IMPACTS OF ENVIRONMENTAL CHANGE ON REPRODUCTION AND DEVELOPMENT IN WILDLIFE

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RESEARCH POSTERS PRESENTED

Steroid exposure has temperature-dependent effects on reproduction in freshwater gastropods

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Within the last decade research has been published indicating that there are reproductive effects in freshwater gastropods exposed to steroids and steroid-mimicking compounds. Oehlmann *et al.* (2006) reported that exposure to Bisphenol A (an oestrogenic compound in vertebrates) increased the reproductive rate of the tropical prosobranch *Marisa cornuarietis*, but only when exposures were made at 20°C (a low temperature for this species that should induce a 'reproductive repose').

Following this, Clarke *et al.* (2009) studied the effects of treated sewage effluent (TSE) on the temperate pulmonate *Planorbarius corneus* in an outdoor mesocosm system. They reported that the exposed snails produced significantly more eggs at certain times of the year, most notably at the onset of autumn. However, as TSE is both warmer and more food rich than natural water bodies, it was determined that similar exposures be made to steroids alone, to establish whether this effect was steroid and / or temperature and resource driven.

Exposures of *P.corneus* to 17β-oestradiol (E2) were made in the mesocosm system, and a similar effect was observed at the onset of autumn to that of Clarke *et al*. (2009). In contrast, when the snails were reproducing maximally during the summer, no such effect was seen. This was also the case in the co-exposed temperate viviparous prosobranch species *Viviparus viviparus*. This suggests that not only is the observed effect steroid driven, but also that it occurs in snails with widely divergent reproductive strategies and only under certain (autumnal) conditions.

References:
Oehlmann, J., Schulte-Oehlmann, U., Bachmann, J., Oetken, M., Lutz, I., Kloas, W. & Ternes, T. (2006): Bisphenol A Induces Superfeminization in the Ramshorn Snail *Marisa cornuarietis* (Gastropoda: Prosobranchia) at Environmentally Relevant Concentrations. *Environ. Health Perspect*. **114:** 127–133.

Clarke, N., Routledge, E., Garner, A., Casey, D., Benstead, R., Walker, D., Watermann, B., Knass, B., Tomsen, A. & Jobling, S. (2009): Exposure to Treated Sewage Effluent Disrupts Reproduction and Development in the Seasonally Breeding Ramshorn Snail (Subclass: Pulmonata, *Planorbarius corneus*). *Environ. Sci. Technol*. **43**: 2092–2098.

Assessing the effects of population inbreeding and EDC exposure in the zebrafish

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Inbreeding may be exacerbated by endocrine disrupting chemicals (EDCs) with the potential to cause reproductive impairment or failure in individuals, resulting in a reduced effective genetic population size. Furthermore, since reproductive traits most closely related to fitness show most

inbreeding depression there is significant potential for compounding effects leading to steeper extinction trajectories in inbred populations. However, potential interactive effects of inbreeding and environmental chemicals have largely been ignored in environmental risk assessment. Whilst some advantage may be gained from selective inbreeding of laboratory strains to purge deleterious alleles to ensure uniformity of response in controls and to reduce the risk of false positives, they may not be representative or protective of populations in the wild.

In a zebrafish (*Danio rerio*) partial life-cycle study we have started to examine the effects of inbreeding and exposure to an aromatase inhibitor (clotrimazole). Two out of 19 inbred families displayed overt morphological deformities but these did not appear to be associated with increased mortality compared to 20 outbred families.

Sex ratio was skewed significantly towards males in both the high-dosed inbred and outbred treatments. This effect is consistent with the mode of action of clotrimazole and a reduction in growth during sexual maturation (between exposure day 26 and 48; i.e. 63–85 days post hatching), smaller size favouring the development of males.

Blood-plasma concentrations of 11-KT (a biomarker of male dominance) were significantly lower in high-dosed outbred males. In this group GSI was significantly higher, but the reason for this has not yet been ascertained.

Based on features of growth and sexual development, our data suggest that inbreds were not more susceptible to the effects of clotrimazole than outbreds. We are now undertaking competitive breeding studies and parentage analyses to assess the relative performance of inbred versus outbred fish that have been exposed to the low clotrimazole dose.

The effects of environmental warming on the reproduction of the three-spined stickleback *Gasterosteus aculeatus* (L.)

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The three-spined stickleback is native to the UK. During the breeding season, the male engages in parental care. His elaborate behaviour includes nest building and aeration of the eggs with a vigorous fanning motion of the fins. The aim of this research was to determine whether reproduction is less successful in warmer waters, either because of higher mortality of the adults or the young, because of reduced parental care, or because increased parental care may be too energetic to be sustainable. Temperatures used were relevant to expected global warming trends.

Wild sticklebacks were collected and placed in either a control or a heated-water group in the laboratory in 2004. Fish in the heated group were subjected to temperatures of either 2ºC above ambient (average 19ºC) or 6ºC above ambient (average 23ºC). All fish were observed directly or indirectly via video recordings across the breeding season. Breeding success was measured by mortality rate of adults, nest-building rate, incubation success, and level of parental care.

The experiments showed that warmed waters impair reproductive success in the three-spined stickleback. Warmer conditions are also likely to make reproduction more difficult in terms of energy expenditure. Populations of three-spined sticklebacks are unlikely to survive well if global warming increases the ambient water temperatures. A reduced or absent stickleback population will affect the health of local ecosystems because of the importance of fish to aquatic systems. Other concerns include the effects of global warming on fish species of economic importance.

Occurrence of reproductive anomalies in the human population around Hindustan Paper Corporation Limited (HPCL), Jagiroad, Assam, India

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Background and Aims:
The presence of various endocrine disruptors in the effluents of pulp and paper industries, and their subsequent effects in both animal and human development and reproduction, have raised concerns in recent times. They are believed to cause different reproductive disorders in males and females. The Hindustan Paper Corporation Limited (HPCL), Jagiroad, Assam, India, has been regarded as one of the leading paper manufacturers in Asia, and it discharges about 50,000-60,000 m^3 effluent water per day. To study the prevalence of reproductive anomalies in the human population, we conducted an extensive survey in the surrounding areas, covering a total population of 46,122 within a 5 km radius of the industry.

Methods:
A specifically designed standard questionnaire was prepared. Data were collected using a systematic and random-sampling technique by directly interviewing individuals. Participants were assured of confidentiality.

Results:
1923 male and 2769 female individuals were interviewed covering a total of 4692 persons, 10.17% of the total 46,122 population. The present survey revealed the occurrence of spermatorrhoea, hydrocele, gynaecomastia, hypospadia, cryptorchidism, and enlargement of prostate in the male population. In the case of male children cryptorchidism and hydrocele were found to be significant. In the female population, cases of stillbirth were reported. Intrauterine death rate and twin birth rate was found to be increased. Occurrence of cases of early puberty was also reported. The sex ratio was 108 male births per 100 female births. The survey also recorded menstrual-cycle irregularities amongst women of different age groups.

Sensitivity of sperm to toxicants and its implications for reproductive strategies in free-spawning marine invertebrates

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The majority of marine macro-invertebrate species reproduce by releasing their eggs and/or sperm into the water column so that fertilization takes place externally. These free-spawning strategists are therefore particularly susceptible to exposure to water-borne contaminants. Successful fertilization is a critical step in the life history of any species, but particularly so for free-spawning marine invertebrates where sperm concentration is often the limiting factor determining fertilization success, which is rarely 100% even under optimal environmental conditions. Here the vulnerability of sperm from the polychaete worm *Arenicola marina* and the bivalve mollusc *Mytilus edulis* to toxicity from a number of environmental pollutants is determined and related to impacts on population fertilization ecology. Exposure to the water accommodated fraction (WAF) of crude oil at concentrations equivalent to 3.8 μgL^{-1} polycyclic aromatic hydrocarbons (PAHs) was found to reduce sperm motility with the equivalent effect of increasing sperm dilution by a factor of 104. This results in significantly reduced sperm:egg collision rates, which negatively impact upon fertilization kinetics and fertilization success, therefore effectively enhancing population sperm limitation. In contrast, the genotoxic PAH benzo(a)pyrene caused significant damage to sperm DNA but did not impact upon sperm swimming behaviour or reduce fertilization success, but did lead to teratogenic effects in resulting embryos and larvae. The significance of these findings are discussed in relation to the selective pressures acting on spawning behaviour and larval competition in free-spawning marine invertebrates living in contaminated environments.

Reproductive physiology offers a mechanism for flexible responses to environmental change

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Global climate change is likely to lead to environmental stress, including shifts in food availability. Reproductive physiology can mediate between environmental signals, such as poor-quality food, and adaptive responses. Insects have the ability to resorb oocytes that are not oviposited. This 'oosorption' is proposed to be an adaptive mechanism to optimize fitness in hostile environments, recouping resources that might otherwise be lost and reinvesting them into future reproductive potential. I examined the reproductive physiology and life-history outcome to poor-quality food in populations of the milkweed bug, *Oncopeltus fasciatus*, that has adapted to live on sunflower seed. Females fed the poor-quality diet of pumpkin seeds had higher levels of ovarian apoptosis (oosorption), lower reproductive output, but no change in lifespan, as expected under the oosorption hypothesis. However, the schedule of reproduction was surprising given the 'wait to reproduce' assumption of oosorption; early fecundity was unaffected. These results set the scene for examining the role of phenotypic and genetic plasticity in reproductive physiology in moderating evolutionary responses to food stress in natural populations of *O. fasciatus* that have adapted to both food-abundant and food-limited environments. Some populations have responded by developing a 'migratory' genetic syndrome. Other populations are nonmigratory but may have genetic syndromes associated with the ability to survive in limited food environments, including oosorption. Further information on how variation among species or populations in their ability to adaptively respond will improve our forecasts for the impact of climate change on biodiversity.

Climate change, juvenile survival and population decline of the Alpine ibex *Capra ibex* population in Gran Paradiso National Park (North-Western Italian Alps)

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Recent studies suggest that temporal variability in demographic parameters of juvenile age classes is responsible for most of the variability in the growth rate of wild ungulate populations. Temporal modifications in these parameters due to exogenous factors, such as climate change, may therefore have a dramatic impact on the population dynamics of these species. Since 1993, when the Gran Paradiso Alpine ibex population reached its peak (4990 ibex counted), the GPNP Alpine ibex population declined by 47%. We show that adult survival and, to a lesser extent, fertility are determined by density dependence and winter snow cover. Despite milder winters since the mid-eighties, possibly due to climate change, while the survival probability of adults increased, survival of kids declined dramatically, passing from an average value of 58% (% of kids which reach the yearling stage in 1981–1990) to an average of 36% in the last 10 years. Furthermore, female productivity (kids/females) declined although not as strongly (43% in 1981–1990 vs 36% in 1999–2008). With a simple simulation model we show how the strong decline in kid survival can explain, to a great extent, the population crash recorded since 1993. On the other hand, the recorded decline in female productivity can not explain the negative population trend in GPNP. We discuss what factors could be responsible for this dramatic decline in kid survival focusing in particular on climate-driven changes in the availability of high-quality forage.

Male reproductive function in the Spinifex hopping mouse *Notomys alexis*: a species of native rodent from the arid environment of Australia

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The Spinifex hopping mouse, *Notomys alexis* is an Australo–Papuan old endemic rodent which is widely distributed in central Australia and shows adaptations for life in the arid environment. Adult males have extremely small testes (ca. 0.2% of body weight), low sperm-production rates, and a poorly developed pampiniform plexus. Are the developing male germ cells resistant to degeneration when animals are exposed to high temperatures? For this, adult male hopping mice (n=5) and laboratory mice (n=5) were exposed to either 37–38°C for 8 hrs per day for each of 3 days or to 23–24°C. Testes were removed 16 hrs after the last heat treatment, thin sections cut and nick labelled with TdT-mediated dUTP (TUNEL) for determining apoptosis. The results showed that in laboratory mice, but not hopping mice, heat exposure significantly reduced testes weights compared to the controls (laboratory mice: mean±s.e.m. 0.07±0.002 g vs 0.1±0.001 g. $P<0.05$; hopping mice: 0.01±0.004 g vs 0.03±0.002 g. $P>0.05$). Nevertheless in both species the heat-treated animals had an increased number of apoptotic spermatogonia and primary spermatocytes in the seminiferous tubules ($P<0.05$). Preliminary observations of hopping mouse testes also showed increased vacuolation in the seminiferous epithelium of heat-treated animals and degenerating seminiferous tubules in at least three animals with reduced numbers of spermatids. We conclude that in hopping mice germ-cell survival during spermatogenesis is reduced and adaptation of this species to the arid environment does not appear to have resulted in any marked reduction in sensitivity of male germ cells to degeneration when animals are exposed to high environmental temperatures.