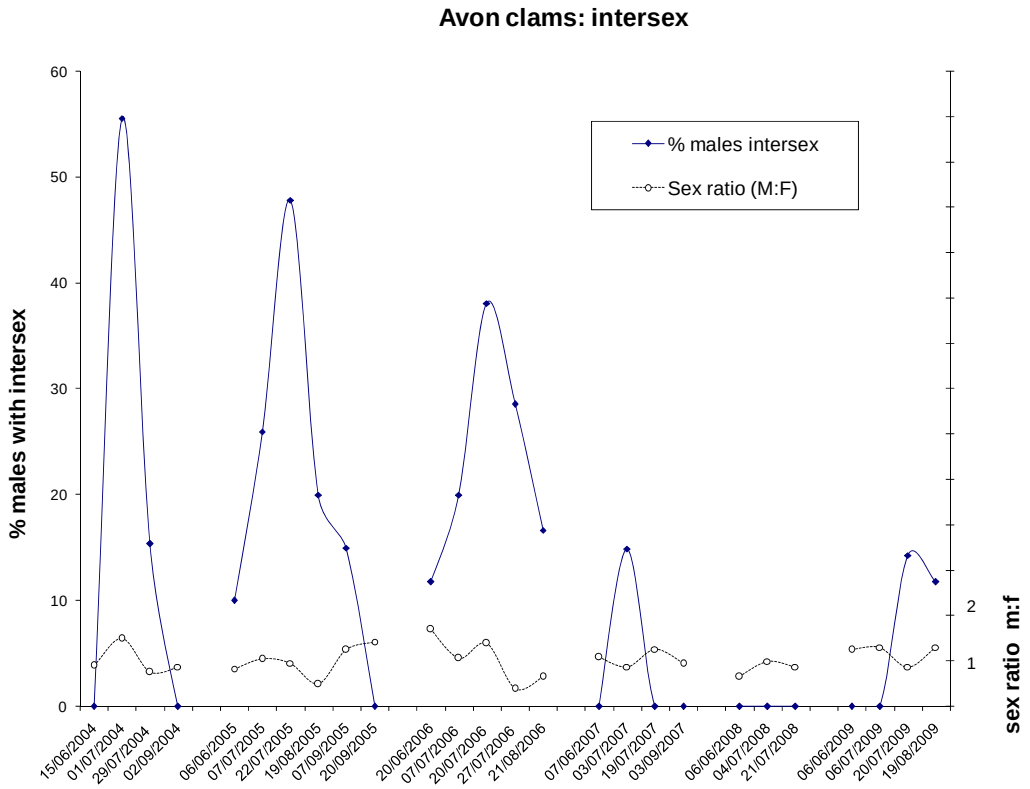

Figure 3 Scrobicularia plana *Milburn Orchard. Sex ratio of populations and percentage of males with intersex in years 2004-2009*

Sex ratios (males:females), though somewhat variable, have not differed significantly from the expected 1:1 since these records began (dotted lines in figure 3). Clearly, in the Avon, however, intersex is a recurring feature in a proportion of males (solid lines in figure 3). The seasonal peak in intersex frequency invariably coincides with sexual maturity in clams, in July.

The intensity of the peak has varied substantially over the last few years. Between 2004 and 2008 there was a marked and systematic decrease in the peak numbers (proportions) of males displaying intersex at Milburn Orchard - from over 50% in 2004 to zero in 2008 (figure 3). This trend appears to have been reversed in 2009 when 15% intersex was again recorded in males (figure 3).

At the more upstream site sampled in the Avon in 2008 - the upstream limit of *S.plana* distribution, 6% intersex was recorded (compared with 0% recorded at Milburn Orchard at that time). However more evidence is needed to establish if there is a definitive axial trend in the estuary.

The cause of intersex and reasons for temporal trends seen at MO are still far from certain, though the absence of industry and relatively low human population in the area led us to hypothesise, initially, that agricultural sources could be a contributory factor. In this context it is noteworthy, that bacteria found in oysters from the lower Avon in the past have also been attributable to cattle.

Consequently, it is intriguing to speculate as to the reasons for the temporal trends (nb decline) in intersex levels in clams since 2004. As far as we are aware, there have been a number of relevant changes in agricultural practice which might have reduced impacts to the estuary. These include significant efforts to install fencing to prevent cattle encroachment directly to the waterway (FWAG) and also a general underlying trend in the South Hams of removing cattle from fields in the winter and keeping them indoors. This is superimposed on a national and regional reduction (>10%) in the dairy herd over the last decade (figure 4). Such changes in farming practice would be anticipated to reduce direct inputs to the estuary from waste products originating from livestock. Cattle (particularly pregnant females), along with other domestic animals, are capable of excreting large amounts of steroid hormones.

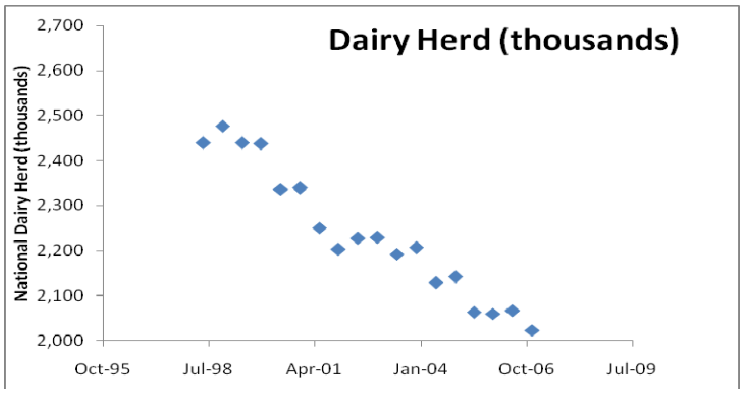

Figure 4. National statistics on dairy herd numbers 1998-2006 (Data source Defra)

Of course there are a number of other potential sources of ED compounds, including not only natural hormones but also various hormone mimics, which could be introduced into the environment from domestic and industrial sources. As far as we are aware there have been few changes to discharges from WwTW (in terms of consented volumes, materials or methods of treatment), which would lead us to suspect that they might be linked to the observed changes in intersex levels in clams. The most likely link for this phenomenon seems, therefore, to be with agricultural sources. However, further evidence of cause and effect, and extent of the condition is needed.

Conclusions and Recommendations

Overall, feminising effects of pollutants on clams *S.plana* in the Avon Estuary appear much less severe now than they were five years ago, when intersex was first observed. Indeed, intersex was virtually absent at Milburn Orchard in 2008, implying the approach of baseline conditions. (Our extended observations elsewhere in the UK reveal that the true background for clams is zero intersex -seen at pristine reference sites, for example in the Outer Hebrides). However, it may be premature to invoke a permanent trend towards better water quality in the Avon, since the improvements in intersex levels appear to have been reversed, to a small extent, over the last year (2009).

Continuing observations of clam populations in the Avon therefore seem warranted in future years, to help confirm trends- and the linkages to possible sources. In this respect it may be valuable to extend sampling to other sites along the length of the estuary (to help identify and distinguish point-sources, as opposed to diffuse sources). It would also be beneficial to compare intersex with other available water quality (including bacteriological analyses of water and oysters). Data on the size and structure of clam populations in the Avon should also be collected, and compared with reference sites to investigate whether shellfish recruitment is being compromised as a result of intersexed males.

Reference

[1]Langston, W.J., Burt, G.R., & Chesman, B.S. (2007). Feminisation of male clams Scrobicularia plana from estuaries in South West UK and its induction by endocrine-disrupting chemicals. Marine Ecology Progress Series, 333, 173-184.

Acknowledgement

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