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Potential Effects of Endocrine Disrupting Compounds on Bivalve Populations in Chesapeake Bay:
A Review of Current Knowledge and Assessment of Research Needs

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Summary

Numerous compounds in the environment interfere with normal endocrine function in humans and other animals. These compounds, which include heavy metals, a wide variety of anthropogenic organic compounds, steroids and steroid-mimicking compounds, are collectively termed endocrine disrupting compounds (EDCs). Over the past 20 years, research on the impacts of EDC exposure has identified a range of effects on growth, development, and reproduction in humans and wildlife.

 Most studies of EDC effects on wildlife have focused on vertebrates. Findings of reduced reproductive success in amphibians, reptiles, birds, and mammals resulting from environmental exposure to EDCs have generated considerable alarm (e.g., Colborn et al. 1996). Though less attention has been focused on invertebrates, it is clear that a wide variety of compounds introduced into the environment by humans can alter normal endocrine function in numerous taxa, affecting growth, development, and reproduction. Most widely noted in this regard has been impairment of the molt cycle in crustaceans due to insecticide exposure. Among mollusks, induction of imposex and intersex in gastropods exposed to tributyltin has been widely studied. Fewer studies have addressed effects of EDC exposure on bivalve mollusks, but there is evidence from several species that a range of compounds can affect sex determination, gonadal and gamete development, egg and sperm viability and function, and larval development. Intergenerational effects, in which exposure of one generation to EDCs reduces the survival of larvae in the next generation, have also been observed.

Viewed in the context of conservation and restoration of bivalve populations already at historic low levels and facing multiple stressors (e.g., Chesapeake Bay oyster populations), environmental pollutants which reduce the reproductive success of individuals can have very pronounced effects on population dynamics. For instance, on oyster reefs where recruitment is intermittent or provided primarily by stocking with hatchery-produced juveniles, exposure to compounds which result in early maturation as females and/or reduce sperm function can lead to reduced fertilization success in the population. Unfortunately, there are large gaps in our knowledge about inputs and fates of EDCs in Chesapeake Bay, and about the impacts these pollutants have on bivalves at the individual, population, and ecosystem levels.

We identify several areas in which critical information is needed to effectively evaluate the magnitude of EDC impacts on bivalves in Chesapeake Bay. Specifically, we recommend seven priority research and development needs: (1) development of an integrated database and communications structure, (2) identification of the major effects and modes of action by EDCs in native bivalves, (3) linkage of effects on individuals at specific stages in the life cycle to population and ecosystem models to evaluate larger-scale impacts of exposure, (4) identification in bivalves of biomarkers indicative of field exposure to EDCs, (5) monitoring of potential EDC source areas, (6) *in situ* field exposure studies, and (7) evaluation of EDC effects on bivalves exposed to multiple stresses characteristic of many Bay habitats.

Introduction

A wide variety of compounds have been shown to interfere with hormonal processes that regulate reproduction, development, and homeostasis in numerous animal species. These endocrine disrupting compounds (EDCs) impact survival, growth, and reproduction (e.g., Colburn et al. 1993, Ankley et al. 1998; Kendall et al. 1998, deFur et al. 1999). Though some of the most compelling cases involve invertebrates, much of the work to date has been conducted on vertebrates, especially oviparous vertebrates affected by estrogenic chemicals. Several factors explain the greater emphasis on effects of EDCs on vertebrates. First, there is the interest in the effects on human health. Second, vertebrates are often larger and more visible components of ecosystems. Finally, the fundamental biology of vertebrate endocrine function is better known and explored than that of invertebrates generally, and bivalve mollusks specifically (see deFur et al. 1999). One of the best illustrations of this vertebrate emphasis is the complete lack of a current textbook on invertebrate endocrinology.

Research on vertebrates has involved both laboratory and field investigations concerning the effects of anthropogenic chemicals on endocrine systems (Kendall et al. 1998, DiGiulio and Tillet 1999, National Research Council (NRC) 1999). Some of the better known examples include fish populations in the Thames River, England, alligator populations in Lake Apopka, FL, and mink and bird populations in the Great Lakes (reviewed in DiGuilio and Tillet 1999).

Guillette and colleagues at the University of Florida (Kendall et al. 1998; NRC 1999; Sparling et al. 2000) investigated the impact of chemical contamination in Lake Apopka on the alligator population. Using a combination of laboratory and field investigations, they found that male alligators in Lake Apopka had underdeveloped reproductive organs and systems and altered hormone levels, limiting the reproductive capacity of the population. Chemical analysis of alligator blood, tissues, and eggs revealed the presence of high levels of DDE, a known endocrine disrupting compound that blocks the action of testosterone. The investigators suggested that exposure to DDE resulted in feminization of male alligators, limiting reproduction and population stability.

Research on Thames River fish by Sumpter (NRC 1999) demonstrated that male fish were developing female secondary sex characteristics. Male fish were also producing vitellogenin, an

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egg yolk precursor protein normally found only in female fish. Estrogenic compounds in the effluent of several sewage treatment plants stimulated estrogenic systems, principally liver cells, to produce egg protein. This line of investigation has been pursued in other river systems, revealing that the phenomenon is widespread and that the hormonally active chemicals causing sexual disruption are found in surface waters throughout the United States (Kolpin et al. 2002).

Over the past decade, a growing body of research has explored the impacts of EDCs on invertebrates (deFur et al. 1999). Much of this work has focused on crustaceans, particularly on the effects of insecticides on ecdysis (e.g., Walker et al. 2005). However, some studies have also reported more direct effects on sexual differentiation and reproductive success (e.g., intersex harpacticoid copepods: Moore and Stevenson 1991, ovarian development in crayfish: Sarojini et al. 1995, embryonic development in mole crabs: Gunamalai et al. 2004).

Among mollusks, the most widely studied effects of EDCs have been those of tributyltin (TBT) causing imposex and intersex in gastropods. Effects were first noted in the neogastropod *Nucella lapillus*, where females were observed to develop a penis (Blaber 1970). Subsequent studies (reviewed in Matthiessen and Gibbs 1998) revealed that this effect was observed following exposure to TBT as low as < 1 ng L^{-1}. To date, over 100 species of prosobranch gastropods have been reported to exhibit imposex (Oehlmann et al. 1991, c.f. Matthiessen and Gibbs 1998). Studies by Gibbs and colleagues (Gibbs et al. 1990, 1991; Gibbs 1996) have shown that exposure to TBT causes oviduct abnormalities and spermatogenesis in females of other neogastropod species. Although there are several competing hypotheses for this effect, including altered biotransformation of testosterone and alterated neuropeptide signaling, no conclusive mechanism has been agreed upon (Spooner at al. 1991, Oberdorster et al. 2005, Horiguchi 2006).

Less attention has focused on the effects of EDCs on bivalve mollusks. Thain and Waldock (1986) reported that exposure to TBT reduced the proportion of a population which developed as males in European flat oysters, *Ostrea edulis,* and reduced larval production. However, no such effects were observed for the Eastern oyster, *Crassostrea virginica* (Roberts et al. 1987). Exposure to estradiol can cause sex reversal from males to females in the Pacific oyster, *C.*

gigas, (Mori 1969), though the opposite effect has been observed in *Placopecten magellanicus* (Wang and Croll 2004). Nonylphenol, a common component in personal care products, affects larval development (Nice et al. 2000), sex ratio and gamete viability (Nice et al. 2003), and sperm motility (Nice 2005) in the Pacific oyster, *C. gigas.*

It is obvious that an environmental pollutant which affects sex determination, sperm motility, and gamete viability has the potential to cause significant population level impacts, particularly when a pollutant is widespread and persistent. These impacts may be especially acute in sessile, free-spawning marine and estuarine bivalves, where fertilization success is highly dependent upon gamete encounter rates in the water column and thus depends upon aggregations of reproductively-capable male and females. Moreover, in many commercially-exploited and coastal bivalve species, local population densities have been reduced by overfishing and habitat degradation to a point where reproductive success is compromised (Arnold 2001, Joaquim et al. 2007). In such cases, even modest effects of EDCs may substantially reduce reproductive success. Protandric hermaphrodites, such as oysters in the genus *Crassostrea*, which generally reproduce as males at a younger age and progressively more as females at older ages, may be at even greater risk of population-level affects associated with low adult densities and EDC exposure. Understanding what, if any, effects EDCs may be having at the population level on commercially-exploited bivalve species has obvious implications for fisheries managers and restoration activities.

Here we review current information on the effects of EDCs on marine and estuarine bivalves which are currently the focus of restoration and conservation efforts, with an emphasis on commercially-exploited species. In doing so, we draw upon published research on these target species and, where appropriate, upon research on freshwater mollusks. We also draw upon presentations of on-going research and discussions from a symposium entitled *Potential Effects of Endocrine Disrupting Compounds on Bivalve Populations in Chesapeake Bay* held as part of the Chesapeake Research Consortium Conference, *Ecosystem Based Management (EBM): the Chesapeake Basin & Other Systems*, in Baltimore, MD on March 23-25, 2009. This symposium brought together shellfish biologists and scientists working on EDC impacts on both vertebrate and invertebrate species to discuss the current state of knowledge regarding impacts of

EDCs on aquatic organisms and the potential impacts of EDCs on bivalve populations in Chesapeake Bay. A list of the symposium participants and the titles of their presentations are given in the Appendix. We couple the findings from this review with examples of demographics from over-exploited and "restored" bivalve populations to identify circumstances under which exposure to EDCs may be of particular concern to fisheries management, conservation, and restoration of marine and estuarine bivalves. Finally, we make recommendations for a research agenda to address critical data needs.

Rationale

Several coincident factors raise the issue of EDC impacts in bivalve mollusk populations, such as oysters in Chesapeake Bay. Oyster populations are in dramatic decline and resist recovery, despite management efforts. While there are several environmental problems known to present challenges to bivalves and to oysters in particular (overharvest, parasitic diseases, habitat degradation), the full scope of causal factors is not understood. Furthermore, water quality monitoring by the U.S. Geological Service (USGS) and others reveals the presence of hundreds of chemicals in surface waters around the U.S., including the Chesapeake Bay watershed (Dorabawila and Gupta 2005, Hartwell and Hameedi 2007, http://toxics.usgs.gov/regional/emc/index.html [Accessed 2/20/2010]). Some of these chemicals are known estrogenic EDCs, and others are known reproductive and/or developmental toxicants. Finally, problems associated with EDCs have been reported in every group of animals investigated to date, including bivalve mollusks, an intuitive result since all animals use chemical signals to regulate biological processes. Recent research on bivalves, summarized below, supports the observation that numerous aspects of bivalve development and reproduction are susceptible to the effects of EDCs. Thus, it is timely and appropriate to examine data gaps and research needs for understanding EDC impacts on bivalves.

The eastern oyster, *C. virginica*, has experienced dramatic declines throughout most of its range along the U.S. Atlantic and Gulf of Mexico coasts during the 19th and 20th centuries due to overfishing, habitat destruction, and disease. Formerly productive populations in the Hudson-Raritan Estuary and Chesapeake Bay have been reduced to < 1% of their former abundances (Rothschild et al. 1994; Kirby 2004). In response to these declines considerable attention has been focused within the past 25 years on conserving and restoring *C. virginica* populations in

many areas, especially along the mid-Atlantic coast. These efforts, which have included the establishment and protection of sanctuary (spawning) reefs and the out-planting of hatchery-produced oysters, have not been clearly demonstrated to result in enhanced recruitment of oysters. The possibility that low recruitment of oysters in many areas of Chesapeake Bay, despite extensive restoration efforts, may be partly the result of reduced reproductive success associated with EDC exposure provided the genesis of this effort to (1) review the current state of our knowledge about EDC effects in bivalve mollusks, (2) assess specifically what is known of potential reproductive impacts of these compounds on oysters, (3) review on-going research on EDCs in the Bay watershed, and (4) identify knowledge gaps and recommend research priorities.

Potential Modes of Action and Effects of EDCs on Bivalves

The bivalve endocrine system does not appear to be as complex as other systems, but a number of endogenous and exogenous chemicals regulate or affect growth, reproduction, and physiology (Krajniak 2000, Cheek et al. 1998). The role that steroid hormones (e.g., estrogens, testosterone) play during reproduction and development in bivalves is poorly understood, but likely differs significantly from that observed in vertebrates (deFur et al. 1999). The lack of an established role for steroid hormones, however, does not indicate an absence of effects from estrogenic compounds as indicated in studies described below.

A number of specific steps in the reproductive process in bivalves may be susceptible to EDCs. Several recent research efforts, notably those of Nice and colleagues (Nice et al. 2000, 2001, 2003, Nice 2005) focus on bivalves, providing important confirmation that EDCs alter bivalve physiology. These effects include, but are not limited to the list that follows.

Sex Determination – Early investigations by Mori and colleagues (Mori 1969, Mori et al. 1972) revealed that injections of estradiol-17β induced sex change from male to female in *C. gigas*. In contrast, Wang and Croll (2006) showed that estradiol injections had the opposite effect on the scallop *Placopecten magellanicus*. Though these studies were directed at investigating normal endocrine system function, they suggest that environmental exposure to estradiol, and potentially other estrogen-mimicking compounds, might have similar effects. Other studies have looked

specifically at environmental exposure to EDCs on sex determination in bivalves. Nice et al. (2003) found that laboratory exposure of *C. gigas* larvae, 7 to 8 days post-fertilization, to seawater with concentrations of nonylphenol at 1 and 100 µg L^{-1} resulted in sex ratios 10 months post-fertilization that were skewed toward females relative to controls. Exposure of adult *O. edulis* to TBT resulted in all animals either developing as males or remaining undifferentiated (Thain and Waldock 1986). Soft shelled clams, *Mya arenaria*, from field sites exposed to TBT have also been shown to have sex ratios skewed towards males relative to ratios in control site populations (Gagné et al. 2003). This latter finding appears to be the result of TBT inhibition of aromates which convert testosterone to estradiol. Thus, the general pattern which has emerged from these few studies is one in which environmental exposure to estrogens or estrogen-mimicking compounds can result in feminization of bivalves, while exposure to compounds which reduce the production of estrogen can result in masculinization.

Gametogenesis and gonadal development – Several studies have demonstrated that estrogens play a central role in the natural gametogenic cycle in oysters, scallops, and clams (Mori 1969, Matsumoto et al. 1997, Gauthier-Clerc et al. 2006). In the soft clam, Gauthier-Clerc et al. (2006) suggest that estradiol-17β and testosterone act as endogenous regulators of gametogenesis, and other studies suggest similar, though species-specific, roles in oysters (Mori 1969) and scallops (Wang and Croll 2006). Environmental exposure to anti-estrogenic compounds has been associated with disruption or reduction in gametogenesis in *M. arenaria* (Gauthier-Clerc et al. 2002, Lindsay et al. 2009) and in the freshwater bivalve *Corbicula fluminea* (Law et al. 2009).

Vitellogenesis - Yolk formation, or vitellogensis, is part of the gametogenic process which appears to be controlled by estrogen in bivalves (Li et al. 1998, Blaise et al. 1999, Gauthier-Clerc et al. 2002). The presence of vitellogenin and vitellogenin-like proteins in male fish has been widely used as an indicator of environmental exposure of estrogen and estrogen-mimicking compounds (Sumpter and Jobling 1995). Blaise et al. (1999) have shown that estrogen (estradiol-17β) and estrogen-mimicking compounds (nonylphenol and pentachlorophenol) induce vitellogenin-like proteins in *M. arenaria* and Li et al. (1998) have shown similar results following exposure of *C. gigas* to estradiol-17β. These studies suggest that altered expression of

vitellogenin–like proteins may represent a useful biomarker of exposure to estrogenic and anti-estrogenic compounds in bivalves (Hellou et al. 2003).

Sperm motility – Most research to date on the effects of EDC exposure on gametes in mollusks have focused on oocytes and egg production, with little attention to effects on sperm. Au et al. (2000) reported that exposure to cadmium and phenol reduced sperm motility in the green-lipped mussel *Perna viridis*. More recently, Nice (2005) has demonstrated that a brief exposure of juvenile oysters to nonylphenol resulted in reduced sperm motility in adults. In these experiments, 3 month-old *C. gigas* were exposed for 72 h to nonylphenol at concentrations of 1 or 100 µg L^{-1}. At sexual maturity four months later, only 30% and 12.5% of these oysters, respectively, were found to have motile sperm compared 100% in control treatments. Since non-motile sperm are presumed to be non-viable with respect to their potential to fertilize eggs, such a dramatic decrease in motility following a short-term exposure suggests that EDCs have the potential to significantly decrease reproductive success in oysters.

Larval growth, survival, and metamorphosis - In addition to altering sex ratio, gametogenesis and sperm motility, exposure to EDCs has been shown to affect the survival, growth and metamorphosis of oyster larvae (Nice et al. 2000, 2001, 2003). Exposure of embryos immediately after fertilization to concentrations of nonylphenol as low as 0.1 µg L^{-1} reduced the growth and survival of larvae (Nice et al. 2000) and exposure of larvae 7 to 8 days post-fertilization resulted in delayed settlement and metamorphosis (Nice et al. 2001). Most strikingly, trans-generational effects have been observed in which the offspring of oysters that were previously exposed to nonylphenol during their larval period have reduced larval survival (Nice et al. 2003).

Life-cycle context

The research conducted to date on the effects of EDCs on vertebrates and invertebrates alike indicate the importance of understanding the impacts of exposure in the context of the organism's entire life cycle (Fig. 1). For many marine benthic invertebrates with planktonic larval stages, this adds an additional layer of complexity in evaluating the impact of EDCs since adult and larval habitats differ substantially. A powerful approach towards addressing this need

is to combine laboratory studies which assess stage-specific exposure effects with stage-specific matrix population models to predict the effect of EDC exposure at the population level. An example of this approach was recently employed by Lindsay et al. (2009) who examined the effects of exposure of larval and juvenile stages of the soft shelled clam, *M. arenaria*, to several pesticides and used their results to parameterize a stage-specific matrix model which simulated population growth and age structure under each of several exposure scenarios. Their results reveal that the effects of pesticide exposure on soft clam populations vary with the stage of exposure and the specific pesticide.

Figure 1. Conceptualized invertebrate life-cycle, emphasizing the need to evaluate EDC effect in a whole life cycle and trans-generational context (source: after deFur et al. 1999).
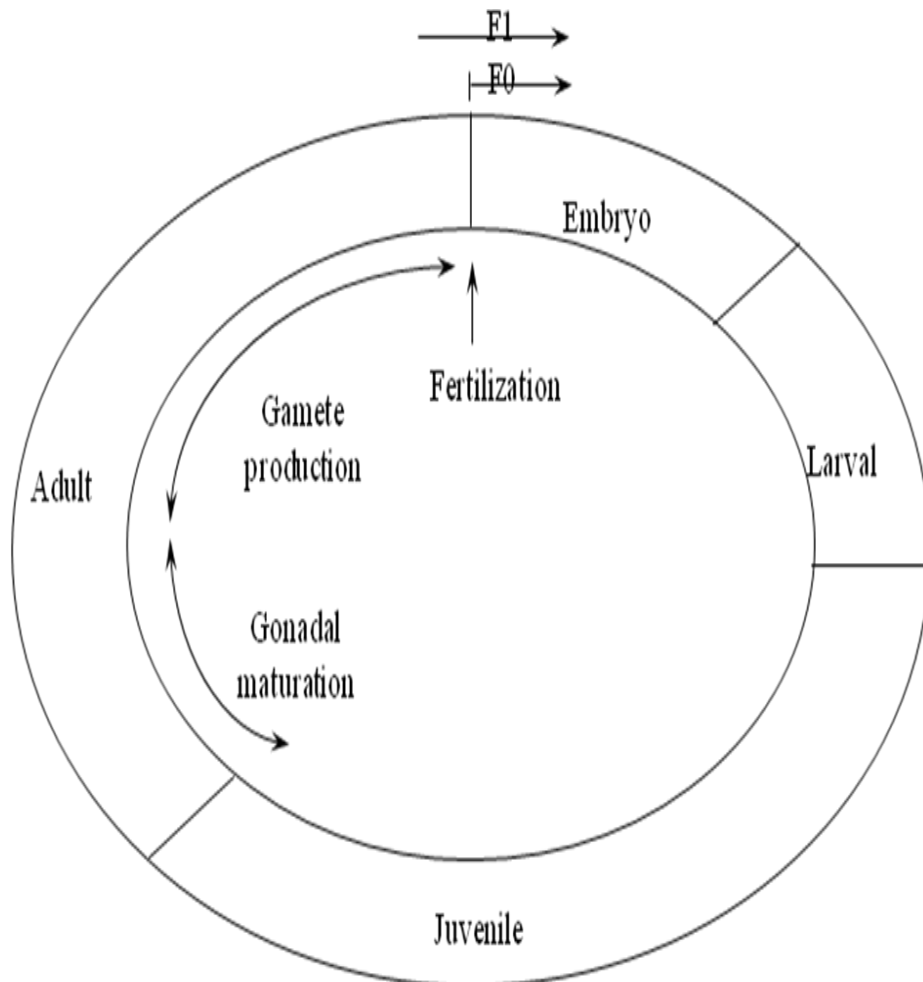
Bivalve demographics in over-exploited and "restored" populations

Attempts to model the demographics of bivalve populations generally begin with a standing stock of adults at T_0 and estimate egg production from empirically-determined size-specific fecundity relationships (e.g., Powell et al. 1992, Ripley and Caswell 2006). More often than not in these models, production of embryos is equated with egg production via a simple relationship in which *embryos = β*eggs*, where *β* is 1 or some lesser fixed value determined from laboratory fertilization studies. Such approaches assume that all or most of the eggs get fertilized. Mann and Evans (1998) apply a density-dependent efficiency term to their oyster demographics model that was derived from experiments with sea urchin gametes (Levitan et al. 1991). Their approach, while an improvement over previous approaches, assumes a 1:1 sex ratio, viable gametes, and motile sperm, all of which may be altered by EDC's.

The life history strategies of free-spawning bivalves are characterized by as much as seven orders of magnitude difference in abundance of various life stages. For instance, an adult eastern oyster *C. virginica* can produce 10^7 eggs per spawn, often with multiple spawns per year and many years of spawning. In a stable population on average only one of these eggs survives to become an adult female (Fig. 2). Most fisheries management and restoration activities focus efforts on processes that most directly affect the survival of settling larvae, juveniles, or adults, without much regard for factors which may be affecting the steepest part of the survival curve. The fact that EDCs can alter egg-to-sperm ratio, swimming speed, and larval growth and survival means that they can potentially have a large impact on population dynamics.

Conservation and restoration context

The fact that bivalve populations which are the focus of current conservation and restoration efforts are generally at

Figure 2. Generalized survival curve for a free-spawning marine bivalve.
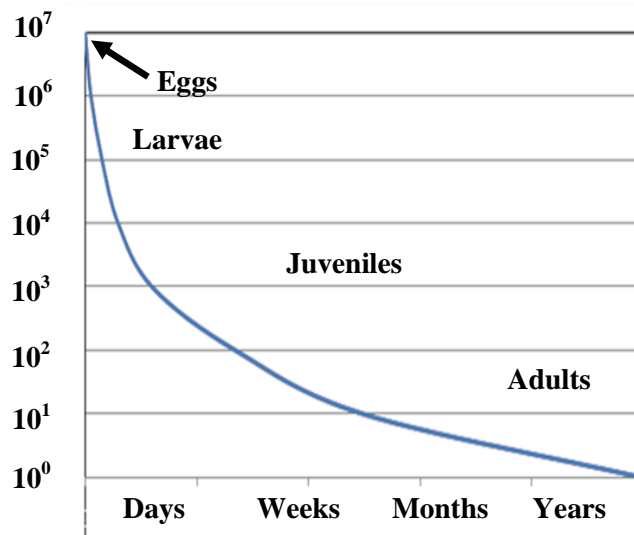
historic low abundances further exacerbates the potential consequences of EDC exposure. Most marine bivalves are free-spawning, releasing gametes into the water column. Low population densities result in low gamete concentrations in the water column which can reduce the proportion of eggs that are fertilized. Since EDC exposure can reduce both the number of males in a population and sperm motility, the combined effects of low population density and such exposure thus have synergistic negative effects on reproductive success.

Among the oysters (family Osteridae), species in the sub-families Ostreinae and Lophinae release sperm into the water column where it can be drawn into the mantle cavity of females where fertilization occurs, while those in the sub-family Crassostreinae are broadcast spawners releasing both eggs and sperm into the water column (Ó Foighl and Taylor 2000). As a broadcast spawner, *C. virginica* reproduction is dependent upon contact between egg and sperm in the water for successful fertilization. Laboratory studies have revealed that in well-mixed conditions over a wide range of gamete concentrations that sperm to egg ratios of 1000:1 or greater are required to achieve high fertilization success in oysters (Luckenbach et al. 2008). Importantly, this finding applies even at high gamete densities with egg concentrations as high as 10^4 eggs mL^{-1} and sperm concentration as high as 10^7 sperm mL^{-1}, indicating that sperm may be limiting even at high gamete levels. Thus, demographic processes or restoration practices which result in skewed sex ratios have the potential to substantially affect reproductive success in oyster populations. For instance, in many areas of Chesapeake Bay, particularly the upper Bay and lower salinity reaches of tributaries in the lower Bay, oyster recruitment is sporadic, with significant recruitment events often occurring only every few years (Kennedy and Breisch 1981). In such cases, the population may be dominated by a single year class, which becomes increasingly skewed towards females as it ages. Though such populations have the potential to produce large numbers of eggs (e.g., $\sim10^{11} - 10^{12}$ eggs $acre^{-1}$) many of these eggs may go unfertilized due to insufficient sperm concentrations.

A similar situation may occur in restoration practices which use hatchery-produced oysters to populate constructed oyster reefs. This approach is particularly popular with community-based oyster restoration projects (e.g., Brumbaugh et al. 2002, http://www.nature.org

/wherewework/northamerica/states/newhampshire/files/nh_oyster_conservationist_final_report.pdf [Accessed 2/3/2010]) as well as larger environmental organizations (http://www.cbf.org/oysters [Accessed 2/3/2010]). For example, in 2005, the Maryland's Oyster Recovery Partnership coordinated the planting of over 168 million hatchery-produced spat on 75.6 hectares in the Chester and Choptank Rivers in Maryland. Of these, only one planting of 6.9 million oysters on 1.3 hectares overlapped with any of the previous plantings made by this group between 1998 and 2004 (Oyster Recovery Partnership 2008). Such planting practices have arisen out of the desire to expand the area covered by restored oyster reefs as rapidly as possible and have assumed that either a sufficient number of males would persist in the population to prevent sperm limitation or that natural recruitment would supplement the number of males in the population.

Recent research and modeling efforts suggest that the assumption that sperm are not limiting reproductive success may be invalid for many restored and unrestored oyster reefs in the Bay. In 2006, Kellogg et al. (2007) surveyed sex ratios on restored oyster reefs at 23 sites in Maryland. Because no significant recruitment (<0.1 recruit m^{-2} yr^{-1}; Paynter unpublished data) or additional plantings had occurred since the initial restoration effort, these sites provided the opportunity to sample field populations of oysters of known age and determine sex ratios in relation to age as well as size (commonly used as a proxy for age). As expected based on previous studies (Galtsoff 1964, Thompson et al. 1996), the proportion of females increased with increasing oyster size and age. Because this pattern raised concerns that sperm limitation could occur on these reefs, Kellogg et al. (2009) created a sperm limitation model by combining this sex ratio data with Luckenbach et al.'s (2008) data on both size-specific fecundity and sperm-to-egg ratios needed to achieve high fertilization success. The resulting model predicts that sperm may become limiting as soon as four years after planting and that sperm limitation is likely to increase with increasing oyster age. It is important to note that this model only predicts the likelihood of sperm limitation as a result of skewed sex ratios and relative gamete concentrations in the population and assumes that the gametes of all oysters are in close proximity. However, fertilization success for broadcast spawning species of invertebrates declines rapidly with increasing distance between adults of opposite sex (Levitan et al. 1991; Pennington 1985). Because many of the historically productive oyster bars in the Bay are characterized by sparse populations (<1 oyster m^{-2}; Jordan and Coakley 2004) and are likely to be dominated by one or a

few age classes due to infrequent and sparse natural recruitment, the potential for reduced reproductive success due to sperm limitation is likely to be even greater on many of these oyster bars.

Within this context it is evident that exogenous factors which alter sex ratios, gamete production, and viability will have even great consequences at the population level than would be expected in otherwise healthy wild populations. However, insufficient data exist at present to predict the exact nature and the scale these impacts.

EDCs in Chesapeake Bay

Chesapeake Bay serves as critical nursery and reproduction habitat for a variety of aquatic species. In the Chesapeake Bay and elsewhere, much concern over chronic chemical contaminants has historically centered around potential effects that toxic compounds may have on sensitive reproductive and developmental processes in resident species. Numerous contaminants present in the Chesapeake Bay sediment, water, and biota (Chesapeake Bay Program 1999) also appear on lists of known or suspected EDCs (Colborn et al. 1993; McLachlan 2001). Initial interest in EDCs arose from observations of reproductive and developmental impairment resulting from exposure to compounds that interact with hormone receptors or hormone-mediated signaling pathways (McLachlan 2001). In particular, a large number of compounds appear to exert effects by stimulating estrogen -mediated pathways (Jobling et al. 2002). Though potential targets for endocrine disruption in Chesapeake Bay and elsewhere extend beyond estrogen-mediated pathways, we will limit our discussion here to the issue of environmental estrogens in Chesapeake Bay.

Environmental estrogens vary greatly in structure and include natural estrogens (e.g., estradiol-17β, estrone), pharmaceuticals (e.g., ethinyl estradiol), organochlorine insecticides (e.g., methoxychlor, DDT, DDE, Kepone), industrial surfactants, and bisphenol A used in plastics (McLachlan 2001; Campbell et al. 2006). Wastewater from wastewater treatment plants (WWTPs) is likely to be a major source for many environmental estrogens entering aquatic ecosystems (Kolpin et al. 2002), including Chesapeake Bay. Induction of the yolk precursor protein vitellogenin has often been observed in male fish of several freshwater species collected

downstream from WWTPs in the UK. These effects are believed to result from exposure to naturally excreted estrogens (e.g., 17β-estradiol, estrone), synthetic estrogen (e.g., ethinylestradiol) and industrial surfactants commonly present in WWTP effluents (Harries et al. 1999; NRC 1999; Sumpter and Johnson 2005). However, we are aware of only one published study that addressed exposure of Chesapeake Bay fish to WWTP effluent (Pait and Nelson 2009). In that study, no significant induction was observed in Atlantic killifish *(Fundulus heteroclitus)* in male individuals collected from several sites in the Bay including sites immediately downstream from WWTPs.

While relatively water-soluble estrogenic compounds (natural and synthetic estrogens, surfactants) likely leave WWTPs via effluent, more hydrophobic contaminants (e.g., organochlorine pesticides, PCBs, brominated diphenyl ethers, and PAHs) tend to accumulate in biosolids (Hale et al. 2001; Hale and LaGuardia 2002). In the US, the majority of biosolids obtained from WWTPs are spread over agricultural land as fertilizer. Relative to investigations of EDCs associated with WWTP effluent, few studies have addressed the potential endocrine disruption resulting from exposure to biosolid-associated contaminants. In one study, exposure of the fathead minnow *(Pimelphales promelas)* to biosolids resulted in induction of CYP1A (a nuclear hormone receptor-mediated process) and DNA damage (Sullivan et al. 2007). In another study, abnormal fetal testis development was observed in sheep raised on pastures that received biosolids as fertilizer (Paul et al. 2005).

In addition to WWTP effluent, environmental estrogens likely enter the Bay via run off from agricultural lands, especially concentrated animal feed operations (CAFOs).   For example, the Delmarva Peninsula is one of the largest and most concentrated poultry producing areas in the country, generating over 600 million birds and 1.6 billion pounds of litter annually (USDA 1997). Disposal of litter is accomplished by application to agricultural fields as fertilizer. Litter contains low levels of many chemicals including steroid hormones that may enter adjacent waterways during rain events (Findlay-Moore et al. 2000). Studies by Yonkos et al. (in press) indicated that laboratory exposures to aqueous extracts of poultry litter resulted in significant induction of vitellogenin in male fish and a gender shift in larval fish following a grow out period. Detectable levels of 17β-estradiol and testosterone were measured in a majority of

drainage ditches and other sites sampled on the Delmarva Peninsula in the spring following poultry litter application to fields. However, no evidence for induction of vitellogenin was found in fish or amphibians collected from these sites (L. Yonkos, personal communication).

Due to the limited number of studies conducted thus far, it is difficult to draw firm conclusions regarding impacts of estrogenic compounds in estuarine portion of the Chesapeake Bay. However, there is currently a great deal of concern regarding possible impacts of these chemicals in upper portions of the Potomac River. During investigations of fish kills and of skin lesions in freshwater fishes in the South Branch Potomac River, scientists at the USGS discovered a high prevalence of intersex (oocytes within testes in smallmouth bass: Blazer et al. 2007, Blazer 2009).   These observations were unexpected as the South Branch drains an area of low human population density, is primarily rural, and land use is dominated by agriculture. Previous reports of intersex in wild fishes were primarily associated with exposure to WWTP effluent where exposure to environmental estrogens is observed (Jobling et al. 2002). The identity and source of putative estrogenic compounds responsible for intersex in the South Branch remains undetermined but is the subject of on-going investigation.

Identification of data gaps and recommendations for research

Despite widespread declines in many commercially- and ecologically-important bivalve species around the world (e.g., MacKenzie 1997a, 1997b, 1997c, Beck et al. in review) and the potential susceptibility of early life stages to EDCs, little is known about how important or widespread the impacts of these compounds are on bivalve mollusk populations.

Managers and scientists continue to face problems that stem from insufficient basic biological information on the hormonal control of growth, reproduction, immune function, and other physiological functions in bivalve mollusks. This information gap is greater for bivalve mollusks than for gastropod mollusks, due in part to research aimed at understanding how TBT impairs reproductive organs in snails. Although no single research program will resolve this information gap in the short term, it is important that researchers, managers, and resource organizations begin addressing key issues. The discussion among participants and attendees at

the CRC Symposium generated a list of data gaps and research needs that will be important for developing a greater understanding of EDC impacts on shellfish populations in Chesapeake Bay and other estuaries in the U.S. Building on these discussions and reports from other working groups on research needs for EDC in the environment (Smithsonian Institution 1997, Joint IPCS-Japan Workshop 2004, European Commission 2007), we developed the following summary of priority needs in support of practical management-based solutions towards potential impacts of EDC's on molluskan bivalves. These research priorities are particularly cognizant of the complexity associated with 1) the impacts of chemicals on multiple life stages of bivalves and 2) the role of these impacts at the population and ecosystem levels. Most of the more specific areas of research address one of these two general information gap categories.

1. **Develop database and communication structures which support exchange of information between researchers and resource managers** – Natural resources managers need more information than ever before to effectively deal with a greater level of ecosystem complexity that includes human factors. As research on EDC impacts on living resources, including bivalve mollusks, proceed in the Chesapeake Bay and vicinity, it will be important at the outset to develop meta-databases which bring together diverse research results from laboratory-based toxicology to field exposures to agricultural run-off studies in a format that is readily accessible to researchers and resource managers. Additionally, there is a need for more frequent workshops on the issue which include researchers and resource managers to examine specific research findings, identify additional research priorities, and develop strategies for reducing putative impacts.

2. **Investigate natural and altered hormonal control of growth, reproductive development, and maturation in bivalves**- Hormonal control of development and maturation, especially in early life stages, is not well understood in bivalves. Advancing our understanding of the natural course of this control and the alterations resulting from environmental chemical exposure is critical to assessing the impacts of EDCs in bivalves. Since hormonal controls and the impacts of EDCs undoubtedly vary throughout the life cycle of bivalves, it is imperative that these investigations address impacts at various stages within the life cycle. There is an expectation that early life stages (i.e., gametes, larvae, and juveniles) may be

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especially sensitive to environmental exposure of EDCs and that impacts at these stages may strongly alter population dynamics. However, it is also reasonable that exposure of adults prior to recrudescence, when the gonads begin to re-differentiate, may affect sex determination and fecundity. Moreover, the observation of multi-generational effects in previous studies on bivalves points to the need to assess the impacts of exposure in parental generations on the survival, growth, maturation, and reproduction of subsequent generations. Studies which address the effects of exposure by potential EDCs on development and maturation of bivalves within life cycle context are thus a high priority need.

3. **Linking life-cycle effects of exposure with population and ecosystem models -** Inferring population-level consequences of individual-level effects on growth, maturation, and reproduction will require incorporation of these effects into demographics models. The incorporation of results from life-cycle exposure experiments into stage-specific matrix population models provides a powerful approach towards achieving this. In addition, ecosystem models which attempt to assess ecosystem functions of growing bivalve populations may need to include constraints on reproduction and population growth in areas with EDC exposures.

4. **Identify measures of EDC stress in bivalves** - One of the key elements in the investigations of EDCs in natural populations of other wildlife species has been the identification of measures of stress from EDCs. These measures include biomarkers of exposure and effects, and indicators of responses to EDCs, such as the imposex in gastropods, vitellogenin in male fish, and alteration of secondary sexual characters. Identifying measures that are indicative of stress related to EDC exposure in bivalves is a high research priority.

5. **Expand monitoring for EDCs in Chesapeake Bay, especially in relation to critical habitats for living resources** – Monitoring efforts in recent years have identified a large number of chemicals in surface waters around the nation, including the Chesapeake Bay and its watershed. Some monitoring in specific local waters reveals the presence of hormonally active chemicals, i.e., estrogenic chemicals. The distribution of these chemicals in the Bay needs to be better characterized, especially in relation to critical habitat for bivalves and other

living resources. Since inputs of these compounds, especially those associated with agriculture and urban storm water runoff, may vary with season and rainfall events, it will be necessary to incorporate temporal variability and pulsed inputs into monitoring programs.

6. **Effects of *in situ* exposures** – To date, most of the observed effects of EDCs on bivalves have come from laboratory studies. While such studies are critical for understanding specific mechanisms and dose response relationships, demonstrable effects from field exposures are necessary for establishing impacts on natural populations. The sampling of wild populations and/or field deployments of bivalves in key habitats should provide important information on biological responses. Studies of the freshwater Asiatic clam, *C. fluminea*, have revealed gonadal atrophy and reduced fecundity following field exposure to PCBs (Law et al. 2009) and reduced fecundity has been observed in Eastern oysters deployed at field sites with suspected EDC contamination (McClellan-Green 2009). Investigations in other water bodies have demonstrated important effects on reproduction and development in bivalves, and similar research has a high potential to offer valuable information in the Chesapeake Bay system.

7. **Effects of interacting multiple stressors –** Natural populations of bivalves in the Chesapeake Bay experience ambient environmental conditions that vary in dissolved oxygen concentration, temperature, and salinity as well as other physical, chemical, and biological conditions. The effects of EDCs and other chemicals on bivalve reproduction are likely to differ under varying levels of stress associated with these conditions. Experimental studies and modeling approaches that incorporate these multiple stressors are needed to develop a comprehensive understanding of the effects of EDCs on bivalve reproduction and population dynamics.

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Appendix. Authors and presentation titles from a symposium on *Potential Effects of Endocrine Disrupting Compounds on Bivalve Populations in Chesapeake Bay* at the Chesapeake Research Consortium conference *Ecosystem Based Management (EBM): the Chesapeake Basin & Other Systems*, March 23 – 25, 2009.

Author(s)	Presentation Title
P.L. deFur	Endocrine disruptors, crustacean molting, and bivalve populations
D. Fisher, L. Yonkos, and P. Van Veld	Effects of natural steroids from poultry litter on aquatic organisms
V.S. Blazer	Potential effects of endocrine-disruptors and other emerging contaminants on fish species in Chesapeake tributaries
G. Scott	Endocrine disruption in invertebrates: Effects of agricultural pesticides and emerging contaminants of concern on molluscs and crustaceans
R.M. Sternberg, A.K. Hotchkiss, and G.A. LeBlanc	A snail's tail: In search of the mechanism for tributyltin-induces imposex
M. Law	Gonadal atrophy in clams induced by PCB exposure: Implications for ecosystem-wide problems?
S.M. Lindsay, J. Chasse, R.A. Butler, W. Morrill, and R.J. Van Beneden	Stage-specific effects of acute exposure to pesticides on the soft shell clam, *Mya arenaria*
P. D. McClellan-Green	EDC effects in bivalves: Are our oysters at risk?
V.S. Kennedy and C.B. McCollough	A survey for potential endocrine disruption effects in bivalves in Maryland's Chesapeake Bay
M.W. Luckenbach, E. North, S. Bonniwell, and R. Mann	Gamete ratio, fertilization success, and potential sperm limitation in oysters
M.L. Kellogg, M.W. Luckenbach, and K.T. Paynter	Potential for population-level impacts of endocrine disrupting compounds: Implications for oyster reef restoration in Chesapeake Bay