biology letters
Biol. Lett.
doi:10.1098/rsbl.2006.0482
Published online

Intersex in the clam *Scrobicularia plana*: a sign of endocrine disruption in estuaries?

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The phenomenon of endocrine disruption is currently a source of growing concern. Feminization of male fish in UK rivers has been shown to occur extensively and has been linked with exposure to endocrine-disrupting compounds present in the environment. Much less is known of the extent and scale of endocrine disruption in estuarine and marine ecosystems, particularly in invertebrates.

We present evidence that intersex, in the form of ovotestis, is occurring in the common estuarine bivalve *Scrobicularia plana*, which is considered to be inherently gonochoristic. We report varying degrees in the severity of ovotestis in male *S. plana*, and have adopted and developed a grading method to assess the extent of this intersex condition.

These findings indicate that *S. plana* offers potential for widespread screening and investigation of endocrine disruption, helping to focus remediatory strategy.

Keywords: endocrine disruption; intersex; ovotestis; *Scrobicularia plana*; estuaries

1. INTRODUCTION

Various natural and man-made compounds in domestic and industrial effluents possess endocrine modulating activity. Endocrine disruption of male fish in rivers and estuaries, caused by (xeno)oestrogens, is well documented with manifestations including the induction of the female yolk precursor protein vitellogenin, and abnormal gonadal development (Purdom *et al.* 1994; Harries *et al.* 1996, 1997; Tyler *et al.* 1998). The appearance of oocytes in the testes (ovotestis—an intersex condition) is regarded as an endpoint of endocrine disruption in male fish and has been linked to the presence of endocrine disrupting compounds which are thought to mimic the actions of the female sex hormone 17-β-oestradiol, and thus disrupt natural hormonal functioning (Bateman *et al.* 2004).

In contrast to fish, unequivocal confirmation of environmentally relevant effects on the reproductive systems of aquatic invertebrates is scarce. Laboratory and field studies suggest that (xeno)oestrogens may have the potential to induce vitellogenin synthesis, or perturb normal developmental pathways, in males of certain invertebrate phyla, and evidence is beginning to emerge of such effects occurring in the field, where organisms may be exposed to oestrogenic compounds on a long-term basis (for review, see Langston *et al.* 2005). Given the current debate over the presence of endocrine disruption in the marine environment, the question of whether anthropogenic forces can influence the sexuality and reproduction of aquatic invertebrates is particularly important, as invertebrates comprise 95% of all animal species and are central to ecosystem function (Defur *et al.* 1999).

Molluscs appear to be a suitable phylum for study in this respect, as illustrated by tributyltin (TBT) research, which established the vulnerability of gastropod species to endocrine disruption in the form of imposex—the imposition of male characteristics in females (Bryan *et al.* 1986; Langston *et al.* 1990; Gibbs 1999). Infaunal bivalves are common molluscs in many estuaries: their sexual identity ranges from strict gonochorism to functional hermaphroditism (Delgado & Camacho 2002) and there is a suggestion that some bivalves may switch readily between states (Mackie 1984). UK populations of the common estuarine bivalve *Scrobicularia plana*, however, are considered to be inherently gonochoristic (Hughes 1971; Ruiz 1993) and their extensive distribution, sedentary lifestyle and deposit feeding habit make *S. plana* a potentially valuable monitoring species.

As a precursor to experimental studies to determine the susceptibility of infaunal bivalves to endocrine disrupting chemicals, and to establish the 'normal' condition and reproductive pattern over seasonal and annual cycles, we conducted a study of *S. plana*, on a predominantly rural south Devon estuary with little industry or urbanization. The current paper describes some important and unexpected observations on the sexuality of these clams.

2. MATERIAL AND METHODS

Batches of 30 *S. plana* were sampled, at roughly four-week intervals, from the Avon Estuary (Ordnance Survey Reference SX683467) between April 2004 and September 2005.

The clams were depurated in 50% seawater for 2 days (to eliminate sediment and grit), before the gonads were excised. A small sample of gonad from each clam was examined under a light microscope to determine gender and reproductive development stage, and to search for any anomalies.

The Chi-squared test was performed on data for gender to reveal any significant departure from unity in the male : female ratio in each batch of *S. plana* sampled.

3. RESULTS

Between the months of October and March, follicles of *S. plana* were empty except for a peripheral layer of primary germ cells. During this stage, it was impossible to determine the gender of individuals. Gametogenesis commenced in April and proceeded in the normal development series (as described by Hughes 1971). Gonad differentiation became evident during June, and by Early July most of the adults had developed gonadal cells, which in the majority of individuals, were either sperm or oocytes (figure 1*a*).

We discovered ovotestis in a small percentage of individuals during the months June to Early September (figure 1*b*). The incidence of ovotestis increased in frequency until spawning commenced (Late July) and receded as the gonads regressed (figure 2). The occurrence of ovotestis was not significantly correlated with clam size/age. Statistical analysis (Chi-squared test) revealed a significant departure from unity ($p<0.05$) in the ratio of (unaffected) males to females

Received 27 January 2006
Accepted 3 April 2006

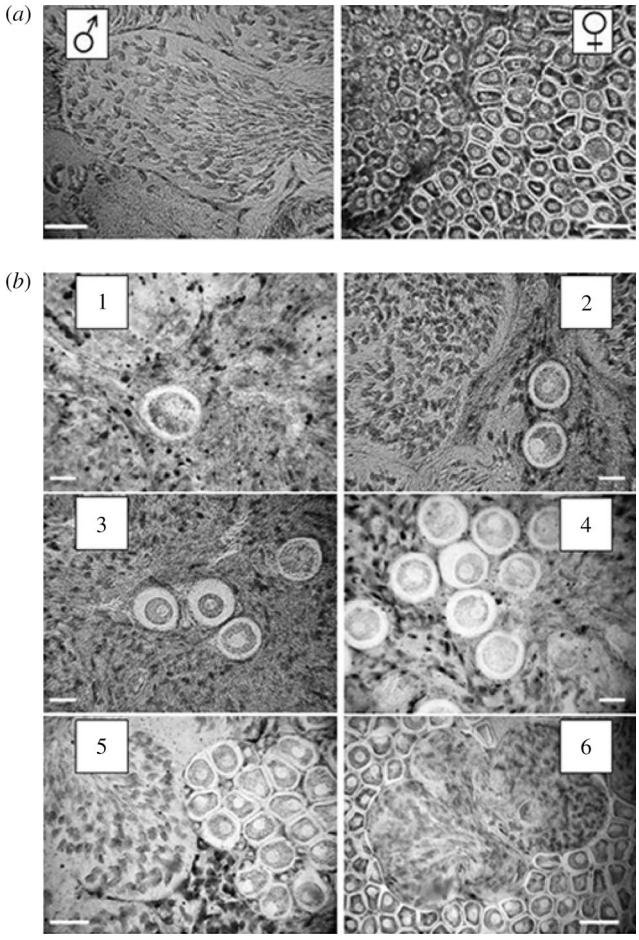

Figure 1. *Scrobicularia plana*. Light micrographs showing: (*a*) appearance of gonadal follicles containing sperm or oocytes in normal male and female, respectively. Scale bars, 120 μm, (*b*) oocyte distribution patterns in gonads of clams exhibiting increasing severity of ovotestis: examples illustrate the intersex index scores 1–6 (see table 1). Scale bars: 1–4, 80 μm; 5–6, 120 μm.

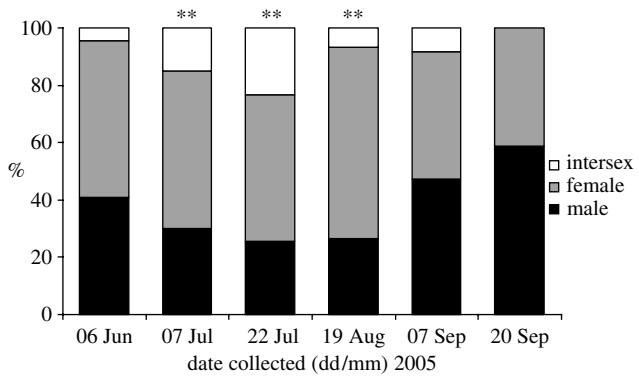

Figure 2. *Scrobicularia plana*. Proportion of male, female and intersex clams found (June–September 2005). ** indicates significant departure from unity in ratio of (unaffected) males to females ($p < 0.05$, Chi-square test).

in *S. plana* collected on 7 and 22 July, and 19 August. On these occasions, the proportion of females remained unaffected at approximately 50%, while the proportion of unaffected males dropped to 35, 33 and 28% (figure 2), implying that intersex is feminization of males.

Males exhibited varying degrees of ovotestis ranging from a single oocyte in otherwise normal testicular tissue, to large regions of mature ovarian tissue interspersed with abnormal testicular tissue (figure 1*b*). In order to describe and assess the severity of ovotestis found in *S. plana* for future comparisons, a ranking system for the evaluation of intersex in a fish species (Bateman *et al.* 2004) was adapted and developed into a scoring index to categorize the different degrees of intersex (table 1).

Table 1. Scoring index categorizing different degrees of ovotestis in the gonads of *S. plana*. (The score is based on the number and distribution pattern of oocytes within a field of view; adapted from Bateman *et al.* (2004).)

distribution type	descsription	score
focal	predominantly male gonad: single oocyte present in a field of view	1
diffuse	predominantly male gonad: greater than one oocyte present in a field of view—no physical association with neighbouring oocytes	2
cluster	predominantly male gonad: 1–4 closely associated oocytes within a field of view	3
zonal	predominantly male gonad: greater than five closely associated oocytes present in a field of view	4
enclosed	predominantly male gonad: follicle(s) containing oocytes	5
advanced	predominantly female gonad: follicle(s) containing bundles of sperm	6

4. DISCUSSION

Despite the fact that *S. plana* is considered to be gonochoristic (Hughes 1971; Ruiz 1993; Sola 1997; Rodríguez-Rúa *et al.* 2003) current results show that intersex, in the form of ovotestis, occurs in the Avon population, albeit at moderate levels (mean = 21% of males examined). It may be that this is the 'normal' condition for these clams; however, since the initial finding, we have sampled at least six sites in other estuaries, where none of the population displays intersex. It is possible, therefore, that (xeno)oestrogens may be responsible: ovotestis in fish has been conclusively linked to the presence of endocrine disrupting compounds (Purdom *et al.* 1994; Jobling *et al.* 1998; Bateman *et al.* 2004), and there is emerging evidence that sex steroids play a role in the regulation of reproduction in bivalve molluscs (for review of relevant literature, see Langston *et al.* 2005). Potential sources in the Avon include effluent from an upstream sewage treatment works (a potential source of steroid oestrogens and other oestrogenic chemicals) or perhaps, owing to the rural nature of the estuary, agriculture. Our investigations are only in the preliminary stages and further rigorous investigation is necessary before effects can be attributed with any degree of certainty.

These findings constitute one of the first indications that endocrine disruption may be a threat to natural invertebrate populations. At present, the causes and consequences of the ovotestis condition in *S. plana* are speculative. However, we do have early

indications from the examination of *S. plana* in 10 other southwest estuaries, that intersex in this species is widespread: 17 out of 23 populations sampled during the height of the reproductive cycle exhibited intersex, with up to 60% of males affected (B. S. Chesman & W. J. Langston 2005, unpublished data).

The incidence of intersex in *Scrobicularia* is sometimes in excess of that observed in wild fish populations whose fertility may be compromised by endocrine disruption (Jobling *et al.* 2002). Population consequences would not be expected occur in species with planktotrophic larvae, such as *S. plana*, unless some barrier to distribution were in operation. However, although *S. plana* is obviously capable of substantial lateral migration, this may occur only rarely in nature, making clam populations susceptible to localized effects (Casagranda & Boudouresque 2005). For example, there is evidence that contaminants, including TBT (a known androgenic compound used in antifouling paints), have impacted on populations of *S. plana* through reductions in larval recruitment (Langston *et al.* 1990; Ruiz *et al.* 1994). *Scrobicularia plana* may, therefore, be vulnerable to the added pressure of endocrine disruption, particularly near to sources of contamination. Bioaccumulation of endocrine disrupting compounds from sediment may exacerbate the risk to these and other deposit feeders. *Scrobicularia plana* clearly offers potential for widespread screening and investigation of endocrine disruption, once we can establish links between sexual development of these bivalves and anthropogenic causes. Further studies are underway to look for wider evidence of reproductive impact.

Thanks are due to Mr G. R. Burt and Ms M. Campbell for assistance with sampling and to Dr R. K. Pipe for advice on histology. The project is supported by funding from Defra under the Endocrine Disruption in the Aquatic Environment programme (EDAQ CTG0301).

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