Hormones and Behavior 55 (2009) 530–537

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Disruption of sexual selection in sand gobies (*Pomatoschistus minutus*) by 17α-ethinyl estradiol, an endocrine disruptor

Minna Saaristo [a,*], John A. Craft [b], Kari K. Lehtonen [c], Heikki Björk [d], Kai Lindström [e]

[a] Department of Bio- and Environmental Sciences, University of Helsinki, P.O. Box 65, FI-00014 Helsinki, Finland
[b] Biological and Biomedical Sciences, Glasgow Caledonian University, Cowcaddens Road, Glasgow G4 0BA, Scotland, UK
[c] Finnish Environment Institute (SYKE), Marine Centre, P.O. Box 2, FI-00561 Helsinki, Finland
[d] Finnish Institute for Verification of the Chemical Weapons Convention, P.O. Box 55, FI-00014 University of Helsinki, Finland
[e] Environmental and Marine Biology, Åbo Akademi University, FI-20500 Turku, Finland

ARTICLE INFO

Article history:
Received 5 December 2008
Revised 21 January 2009
Accepted 21 January 2009
Available online 30 January 2009

Keywords:
Endocrine disruptor
Sexual selection
Reproductive behavior
Sand goby
Pomatoschistus
Molecular biomarkers

ABSTRACT

In aquatic environments, endocrine disrupting chemicals (EDCs) that interfere with the reproductive physiology of males form a threat to the reproduction of populations. This is often manifested as decreased sexual performance or sterility among males. We show that exposure to EDCs can directly affect the mating system of a marine fish, the sand goby (*Pomatoschistus minutus*). We exposed males for 1 to 4 weeks to two different concentrations (5 ng L^{-1} and 24 ng L^{-1}) of 17α-ethinyl estradiol (EE2); a synthetic compound mimicking estrogen and a water control. The sand goby exhibits a polygynous mating system, in which male mating success is typically skewed towards the largest males, resulting in strong sexual selection for increased male size. Our experiment shows that when males have been exposed to EE2, male size has a smaller effect on mating success, resulting in weaker sexual selection on male size as compared to the control. There was an interaction between treatment and exposure time on the expression of vitellogenin and zona radiata protein mRNAs. Males exposed to high EE2 reached much higher expression levels than males exposed to low EE2. Of the somatic markers, the hepatosomatic index was lower in males exposed to high EE2 than in the low EE2 and control males. Our results suggest that exposure to EDCs can have effects on the mating system before physiological changes are observable. These effects can be of profound nature as they interfere with sexual selection, and may in the long run lead to the loss of traits maintained through sexual selection.

Introduction

Research on endocrine disrupting chemicals (EDCs) and their effects on wildlife at the molecular and physiological level has increased considerably during the last decades (Sumpter and Jobling, 1995; Desbrow et al., 1998; Kramer et al., 1998; Andersson et al., 2007). However, EDCs are also known to result in behavioral changes and it has recently been suggested that more research should focus on these effects (Clotfelter et al., 2004). With regard to marine and freshwater organisms, few studies have investigated the effects of EDCs on reproductive behavior with only a limited number of studies on fish (Bayley et al., 1999; Bjerselius et al., 2001; Wibe et al., 2002; Ros et al., 2004; Martinovic et al., 2007). Obviously, behavioral studies are more challenging to carry out and interpret than classical toxicity tests, but a single behavioral parameter is in many ways much more comprehensive than a physiological or biochemical parameter considering its ecological relevance (Jones and Reynolds, 1997). In addition, a change in the reproductive behavior of an organism is a good signal of very early-stage alterations in molecular traits (Smith and Logan, 1997). An alteration in reproductive behavior is believed to be of particular ecological relevance since this, in most cases, will result in direct effects on reproductive success (Wibe et al., 2002).

Reproductive behavior is usually hormonally regulated and disruptions in this regulation may lead to severe behavioral changes that disrupt intra- and intersexual communication. As this communication breaks down, one may expect that it changes the species' mating system, which can be defined as the distribution of mating success among individuals, and hence the operation of sexual selection (Reynolds, 1996). Eventually these disturbances may lead to a breakdown of sexual selection mechanisms (Andersson, 1994), which can have profound future consequences for populations, communities and the entire local ecosystem (Jones and Reynolds, 1997; Seehausen et al., 1997).

In the present study, we used the sand goby (*Pomatoschistus minutus*) as a model organism to test how EDCs affect the mating system of a marine fish species. The sand goby is a small (42–58 mm in length) marine fish with a one-year life cycle (Healey, 1971). It feeds on zooplankton and benthic invertebrates and occurs over a wide salinity and temperature range. The sand goby has a resource-defense

* Corresponding author.
E-mail address: Minna.Saaristo@helsinki.fi (M. Saaristo).

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doi:10.1016/j.yhbeh.2009.01.006

mating system and male parental care: the male builds a nest under a suitable substrate, attracts females by courtship and tends the eggs until they hatch. A male's nest usually contains eggs from several females (Jones et al., 2001) and females can spawn with several males over the season. Nest availability and size determines the distribution of male mating success and sexual selection in the sand goby (Forsgren et al., 1996; Lindström and Seppä, 1996). In addition to male–male competition (Lindström, 1988, 1992), female mate preference is an important process determining male mating success. Female sand gobies show mate preferences based on a number of male traits such as size, color and behavior (Forsgren, 1992; Lindström et al., 2006).

To study the effects of estrogenic compounds on the mating system of sand goby we chose the pharmaceutical 17α-ethinyl estradiol (EE2) as our model compound. EE2 is used in oral contraceptives and it is more persistent in the environment than natural steroids (Jürgens et al., 1999; Young et al., 2004). EE2 exhibits much lower aerobic biodegradation than E2: the half life of EE2 is 17 d, as opposed to 1.2 d for E2 in the River Thames in spring 2000 (Jürgens et al., 2002). It has been shown to induce vitellogenin (Vtg) production at concentrations as low as 0.1 ng L^{-1} (Purdom et al., 1994) and cause collapse of wild fathead minnow (*Pimephales promelas*) populations after 2-year chronic 5 ng L^{-1} exposure (Kidd et al., 2007). Prolonged exposure to EE2 (6 months at 6 ng L^{-1}) under laboratory conditions also resulted in significant reduction of reproductive success in sand goby (Robinson et al., 2003). EE2 has been detected in ecologically relevant concentrations (<5 ng to 15 ng L^{-1}) from sewage effluents, surface and river waters, and activated and digested sludge (Baronti et al., 2000; Johnson and Sumpter, 2001; Muller et al., 2008).

To ensure that EE2 exposure was having a physiological response on the fish, two molecular biomarkers were used in this study. Vitellogenin (Vtg) is an egg yolk precursor protein (Mommsen and Walsh, 1988) and the classical biomarker for estrogenic exposure in male fish. Because males express estrogen receptors in liver, exposed animals produce Vtg which can be conveniently detected in sand goby by the appearance of Vtg mRNA (Kirby et al., 2003; Robinson et al., 2003). Zona radiata protein (Zrp) is a protein which forms the inner core of the eggshell (Oppen-Berntsen et al., 1992) and like Vtg is produced in the liver of male fish exposed to estrogens and can also be detected at the mRNA level (Robinson et al., 2003). Vtg and Zrp have been widely used as sensitive biomarkers of exposure to EDCs in both monitoring and laboratory testing (Arukwe and Goksoyr, 1998; Christiansen et al., 2000; Arukwe et al., 2000; Kwak et al., 2001; Boon et al., 2002; Robinson et al., 2003), but it has been suggested that Zrp may have greater sensitivity in some species (Arukwe et al., 2000).

We exposed sand goby males to two concentrations of EE2 (intended concentrations of 25 ng and 50 ng L^{-1}, but when measured from the seawater in the exposure tanks 5 and 24 ng L^{-1}) and then allowed four males at a time to compete for matings in experimental pools. The aims of this study were (1) to test if exposure to EE2 affects the mating system of the sand goby, and (2) to compare two ways of detecting EE2 exposure: molecular biomarkers (Vtg and Zrp) and behavioral studies. We expected EE2 exposure to change the mating system of the sand goby, because in another study (Saaristo et al. in preparation) we found that exposed males were less competitive and less preferred by females than control males.

Material and methods

Study site and exposure setup

The study was carried out at the Tvärminne Zoological Station, southern Finland, during May–July 2006, which corresponds to the main breeding season of sand gobies in the Northern Baltic. The fishes used in the experiments were caught at a nearby natural breeding site using a hand trawl, brought back to the lab and sorted into males and females. Only males were exposed to EE2. Before the males used in the experiments were introduced into the exposure tanks they were individually marked using injected elastomeric colors (Northwest Inc). Before marking they were anaesthetized in benzocaine. The males were then randomly divided among 6 different exposure tanks (80×80×40 cm) and two tanks were assigned to each of three different exposure treatments: 1) high concentration of EE2 (actual 24, intended 50 ng L^{-1}), 2) low concentration EE2 (actual 5, intended 25 ng L^{-1}) and 3) water control (no exposure to EE2). All tanks were provided with a flow-through of fresh seawater. The water to the exposure tanks was led through a mixing tank into which EE2 was pumped from stock solution using peristaltic pumps (Watson Marlow). From there the water was channeled into the exposure tanks using silicon tubing. The flow of water was kept similar for all tanks using flow meters (Kyrömäki) equipped with adjustable valves. The males used in the experiment were exposed for 1 to 4 weeks before introducing them to the mating system experiment. The first subset of fish was taken after 8 days of exposure and the following subsets every fourth day until the day 31. During the exposure the fishes were fed *ad libitum* with live *Mysis* spp. and frozen chironomid larvae.

Mating system experiment

The design follows a previous study (Lindström and Seppä, 1996) in its general layout. The experiment was carried out in clean seawater in order to prevent any EE2 effects on females. The experimental pools (80×80×40 cm) were equipped with a continuous flow-through of seawater. Each pool was provided with four clay flowerpots (10 cm in diameter) as nest sites. The inside of the each flowerpot was lined with a transparent film cut to fit the nest dimensions. Female sand gobies attach their eggs to the inner, upper surface of the nest in a single layer when spawning. The film can then be removed and photographed in order to count the number of eggs. After the nest sites were added to each pool, four randomly selected males were introduced into each and they were allowed to occupy and build nests

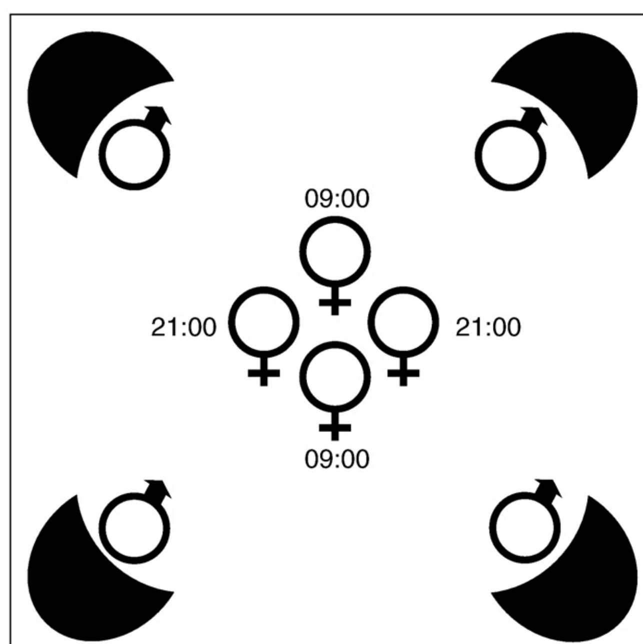

Fig. 1. Design for mating system experiment. Each pool had four nest sites (similar in size), and each nest had a transparent film lining inside. Four males of different size, but from same treatment, were placed to the pool in the evening. After 24 h of nest building a sexually mature female was added every 12 h until 4 females had been introduced.