Chapter 5
Reproduction

Abstract Reproduction is obviously a very important endpoint, since impaired reproduction can have rapid repercussions at the population level. Life-cycle characteristics of different organisms are a major factor in determining their vulnerability to particular contaminants. There are many ways in which reproduction can be affected, but one of particular concern is by very low levels of some environmental chemicals that can interfere with the endocrine system, termed endocrine disruption. Contaminants can also directly affect gametogenesis, mating, and fertilization. These various stages of the reproductive process are clearly connected to one another.

Keywords Endocrine • Fertilization • Gametogenesis • Mating • Vitellogenin

This chapter and the three to follow cover life cycle functions; separate chapters cover reproduction, embryonic development, larval development, and subsequent developmental processes. The present chapter is subdivided into sections dealing with Endocrine Effects, Gametogenesis and Fecundity, and Mating and Fertilization. All these processes are continuous, and the subdivisions between sections (and chapters) are not totally distinct from each other. It is clear that effects at one stage can produce subsequent effects at later stages.

5.1 Endocrine Effects

The endocrine system regulates all biological processes in the body, including metabolism, development of the nervous system, and the growth and function of the reproductive system. The female ovaries, male testes, and pituitary, thyroid, and adrenal glands are major constituents of the vertebrate endocrine system. These glands produce hormones that circulate in the blood and interact with cells by binding to special proteins called receptors, which are specific for each

J.S. Weis, *Physiological, Developmental and Behavioral Effects of Marine Pollution*,
DOI 10.1007/978-94-007-6949-6_5,

hormone. When enough binding sites are occupied, a message is passed on to the target cell nucleus stimulating genes that result in physiological changes regulating metabolism, development, growth, reproduction etc.

For the past 20 or so years, there has been considerable attention devoted to effects of very low levels of contaminants on the endocrine system; chemicals having such effects (and there are many) have been called "endocrine disruptors." Some of the concern is because hormones have effects at extremely low concentrations in the body, and endocrine-disrupting chemicals similarly can produce effects at levels far below those that had previously been considered safe. Since hormones are already present in the body in biologically active concentrations, additional exposure to relatively small amounts of hormonally active substances can disrupt the proper functioning of the endocrine system. In some cases, these effects disappear at higher concentrations, being an exception to general dose–response relationships in toxicology. Another cause for concern is that exposures during early life stages (e.g. embryos) can produce delayed effects that become apparent only when the organism is mature and theoretically ready to reproduce.

The bulk of research on endocrine disruption has focused on sex steroids. The two main classes of sex steroids are androgens and estrogens, which include testosterone and estradiol. In general, androgens are considered "male sex hormones" since they have masculinizing effects, while estrogens are considered "female sex hormones" although all types are present in each sex, but at different levels. Some chemicals mimic a natural hormone, fooling the body into over-responding to the stimulus, or responding at inappropriate times. Other endocrine-disrupting chemicals block the effects of a hormone from certain receptors, stimulate or inhibit the endocrine system causing overproduction or underproduction of hormones. Environmental chemicals can be estrogen mimics, anti-estrogens, androgen mimics, and anti-androgens. Endocrine disrupting chemicals may resemble sex steroids structurally. These chemical properties allow them to bind to hormone-specific receptors on the cells of target organs. This binding may result in activating the cell inappropriately or blocking natural hormones from binding. Among xenoestrogens are widely used industrial compounds, such as PCBs, Bisphenol-A, and phthalates. They can cause an estrogen-like response at the wrong time or in the wrong amounts in both sexes, and can enhance female traits in males (feminize). Hormone blockers include drugs like tamoxifen, a specific antiestrogen used to treat breast cancers that need estrogen to grow. DDE, a breakdown product DDT, blocks androgen receptors so testosterone cannot bind.

5.1.1 Crustaceans

In crustaceans ecdysteroids and terpenoids play major roles in regulating development, growth, maturation, and reproduction. Laboratory studies have demonstrated the susceptibility of crustaceans to endocrine disruptors, and studies have shown endocrine disruption in field populations, though the causal link between abnormalities and particular environmental chemicals is often lacking. Indicators of reproductive endocrine disruption (e.g., intersexuality) allow investigation of the degree to which endocrine disruptors are affecting populations (LeBlanc 2007), yet there are few studies that examine gonadal development, intersex or other direct reproductive endocrine effects. Ecdysteroids regulate aspects of embryo development, molting, and reproduction, so chemicals that interfere with ecdysteroid signaling can produce effects. Chemicals with anti-ecdysteroidal activity can function as ecdysteroid synthesis inhibitors or ecdysteroid receptor antagonists. Such chemicals include many of the classic estrogen receptor agonists (e.g. bisphenol a, DDT metabolites, nonylphenol). Many of these endpoints are appropriate to chapters on embryo and larval development, but will be described here. Alterations in molt frequency can indicate disruption of ecdysteroid signaling, but effects of chemicals on molting will be discussed in the chapter that covers growth (Chap. 8).

Pesticides and Contaminants of Emerging Concern

Methyl farnesoate is a major terpenoid hormone of crustaceans. Many laboratory studies have been performed with insect growth- regulating insecticides (McKenney 2005) which function as methyl farnesoate mimics. Metamorphic success of decapods is among the most sensitive endpoints affected by insect growth regulators. Delayed metamorphosis or metamorphic abnormalities caused by exposure to insect growth regulators have been reported in shrimp, crabs, and lobsters (Christiansen et al. 1977a, b; McKenney and Celestial 1993; Celestial and McKenney 1994; Cripe et al. 2003). These will be discussed in greater detail in Chap. 7, on larval development. Estrogens themselves (17b-estradiol, diethylstilbestrol, 17a-ethinyl estradiol, estrone) can elicit effects on crustaceans including altered gonadal development in amphipods (Segner et al. 2003), and reduced vitellin levels in mysids (Ghekiere et al. 2006).

Metals

There has been very little work on endocrine-disrupting effects of metals on crustacean reproduction. Tributyltin exposure of hermit crabs, *Clibanarius vittatus* females caused disorganization and atrophy of the ovaries, which would impair reproduction (Sant'Anna et al. 2012). This chemical has profound endocrine disrupting effects on females of many groups of organisms, described below for mollusks and fishes.

Polluted Environment

There have been reports of intersex individuals of many species, but they have not been generally correlated with particular pollutants. Copepods *Paramphiascella* *hyperborean, Stenhelia gibba,* and *Halectinosoma* sp. collected near sewage outfalls had elevated incidence of intersex (Moore and Stevenson 1991, 1994). This similar effect in different species in the same vicinity suggests an environmental cause, but no clear relationship could be established between sewage effluent and incidence of intersex (Moore and Stevenson 1994).

5.1.2 Mollusks

Metals

TBT

One of the earliest reported examples of endocrine disruption found in the field was a condition called *imposex* in female gastropods exposed to very low levels of tributyltin (TBT), a commonly used constituent of antifouling paints on vessels until the 1980s, when it was restricted or banned in many countries. Affected female snails developed male structures including a penis and vas deferens, which could block the passage of eggs down the oviduct preventing egg deposition and causing reproductive failure. Many dogwhelk, *Nucella lapillus,* populations had females in which vas deferens overgrew the female opening, rendering the female sterile (Gibbs and Bryan 1986); this was associated with population declines around Southwest England (Bryan et al. 1986). Population recovery occurred only after restrictions on the use of TBT came into effect. While some species recovered quickly after TBT use was restricted, in *Nassarius reticulatus*, tissue levels of TBT in polluted sites dropped by 5–10 times between 1987 and 1993, but the rate of imposex declined very slowly; this was attributed to longevity of the snails and limited recruitment of less-affected females (Bryan et al. 1993). Even in 2011, however, Qiu et al. found that imposex remained severe in *Thais clavigera* from Victoria Harbour and other sites with extensive shipping activities. Imposex severity (measured by relative penis length in females) was correlated with tissue concentrations of TBT. High levels of imposex were also found in *Thais biserialis*, *T. brevidentata*, and *T. kiosquiformis* in many coastal sites in Ecuador (Castro et al. 2012). Butyltin compounds (TBT, dibutylin -DBT, and monobutylin -MBT) were found in sediments. TBT does degrade over time, but not as rapidly as was originally thought. Although BT degradation suggested an older input of TBT, the high imposex levels suggest that restrictions on TBT were still not effective in Ecuador.

Abidli et al. (2011) studied testosterone and estradiol in two gastropods, *Hexaplex trunculus* and *Bolinus brandaris*, to clarify the impact of TBT on free and esterified steroids. Two months exposure to 50 ng l^{-1} induced imposex. Testosterone and estradiol were present in free and esterified forms in the digestive gland-gonad complex. In female *B. brandaris*, 50 ng TBT l^{-1} elevated free testosterone and decreased the esterified form. However, in female *H. trunculus*, TBT elevated both the free and esterified form of testosterone.

5.1.3 Fishes

Fishes have considerable sexual plasticity. Although the sexes are usually separate, there are some functional hermaphrodites (e.g., some Serranidae and Sparidae). In some species, individuals can change functional sex in response to social and environmental cues. There are examples of both protandrous (male first) and protogynous (female first) groups, e.g., bluehead wrasses and clownfish, respectively, but increasing numbers of species are being found to have this ability. It has become clear that this natural hormonal balance may be disturbed by chemicals with hormone-like activities. Fish are particularly vulnerable to potential endocrine-disrupting chemicals (EDCs) in surface waters.

The egg protein, vitellogenin (VTG), is produced in the female's liver and transported to the ovaries, where it is added to egg yolk prior to ovulation. Synthesis of this protein is regulated by estrogens and thus can serve as a marker of exposure to environmental estrogens. VTG induction in male fish (which have very low endogenous estrogens, but whose livers nevertheless are able to synthesize VTG in response to exogenous ones) is an effect of estrogenic contamination; numerous studies have measured this biomarker in field populations. VTG induction in males is an excellent biomarker of exposure to estrogens acting via hepatic estrogen receptors. Other commonly observed responses are intersex individuals and altered sex ratios. Biochemical changes in sex hormones or enzymes involved in their synthesis are also frequently studied. Numerous bioassays have been developed using steroid hormones and VTG as end points (MacLatchy et al. 2003).

An analogous biomarker to VTG that is sensitive to androgens has been developed in the three-spine stickleback (*Gasterosteus aculeatus*) (Katsiadaki et al. 2002). This is the protein *spiggin*, which is produced in the male kidney and secreted for use as a glue during nest construction. Spiggin is produced in females only after exposure to exogenous androgens.

Metals

Thomas (1989) examined effects of Cd on the reproductive endocrine function in female Atlantic croaker (*Micropogonias undulatus*) Fish were exposed to 1 mg l^{-1} Cd for 40 days during the period of ovarian recrudescence. Exposure accelerated ovarian growth and elevated plasma estradiol concentrations, suggesting a stimulation of vitellogenesis. He also observed an increase in the spontaneous secretion of gonadotropin (GTH) from pituitaries of Cd-exposed fish *in vitro*. Depew et al. (2012) reviewed effects of dietary mercury on fish and found that adverse effects on behavior had a wide range of effective dietary concentrations, but generally occurred above 0.5 μg g^{-1} wet weight. In contrast, effects on reproduction (generally endocrine effects) occurred at dietary concentrations that were much lower (<0.2 μg g^{-1} wet wt). Tributyltin (TBT) produces masculinization in fish, reminiscent of effects in invertebrates. Genetically female Japanese flounder

(*Paralichthys olivaceus*) were fed an artificial diet containing tributyltin oxide (TBTO) at concentrations of 0.1 and 1.0 μg/g diet from 35 to 100 days after hatching, which includes the sex differentiation period (Shimasaki et al. 2003). The ratio of sex-reversed males increased to 25.7 % in flounder fed the 0.1 μg/g diet and to 31.1 % in those fed the 1.0 μg/g diet compared with the control (2.2 %). TBT's breakdown product, dibutyltin (DBT), also can act as a masculinizing agent in fish (McGuiness et al. 2012). Zhang et al. (2013) investigated effects of TBT on ovarian lipid accumulation and testosterone esterification in rockfish (*Sebastiscus marmoratus*). After exposure to TBT (0, 1, 10 or 100 ng l^{-1} as Sn) for 48 days, there was delay in oogenesis, a decrease of neutral lipid droplets in the ooplasm of ovaries. Exposure also induced an increase of interstitial ectopic lipid accumulation and total lipids in ovaries. A decrease of serum T3 and T4 (triiodothyronine and thyroxine) concentrations (at 10 and 100 ng l^{-1}) was a possible cause for the lipid responses. In addition, the percentage of testosterone in esterified form was decreased in the ovaries by TBT, which might be a mechanism by which free testosterone levels increased. The accumulation of ectopic lipids and increase of free testosterone in ovaries could impact ovarian functions and oocyte development.

Organics

Chlorinated Chemicals

PCBs and dioxins have frequently been associated with endocrine disruption. Thomas (1989) examined effects of the PCB mixture Aroclor 1254 on the reproductive endocrine function in female Atlantic croaker (*M. undulatus*). Fish were fed PCBs in the diet (0.5 mg/100 g body wt/day) during the period of ovarian recrudescence, which impaired ovarian growth and decreased plasma estradiol. Pituitaries from treated fish decreased their spontaneous secretion of gonadotropin *in vitro*. Loomis and Thomas (1999) identified an estrogen receptor in the testis in Atlantic croaker. Xenoestrogens, including DDT, chlordecone (Kepone), nonylphenol, and PCBs, bound to this receptor with relatively low binding affinities, 10^{-3} to 10^{-5} that of estradiol. Khan and Thomas (1992, 1997, 2001, 2006) accumulated evidence of the involvement of PCBs in disruption of the serotoninergic systems in fish brains that regulate reproductive hormones. In male *M. undulatus*, exposure to Aroclor 1254 during gonadal recrudescence caused a significant decline in 5-HT (serotonin) and DA (dopamine) and an increase in their metabolites (Khan and Thomas 1997). The reduction in 5-HT led to an inhibition of luteinizing hormone (LH) secretion and an absence of gonadal growth, since 5-HT stimulates LH secretion in this species (Khan and Thomas 1992, 1997; Khan et al. 2001).

Dioxins, furans and dioxin-like polychlorinated biphenyls (PCBs) were analyzed in muscle of yellow phase European eel (*Anguilla anguilla*) from 38 sites (Geeraerts et al. 2011). In most sites, eels had levels considered detrimental for reproduction; these chemicals were suggested as factors contributing to the population decline of this species.

Oil and PAHs

Oil has major impacts on fish embryos that may be manifested in adult stages as endocrine disruption. Pink salmon that had been exposed as embryos to *Exxon Valdez* oil and survived to migrate to the ocean, returned from the sea at only half the rate of control fish (Heintz et al. 2000). These returning adults showed reproductive impairment and their embryos had reduced survival. Thus, the second generation was affected by the sublethal exposures their parents had had as embryos and fry (Peterson et al. 2003).

Specific PAHs may act as endocrine disruptors. Dong et al. (2008) hypothesized that altered expression of genes for the P450 enzyme aromatase could be responsible for reproductive dysfunction caused by Benzo(a)Pyrene (BaP). Aromatase is involved in steroid balance by converting androgens into estrogens. CYP19A1 expression decreased after BaP exposure in 3-month-old *Fundulus* immature oocytes, but BaP did not affect its expression in adult oocytes. In embryo brains, BaP significantly decreased CYP19A2, and in adults, CYP19A2 expression was decreased in the pituitary and hypothalamus. The study gives insights into molecular mechanisms of action of BaP.

Following the *Exxon Valdez* oil spill in 1989, Sol et al. (2000) studied the effect of oil on reproductive parameters in wild populations of female dolly varden, yellowfin sole, and pollock. Exposure to oil was the highest in the first year and decreased in subsequent years of sampling. A higher proportion of dolly varden sampled in 1989 had depressed plasma estradiol-17β compared to the fish in 1990.

Contaminants of Emerging Concern

Some studies have exposed animals to hormones themselves instead of hormone mimics, often ethynylestradiol (EE_2), since this estrogen (used in birth control pills) is released from sewage treatment plants. In some cases, EE_2 has been used to develop a bioassay for contaminants with estrogenic effects. *F. heteroclitus* exposed to 17α-ethynylestradiol showed decreased plasma reproductive steroid levels, decreased gonadal steroid production, increased plasma VTG, decreased fecundity and impaired fertilization. In exposed males, testosterone production decreased, indicating effects on the steroidogenic pathway. Hepatic transcript levels of estrogen receptor alpha (ERα) and VTG increased in treated males, an estrogenic response (Hogan et al. 2010). Recrudescing *F. heteroclitus* were exposed to EE_2 for 7–15 days (MacLatchy et al. 2003). At high EE_2 (>250 ng l^{-1}), males had depressed androgen synthesis and plasma steroid levels and females had depressed gonadal production and circulating E_2; however, <100 ng l^{-1} EE_2 increased gonadal production and plasma E_2. Male and female plasma VTG responded in a concentration-dependent fashion, with the low effect concentration being 1 ng l^{-1}.

Loomis and Thomas (2000) studied short-term effects of estrogens and xenoestrogens on androgen production by testicular tissue from the Atlantic croaker (*M. undulatus*). Incubation of testicular tissue with estradiol (37 nM to 37 μM) decreased gonadotropin-stimulated 11-ketotestosterone (11-KT) production. The effect was specific for estrogens; progesterone, cortisol, or the synthetic androgen mibolerone did not alter 11-KT production at similar concentrations. Diethylstilbestrol, the antiestrogen ICI 182,780, and several xenoestrogens including Kepone (chlordecone), 4-nonylphenol, and a hydroxylated PCB metabolite also decreased gonadotropin-stimulated 11-KT production. The action of estradiol was rapid (<5 min) and was not blocked by actinomycin D or cycloheximide (inhibitors of transcription and translation, respectively) demonstrating that estrogens (and also probably xenoestrogens) act on the cell surface via a nongenomic mechanism to alter androgen production. However, genes normally induced by estradiol (E_2) in female fish, those for VTG and zona radiata proteins, are inducible in males exposed to estrogenic chemicals. Male sheepshead minnows (*Cyprinodon variegatus*) were exposed to both E_2 and para-nonylphenol (NP), to determine a dose–response (Knoebl et al. 2004). Quantitative real time PCR measured mRNA for the genes. Both E_2 and NP elicited a dose-related increase in all of the mRNAs tested. Hogan et al. (2008) also examined genetic responses as well as hormonal ones. To determine the sensitivity of genes to induction by hormones, male and female three-spine sticklebacks (*G. aculeatus*) were exposed to 1, 10 and 100 ng l^{-1} of methyltestosterone (MT) or estradiol (E2). Spiggin induction in females, and VTG induction in males were both detectable at 10 ng l^{-1} of either hormone. Gonadal steroid hormone production was measured in exposed fish to compare gene expression endpoints to an endpoint of hormonal reproductive alteration. Reduction in testosterone production in ovaries at all three MT exposure concentrations, and ovarian estradiol synthesis at the 100 ng l^{-1} exposure were observed *in vitro* for both hormone exposures.

Pharmaceuticals that are not designed for reproductive functions may also interfere with androgen synthesis. The *in vitro* interference of fibrate (gemfibrozil, clofibrate, clofibric acid), anti-inflammatory (ibuprofen, diclofenac), and anti-depressive (fluoxetine, fluvoxamine) drugs with key enzymes – C17,20-lyase and CYP11β – that are involved in androgen synthesis in gonads of male fish were investigated by Fernandes et al. (2011). Fluvoxamine and fluoxetine were the strongest inhibitors of C17,20-lyase and CYP11β enzymes at concentrations of 321–335 and 244–550 μM, respectively.

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and the active ingredient of Prozac. Usually detected <1 μg l^{-1}, fluoxetine and its metabolite norfluoxetine bioaccumulate in fish, particularly in the brain. In the Atlantic croaker *Micropogonias undulatus*, serotonin is involved in the neuroendocrine stimulation of reproduction by increasing LH release (Khan and Thomas 1992, 1994).

EE_2 exposure of juveniles can have delayed effects. Maunder et al. (2007) exposed juvenile sticklebacks to 1.75 and 27.7 ng l^{-1} EE_2 for 4 weeks post-hatch and reared them in clean water until they matured. Exposure to the higher concentration caused the occurrence of ovotestis in males, which had less intense nuptial coloration, built fewer nests, in which fewer eggs were deposited. The group exposed to 1.75 ng l^{-1} also built significantly fewer nests than controls.

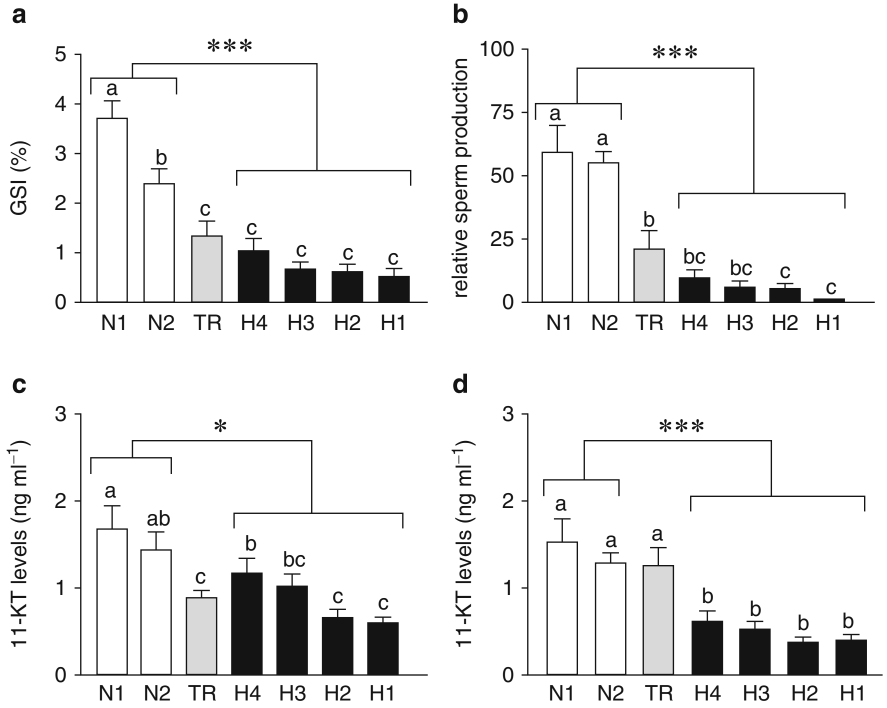


Fig. 5.1 Testicular development and endocrine function in male croaker collected from normoxic (N1, N2) and hypoxic (H 1,2,3,4) sites. (**a**) GSI (testicular growth). (**b**) Relative sperm production. (**c**, **d**) Plasma 11-KT levels. * = significant differences between normoxic and hypoxic sites. *** = highly significant differences between normoxic and hypoxic sites. Individual site differences indicated with different letters ($p < 0.05$) (Reprinted from Thomas et al. 2007: 2696, courtesy The Royal Society)

Hypoxia

It is interesting that exposure to low DO can also trigger reproductive endocrine disruption. Chronic environmental exposure of Atlantic croaker (*M. undulatus*) to hypoxia suppressed ovarian and testicular growth, associated with impairment of reproductive neuroendocrine function and decreases in hypothalamic serotonin (5-HT) and activity of the 5-HT biosynthetic enzyme, tryptophan hydroxylase (Thomas et al. 2007) (Fig. 5.1). Pharmacological restoration of hypothalamic 5-HT levels restored neuroendocrine function, indicating that the serotonergic neuroendocrine pathway is a major site of hypoxia-induced reproductive disruption.

Thomas et al. (2006) studied effects of 10 week exposures to low DO (2.7 and 1.7 mg l^{-1}) on reproductive responses in female *M. undulatus* in the laboratory, and in fish from hypoxic sites. Exposure to moderate hypoxia during ovarian recrudescence, both in the laboratory and field, impaired ovarian growth and decreased production of mature oocytes, associated with decreases in the estrogen signaling pathway for production of VTG. The results indicated that endocrine and morphological biomarkers are sensitive to moderate hypoxia, and are early warning indicators of reproductive failure. Sustained diel exposure to hypoxia was associated with smaller gonadosomatic index (GSI, gonad mass/body mass) and lower sex steroid concentrations in wild Gulf killifish (*F. grandis)* (Cheek et al. 2009). Testes and ovaries were significantly smaller under both moderate (2.61 mg l^{-1}, 0.6 h day^{-1}) and severe (0.93 mg l^{-1}, 3.4 h day^{-1}) diel hypoxia. Male 11-KT concentrations were lower under moderate hypoxia, while both testosterone (T) and 11-KT were significantly reduced under severe diel hypoxia, which may affect reproduction by inhibiting steroidogenic enzymes in the gonad. Reproductive success, growth, and physiological status under longer hypoxic episodes (5 h daily for 30 days) were examined by Cheek (2011). Growth, GSI, steroid hormone levels, and fertilization rate were unaltered by exposure to diel hypoxia, but at sites with diel hypoxia egg production was 50–85 % lower than at sites in the same estuary without daily hypoxia.

Climate Change

Susceptibility of *F. heteroclitus* to EE2 exposure, as indicated by increases in VTG gene expression changed with temperature. Liver *vtg1* mRNA was induced in males exposed to EE2. Males acclimated to 26 °C and exposed to 250 ng l^{-1} EE2 produced 3.5-fold more *vtg1* mRNA than EE2-exposed males acclimated to 10 °C, suggesting that they are more susceptible to EE2 under temperature increases that are expected with warming of coastal waters (Chandra et al. 2012).

Polluted Sites

Animals in polluted estuaries are exposed to complex mixtures of xenobiotics which can alter normal reproduction. Many effects have been reported in flatfish, which spend their lives in close contact with contaminated sediments. Lye et al. (1998) reported VTG induction and testicular abnormalities in male flounder (*Platichthys flesus*) from near a sewage discharge in NE England. More detailed surveys (Matthiessen et al. 2002) showed that VTG induction is widespread in the males of this species. It is worth noting that reduced VTG has occasionally been reported in females (e.g. Casillas et al. 1991) from contaminated sites, which might be attributable to antiestrogen or androgen exposure or to generalized stress, causing lower VTG synthesis, which could reduce normal egg development. Female sole from contaminated sites also have lower estrogen levels and inhibited ovarian development (Johnson et al. 1988). Significant levels of VTG were found in male English sole from several urban sites, with especially high numbers in Elliott Bay, along the Seattle Waterfront (Johnson et al. 2008). At the sites with male VTG production, the timing of spawning appeared altered.

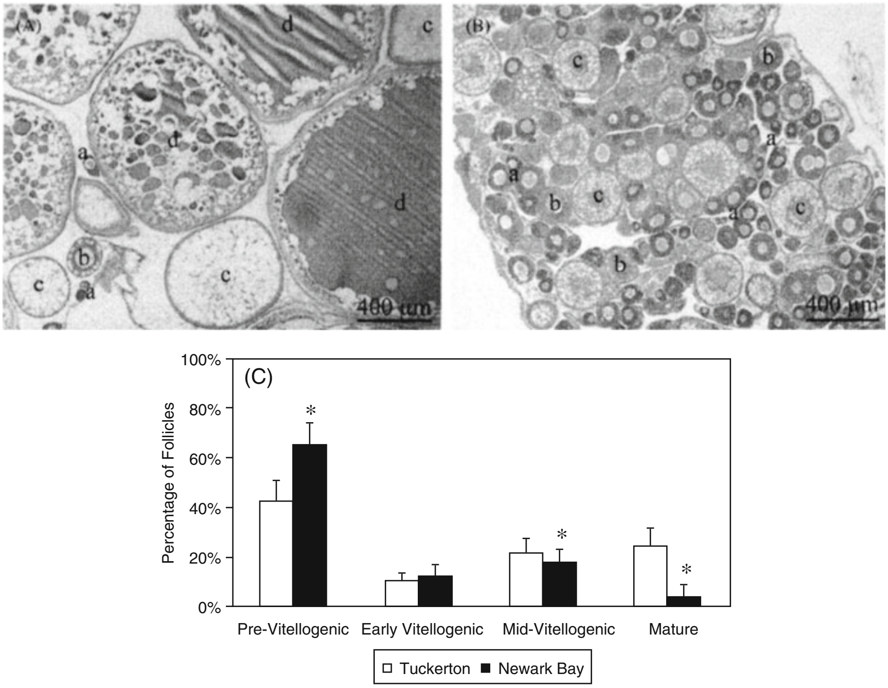


Fig. 5.2 Representative photomicrographs of (**a**) Tuckerton (reference site) and (**b**) Newark Bay ovaries. Previtellogenic (*a*), early vitellogenic (*b*), mid-vitellogenic (*c*) and mature follicles (*d*) are shown. (**c**) Ovarian follicle developmental stages. Mean ± SD. * = significantly different from Tuckerton, $p < 0.05$ (Reprinted from Bugel et al. 2010: 188, courtesy Elsevier Publishing Co)

Experimental exposure to pulp-mill effluent depressed testosterone in *F. heteroclitus* (Dubé and MacLatchy 2000), possibly via action on pituitary GTH secretion. Bugel et al. (2009) found impaired reproductive health in both sexes of *F. heteroclitus* in industrialized Newark Bay (NB), New Jersey. Males had decreased gonad weight, altered testis development and decreased gonadal aromatase mRNA expression; females had decreased gonad weight, inhibited gonadal development, decreased hepatic VTG production, and increased mRNA expression of gonadal aromatase, as well as fewer mature follicles (Fig. 5.2). NB fish also had reduced fecundity and lower hatching success, as well as lower mass and yolk-volume of eggs. Circulating 17β-estradiol levels in NB females were eight-fold lower than females from the reference site (Bugel et al. 2011).

Sewage plant effluent with anti-androgenic activity affected reproductive physiology and behavior of three-spined sticklebacks (*G. aculeatus*) exposed for 21 days. Levels of spiggin and VTG were unaffected, but male reproductive behavior was impaired (Sebire et al. 2011). Males in full strength effluent built fewer nests, and courtship behavior was reduced in 50 and 100 % effluent treatments. This is another example of behavior being more sensitive than the biochemical biomarkers. Wild gudgeon were collected upstream and downstream of urban and pharmaceutical manufacture effluents by Sanchez et al. (2011). Fish downstream of the effluent exhibited endocrine disruption including VTG induction, intersex and male-biased sex-ratio. These effects were associated with reduced population density.

5.1.4 Other Taxa

Polychaetes

Lewis and Watson (2012) have encouraged increased focus on reproductive endpoints in polychaetes. They suggest the following be investigated: (1) reproductive endpoints for the traditional 'model' species and those that have different reproductive traits to ensure broad ecological relevance; (2) Nereids and *Arenicola marina* be used to investigate the interaction of pollutants with the endocrine/environmental control of reproduction; (3) Use of polychaetes to assess male ecotoxicity effects; and (4) Assess emerging pollutants with reproductive endpoints. Long-term exposure to Cu-spiked sediment had deleterious effects on sperm and egg production in *N. virens* (Watson et al. 2013). Differences in the number of normal embryos produced by eggs fertilized with sperm from exposed males showed that sperm were more susceptible to toxicity, although eggs were also affected at higher concentrations.

Marine Mammals

Marine mammals are frequently top carnivores, and as such accumulate very high concentrations of chlorinated chemicals that can be endocrine disruptors. Although experimental studies are rare, there have been many correlative studies of which a few are presented here. Relationships between organochlorine compounds such as PCBs, DDTs, hexachlorobenzene, and oxychlordane, and hormones in Arctic mammals imply that these chemicals pose a threat to endocrine systems of these animals. The most pronounced relationships were reported with the thyroid hormone system, but effects are also seen in sex steroid hormones (Jenssen 2006). Pseudo-hermaphroditism in polar bears is thought to be an effect of EDCs. Over the past several decades, female polar bears at Svalbard, Norway have been reported with both female and male genitals (Wiig et al. 1998). The pseudo-hermaphrodites were genetically females but also had small penises. California sea lions had premature births, associated with accumulation of organochlorines (DeLong et al. 1973). Harbor seals from the Dutch Wadden Sea had low reproductive success and declining population numbers that were attributed to the impact of PCBs. Experimental studies showed that female harbor seals fed fish from the polluted Wadden Sea had a lower reproductive success (50 %) than seals fed less-contaminated fish. Implantation failure was found to be associated with reduced levels of 17β-estradiol (Reijnders 1986) induced by EDCs. Increasing levels of PCBs and DDE in the blubber of Dall's porpoises were found to have a negative association with testosterone in blood, which decreased in a statistically significant way with increasing DDE concentrations (Subramanian et al. 1987). These results collectively suggest that current levels of persistent organochlorines are causing an imbalance in sex hormones and subsequent reproductive abnormalities in marine mammals. Recent reduction of chlorinated organic chemical levels was correlated with improved reproductive status (Roos et al. 2012). In female sea otters, reproduction increased after 1990. In grey seals, pregnancy rate increased 1990–2010 and uterine obstructions ceased after 1993. The frequency of uterine tumors was highest 1980–2000. Organochlorine concentrations decreased at annual rates of between 3.5 and 10.2 %. The estimated mean concentration (mg/kg) for total-PCB decreased from 70 to 8 (otters), and from 110 to 15 (seals). The corresponding concentrations for ΣDDT decreased from 3.4 to 0.2 (otters), and from 192 to 2.8 (seals).

Corals

The scleractinian coral, *Oculina patagonica*, inhabiting contaminated vs. uncontaminated reference sites in the Mediterranean was investigated by Armoza-Zvuloni et al. (2012), who found significantly higher steroid levels in water and coral tissue from contaminated sites, suggesting that corals accumulate steroids from the surrounding waters. Despite their higher steroid levels, corals from the contaminated sites showed reproductive potential comparable to those of the reference sites.

5.2 Gametogenesis and Fecundity

Gametogenesis is the production of gametes. Spermatogenesis, which takes place in the testes, is the process by which male primordial germ cells called spermatogonia undergo meiosis, halving their number of chromosomes. The initial cells in this pathway are primary spermatocytes, which divide into two secondary spermatocytes, each of which divides into two spermatids. Thus, each primary spermatocyte gives rise four spermatids. These undergo development (spermiogenesis) into mature spermatozoa (sperm cells) under the influence of testosterone, by growing a tail (flagellum), and developing a thickened mid-piece, where mitochondria concentrate. Spermiogenesis also involves nuclear condensation, formation of the acrosomal cap from the Golgi apparatus, and removal of unnecessary organelles.

Oogenesis is the comparable process in females, Primary oocytes enlarge and begin to undergo meiosis. The primary oocyte is a very large cell containing many nutrients that will be important for the early embryo. It undergoes the first meiotic division, producing a secondary oocyte and another small cell called the first polar body. During cell division, most of the cytoplasm of the primary oocyte moves to the secondary oocyte. The first polar body may undergo a second meiotic division and its daughter cells degenerate. The secondary oocyte undergoes a second division, producing another polar body and one final ovum. The asymmetric division insures that the ovum retains most of the yolk. The mechanisms of oogenesis vary more between species than for spermatogenesis. For example, in most mammals only a few eggs are produced during an individual's lifetime whereas in other species such as fishes or sea urchins, thousands of eggs can be produced routinely. In the species that produce thousands of eggs, oogonia are stem cells that proliferate throughout the lifespan of the organism. In species that produce fewer eggs, the oogonia divide to form a limited number of egg precursor cells.

As animals approach the mating season, the gonads grow relative to the rest of the body. A measurement of the relative size of gonads is the gonosomatic index (GSI), a reduction of which is a frequently measured response to contaminants.

Observations of endocrine effects lead to the question of whether marine organisms that have experienced disturbances including estrogen alteration, VTG induction, perturbed steroid levels, intersex, etc. have been reproductively compromised, and whether populations are potentially at risk. It is also possible that toxicants can exert direct effects on developing gametes directly, without involving hormones.

5.2.1 Crustaceans

Metals

Hg and Cd, especially when acquired through food, produced decreases in ovarian development, egg production, yolk content of eggs, and hatching rates in the copepods *Acartia hudsonica* and *A. tonsa*. Exposure to dissolved Cd had no effect, but dissolved Hg did affect egg production (Fig. 5.3) (Hook and Fisher (2001). Different exposure routes produced different metal distributions: after water exposures most accumulation was in the exoskeleton, while dietary exposures caused most accumulation in internal organs, which is more likely to produce toxicity. Decreased reproduction was seen at metal concentrations only slightly higher than levels in coastal waters.

Effects of elevated Cu on egg production in the amphipod *Corophium volutator* were studied by Eriksson and Weeks (1994). The amphipods were exposed for 14 days to <0.1, 50 and 100 μg Cu l^{-1} resulting in increased total body Cu and reduced egg production. The effects of TBT on reproduction are not restricted to mollusks. While male hermit crabs *Clibanarius vittatus* exposed to TBT in the laboratory for 9 months showed no effects, exposed females displayed disorganization and atrophy of their ovaries, thus affecting reproduction (Sant'Anna et al. 2012). The amphipod, *Caprella danilevskii*, was exposed to TBT over a generation. Marked delays in growth and molting during the early developmental stages and maturation occurred at 100 and 1,000 ng l^{-1}. Inhibition of maturation and reproduction such as a decrease in the number of juveniles hatched was apparent in 10 and 100 ng l^{-1} (Ohji et al. 2003).

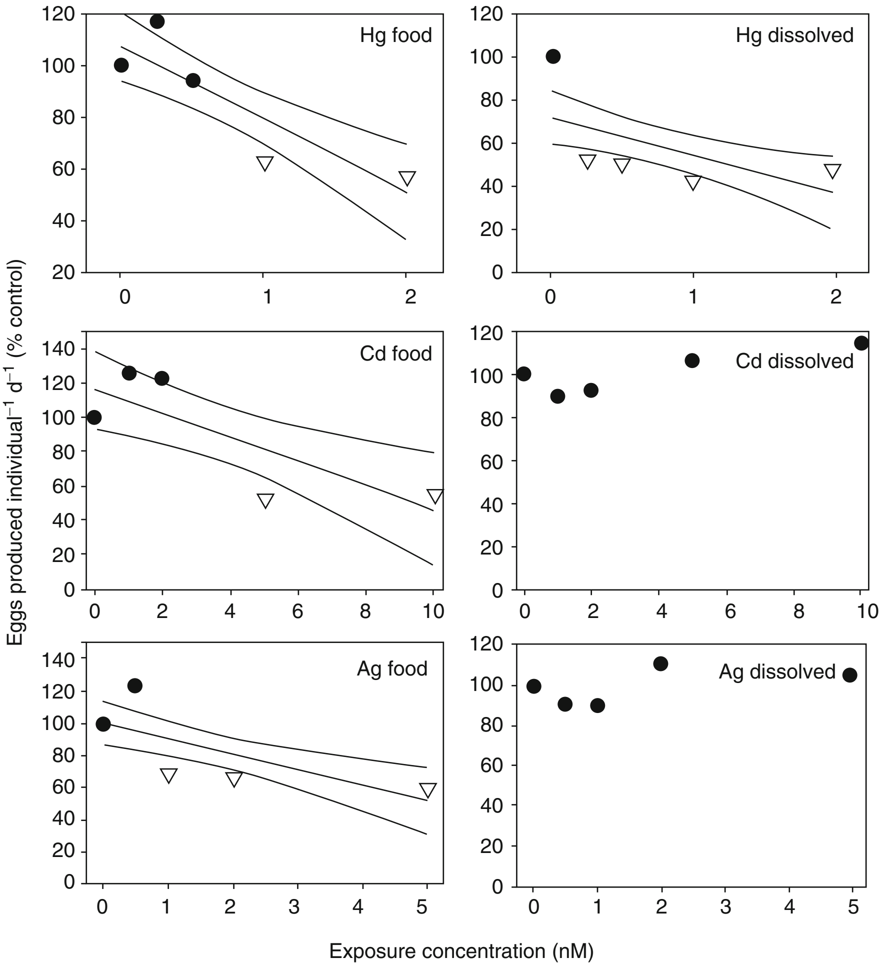


Fig. 5.3 Egg production individual^{-1} day^{-1} as % control in *Acartia tonsa* and *A. hudsonia* following exposure to dietary (*left*) or dissolved (*right*) Ag, Hg, and Cd. Egg production significantly lower than controls when food exposed to 1 nM Hg or 5 nM Cd (*open triangles*). Egg production lower than controls at 0.25 nM dissolved Hg. *All lines* shown are significant ($p < 0.05$) (Reprinted from Hook and Fisher 2001: 1134, courtesy of Springer Publishing Co)

Organics

Male grass shrimp (*P. pugio*) exposed to 63 μg l^{-1} pyrene had delayed molting and time until reproduction, as well as elevated ethoxycoumarin *o*-deethylase (ECOD) activity. Pyrene did not affect females, but their offspring had elevated mortality (Oberdörster et al. 2000). The authors hypothesized that vitellin binds pyrene, making it unavailable to adult females, resulting in maternal transfer of pyrene to the embryos. This would account for the lack of effect on females, and reduced survival of their offspring.

Jensen and Carroll (2010) examined reproduction of *Calanus* spp. exposed to the WSF of crude oil. While egg production in *C. glacialis* exposed to 10.4 and 3.6 μg l^{-1} was unaffected, after eggs were transferred to clean seawater, hatching success was significantly lower in the high dose group. Exposure of *Tigriopus japonicus* to PCB 126 (3,3′,4,4′,5 pentachlorophenol) resulted in increased sensitivity in successive generations of this copepod. While body size was most sensitive, reproduction and intrinsic population growth were reduced at 1 μgl^{-1} in the F1 generation (Guo et al. 2012).

Acidification and Climate Change

Fitzer et al. (2012) determined reproductive response (naupliar production and growth) of the copepod *Tisbe battagliai* over three generations at pH 7.67, 7.82, 7.95, and 8.06. Naupliar production increased at pH 7.95, followed by a decline at pH 7.82, the increase at 7.95 attributed to hormesis. A multi-generational model predicted a gradual decline in naupliar production and growth over the next 100 years. Effects of seawater pH levels (8.2, 7.6 and 6.9) on the reproduction of *Calanus glacialis*, an Arctic copepod, were examined (Weydmann et al. 2012). Low pH did not affect egg production, but pH 6.9 delayed hatching and reduced hatching success. The results indicate that copepods are not very susceptible to acidification. However, studies have been over short periods and have only considered impacts of elevated CO_2. Authors encouraged long-term exposures examining synergistic effects of acidification and warming. Bergey and Weis (2008) observed a much longer breeding season for fiddler crabs (*Uca pugnax*) in New Jersey (US) compared to what had been reported in the 1970s. It was suggested that climate change might have been responsible for the lengthened breeding season.

Hypoxia

Wiklund and Sundelin (2001) investigated effects of hypoxia on reproductive variables in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* including unfertilized/ undeveloped eggs, dead eggs and females carrying a dead brood. Low oxygen (2–6 mg O_2 l^{-1}), resulted in more females carrying dead broods; females exposed to hypoxia had a lower fertility rate than controls. Similarly, the amphipod *Melita longidactyla* was impaired by moderate DO levels (3.5–4.5 mg O_2 l^{-1}), higher than levels considered hypoxic (2.8 mg O_2 l^{-1}). Negative growth and decreases in respiratory energy expenditure were noted after exposure to moderately low DO for 3 weeks. Complete reproductive failure occurred after exposure to 3.5 mg O_2 l^{-1} for 1 month, but no significant effect on percentage copulation, number of broods and offspring or fecundity was seen at 4.5 mg O_2 l^{-1}, indicating that reproductive impairment occurs below 4.5 mg O_2 l^{-1} (Wu and Or 2005). In contrast, Brouwer et al. (2007) found that chronic hypoxia appeared to enhance grass shrimp (*P. pugio*) reproduction. Females exposed to 2.5 mg O_2 l^{-1} had higher fecundity, and a greater percentage produced repeated broods than normoxic shrimp. The hypoxic shrimp took longer to produce their first brood than controls, but starved larvae from hypoxia-exposed mothers lived longer than those from controls. Shrimp exposed to severe hypoxia (1.5 mg O_2 l^{-1}) also had higher fecundity than controls, although embryos from hypoxia-exposed mothers took longer to hatch than control embryos. This species lives in eutrophic estuaries and seems to be quite resilient to hypoxia.

Polluted Sites

Egg membranes of the mole crab *Emerita analoga* near the San Onofre nuclear plant ruptured soon after egg extrusion (Siegel and Wenner 1984). These females had a smaller size at the onset of egg production and a later onset of reproduction than in areas north or south. This may have been due to a failure of overwintering (Wenner 1988). When crabs from the affected area were brought into the laboratory they extruded eggs that developed normally, but their molt rate and molt increment were depressed.

Exposure of copepods, *Tigriopus californicus*, to contaminated sediments from Puget Sound resulted in delays in the period of peak reproduction and reductions in the total number of nauplii produced by each female (Misitano and Schiewe 1990).

5.2.2 Mollusks

Metals

The giant sea scallop, *Placopecten magellanicus* was exposed to Cu or Cd at 20 μg l^{-1} for 7 weeks. In scallops undergoing early gametogenesis, Cd promoted early gamete maturation, while Cu inhibited it (Gould et al. 1985). In scallops with fully differentiated gonads, however, Cu induced gonad regression. Gonads accumulated high levels of the metals. *Mya arenaria* were collected at different sites along the St. Lawrence estuary. Near an active harbor, clams had high levels of TBT and DBT in gonads, along with a lower gonadosomatic index, low progesterone levels and delayed sexual maturation compared to the reference site. Sites with intermediate levels of TBT exhibited intermediate responses of hormones and maturation stages (Siah et al. 2003).

Organics

Dioxin (TCDD)

2,3,7,8-TCDD alters normal development of reproductive organs and early development in bivalve mollusks at 2–20 pg g^{-1} wet weight. In both *C. virginica* and *M. arenaria*, 2,3,7,8-TCDD accumulates in gonads, and in oysters 10 pg g^{-1} caused histopathological lesions by day 14 of gametogenesis in both sexes, resulting in complete inhibition of gonadogenesis. A total body dose of 2 and 10 pg g^{-1} caused abnormal gametogenesis in female and male oysters, respectively, including incomplete oocyte division, inhibition of oocyte growth and maturation, unsynchronized sperm development, and inhibition of spermatogenesis (Wintermyer and Cooper 2007). The sensitivity of gonad maturation is likely due to disruption of cross-talk between steroid, insulin, and metabolic pathways involved in gonad differentiation. Altered gonad development and decreased veliger larval survival can partially explain the lack of self-sustaining bivalve populations in 2,3,7,8-TCDD-contaminated estuaries (Cooper and Wintermyer 2009).

Oil

Mytilus edulis were exposed to dispersed crude oil (0.015–0.25 mg l^{-1}) by Baussant et al. (2011). After 1 month in 0.25 mg l^{-1}, alkali-labile phosphates and the volume and density of atretic oocytes in females were elevated, indicating that oil affected VTG-like proteins and gamete development. Parental oil exposure did not affect subsequent fertilization success, but caused slower development, abnormalities, and reduced larval growth, effects that were enhanced when larvae were raised at 0.25 mg oil l^{-1}.

Pesticides

Akcha et al. (2012) investigated effects of the herbicides glyphosate and diuron on oyster gametes and embryos. Glyphosate had no effect, while diuron significantly affected embryo-larval development from the lowest tested concentration of 0.05 μg l^{-1}, an environmentally realistic concentration. The alkaline comet assay showed diuron had genotoxic effects on sperm at the lowest tested concentration, but did not effect sperm mitochondrial function or acrosomal membrane integrity.

Contaminants of Emerging Concern

Pang et al. (2012) compared effects of CuO nanoparticles with aqueous Cu ($CuCl_2$). They added copper to the sediment as aqueous Cu, nano- (6 nm) and micro- (<5 μm) CuO particles and examined effects on the deposit-feeding snail, *Potamopyrgus antipodarum.* After 8 weeks of exposure to nominal concentrations of 30, 60, 120 and 240 μg Cu g^{-1} dry weight sediment, nano-CuO had greater effects on reproduction than copper added as either micro-CuO or aqueous Cu.

Polluted Sites

Tlili et al. (2011) noted differences in the gametogenic cycle of the clam *Donax trunculus* from polluted and unpolluted sites. The spawning period began in March and was maximum in May at both sites, but the percentage of spawners was higher and the spawning period was shorter at the polluted site. Energy reserves (glycogen, lipids) were lower in clams from the polluted site, suggesting that energy was being shunted to deal with the chemical stress. Scallops *Mizuhopecten yessoensis* from six stations in Peter the Great Bay (Sea of Japan) were studied (Vaschenko et al. 1997). Those from polluted sites had retarded gametogenesis, oocyte resorption, and autolysis of spermatozoa. They had more hermaphrodites, decreased fertilization success, reduced percent of normal larvae, and retardation of larval growth. Clams, *Potamocorbula amurensis* from a silver-contaminated site in San Francisco Bay had a low percentage of reproductive individuals, <60 %. When Ag tissue concentrations decreased, the proportion of reproductive individuals increased to 80–100 % (Brown et al. 2003). There was a negative correlation of tissue Ag and percent of reproductive individuals (Fig. 5.4), and no correlation with other environmental variables.

5.2.3 Fishes

Organics

Pesticides and Industrial Chemicals

Exposure to Kepone or *o,p′*-DDD (100 nM–100 μM) prevented most of the oocytes of *M. undulatus* from completing germinal vesicle breakdown (GVBD); many were arrested at the lipid coalescence or germinal vesicle migration stage after exposure to100 μM (Ghosh and Thomas 1995). In addition, clearing of the ooplasm, oil droplet formation and hydration were incomplete in oocytes that did undergo GVBD. The pesticides inhibited GVBD in a concentration-dependent manner. Exposure to either pesticide for as little as 1 min could block GVBD. Washing the follicle-enclosed oocytes after exposure restored their ability to undergo GVBD.

Paclobutrazol (PBZ), a triazole-containing fungicide, is widely used in agriculture. Li et al. (2012) investigated effects of PBZ at environmentally relevant concentrations on testicular development in male rockfish *Sebastiscus marmoratus.* Exposure to 10, 100 and 1,000 ng l^{-1} for 50 days did not alter the GSI, but reduced the number of mature sperm and late stage spermatocytes in the testes.

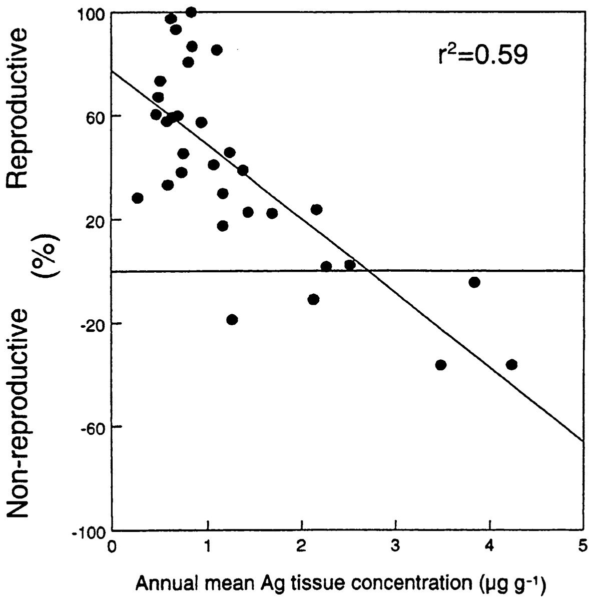


Fig. 5.4 Correlation between annual proportions (%) of reproductive clams (*Potamocorbula anurensis*) with annual mean Ag concentration at four sites. Y-axis = central tendency of the reproduction data: the proportion of clams that were reproductively active (% active + % ripe + % spawned) minus the proportion that were non-reproductively active (% inactive + % spent) (Reprinted from Brown et al. 2003: 110, courtesy Taylor & Francis)

Juvenile Atlantic cod (*Gadus morhua*) were fed different alkyl phenols (APs) (4-*tert*-butylphenol, 4-*n*-pentylphenol, 4-*n*-hexylphenol and 4-*n*-heptylphenol) for 20 weeks during vitellogenesis (Meier et al. 2011). While 60 % of the females and 96 % of the male controls were mature at the end of the experiment, exposure to APs and E_2 had different effects depending on the developmental stage. Juvenile females advanced into maturation, but gonad development was delayed in maturing females and males. The AP-exposed groups (>4 μg/kg body weight) had increased numbers of mature females, suggesting that AP-exposure affects the timing of onset of puberty at extremely low concentrations.

Sea-Nine® 211 (4,5-dichloro-2-*n*-octyl-3(2H)-isothiazolone) is widely used as an antifouling biocide after the banning of TBT. Ito et al. (2013) found testicular toxicity in mummichog *Fundulus heteroclitus*, after 28-days exposure. Although Sea-Nine® 211 did not affect germ cell proliferation, the number of apoptotic spermatocytes was increased in 1.0- and 3.0-μg l^{-1}-exposed groups. The numbers of cysts expressing caspases 2, 3, 6, and 8 (apoptosis-associated proteins) were increased in the 1.0-μg l^{-1} group, and the signal intensity of an anti-apoptotic protein Bcl-xL was reduced in a dose-dependent manner. This suggests that Sea-Nine® 211 induces apoptosis in the testicular germ cells of mummichogs via a caspase-dependent pathway.

Oil/Hydrocarbons

When female winter flounder (*Pseudopleuronectes americanus*) and their developing oocytes were exposed to #2 fuel oil at 100 $\mu g\ l^{-1}$, there was delayed hatching, reduced viable hatch, and increased incidence of malformations. Larvae raised in clean water after exposure only during parental gametogenesis had elevated mortality and slower growth (Kuhnhold et al. 1978).

Produced water discharged from offshore oil industry activities contains toxic substances including PAHs. Reproductive biomarkers were studied by Sundt and Bjorkblom (2011) in prespawning Atlantic cod (*Gadus morhua*) exposed for 12 weeks. Results showed that exposure to sufficiently high levels of PW produced an increase in VTG levels in females, as well as impaired oocyte development and reduced estrogen levels. In males testicular development was altered, showing a rise in amount of spermatogonia and primary spermatocytes and a reduction in mature sperm in the exposed fish compared to control.

Sun et al. (2011) investigated effects of phenanthrene (PHE) at environmentally relevant concentrations on testicular development in *Sebastiscus marmoratus*. After 50 days exposure, the GSI and percentage of sperm produced showed a U-shaped dose response. The levels of gonadotropin releasing hormone, follicle-stimulating hormone, luteinizing hormone mRNA, 17β-estradiol, and γ-glutamyl transpeptidase activity all showed a U-shaped dose responses, which demonstrated the U-shaped effects on spermatogenesis. A U-shaped dose–response curve is well recognized as a hormetic phenomenon. PHE accumulation in the brain also showed an inverse U-shaped increase.

Climate Change

Since rising temperatures are changing the phenology (timing) of reproduction in many taxa, it is not surprising that effects are seen in fishes. Effects were reviewed by van der Kraak and Pankhurst (1997). There have been many more reports since then. Recently, Zucchetta et al. (2012) studied relationships between changes in water temperature and the timing of reproductive investment of the grass goby *Zosterisessor ophiocephalus* in the Venice lagoon. A time series of the monthly GSI was coupled with thermal profiles of water temperatures from 1997 to 2010. Reproductive investment was positively affected by warmer water, in terms of monthly thermal anomalies and cumulative degree days. A predictive model to assess the shift of reproductive peaks in response to thermal fluctuations indicated that in warmer years, the reproductive peak occurred earlier than during colder years.

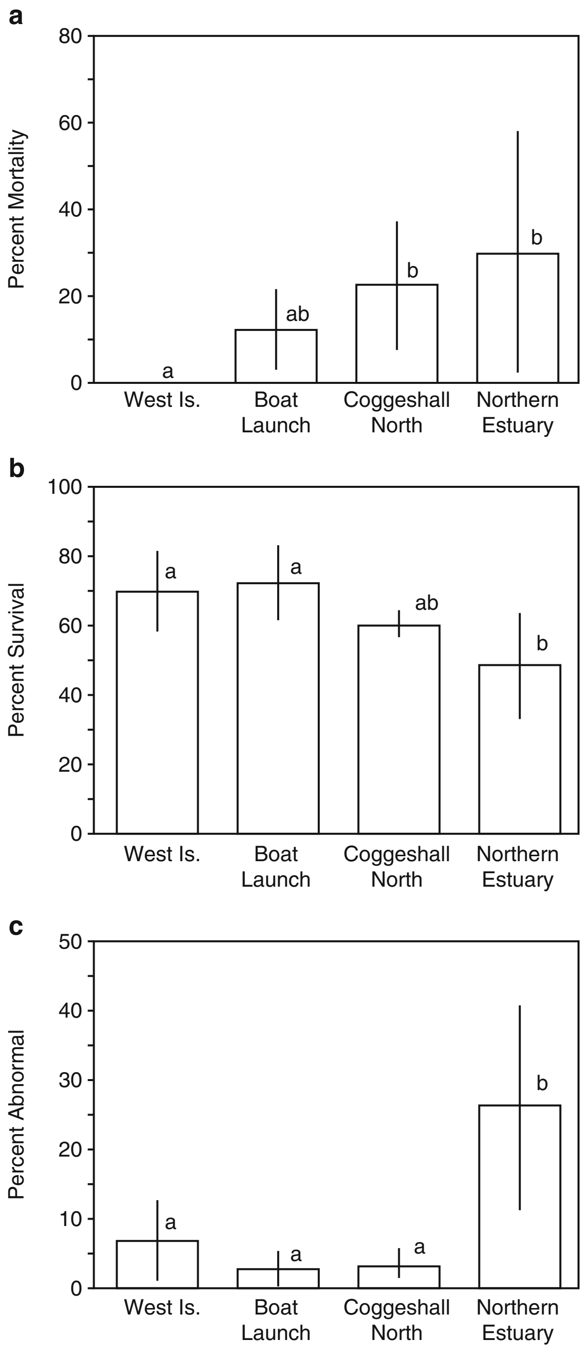
Polluted Sites

A number of field studies have linked reduced hatching success and fry survival to increased levels of lipophilic contaminants in eggs. For example, elevated PCBs in Baltic flounder (*P. flesus*) ovaries were correlated with impaired egg development and fry survival (Westernhagen et al. 1981); a similar study of Baltic herring (*Clupea harengus*) found that ovarian DDE residues of >18 ng g^{-1} wet wt. or PCB residues >120 ng g^{-1} wet wt. were associated with reduced viable hatch (Hansen et al. 1985). High larval mortality and reduced hatching success in Baltic cod (*Gadus morhua*) have also been associated with organochlorines (Petersen et al. 1997). Annual investigations of the health status of female perch (*Perca fluviatilis*) from the Baltic Sea were undertaken by Hansson et al. (2006). Fish were sampled at three coastal sites in Sweden: two in the Baltic Proper and one in the Bothnian Bay. In all, 19 biochemical, physiological and histopathological variables were measured. The most important observation was decreased gonadosomatic index (GSI) in the Baltic proper. The reduced gonad size indicates that unidentified pollutants affect reproduction even in a reference area in the Baltic proper.

Cross and Hose (1988) and Hose et al. (1989) found that a population of white croaker (*Genyonemus lineatus*) from a DDT-contaminated site had early oocyte destruction, preovulatory atresia, lower fecundity and/or spawning inducibility, and lower fertilization success than reference populations. Similarly, flatfish (*P. bilineatus* and *P. vetulus*) from contaminated areas of Puget Sound have precocious sexual maturation, retarded gonadal development, reduced egg weight, and reduced spawning success (Johnson et al. 1998). These fish are contaminated with a variety of chemicals, including aromatic hydrocarbons and PCBs, which are potential causative agents, either as antiestrogens (PAHs) or estrogen-mimics (some PCBs). These English sole have reduced viability of eggs and larvae (Casillas et al. 1991). Starry flounders (*Platichthys stellatus*) from polluted San Francisco Bay had reduced embryo development and hatching success, associated with PCBs in the eggs (Spies et al. 1985). Winter flounder (*P. americanus*) from industrial areas in Boston Harbor had reduced egg size, fertilization success, viable hatch and larval size compared to reference fish (Nelson et al. 1991). *F. heteroclitus* from the PCB-contaminated New Bedford Harbor (MA) produced as many eggs as those from reference sites, but their progeny had reduced survival and increased malformations (Black et al. 1998a) (Fig. 5.5). When adult females were given IP injections of PCBs, egg production was reduced by 77 % at the highest dose (19*u*g PCB per gram of dry liver) (Black et al. 1998b).

Mummichogs near a bleached kraft pulp mill in Canada had delayed gonadal maturation and reduced egg size (Leblanc et al. 1997), but higher fecundity and GSI at their reproductive peak than fish at the other sites. Nesting plainfin midshipman *Porichthys notatus* were collected from areas with low and high contamination on Vancouver Island, British Columbia. Males in contaminated areas had more testicular asymmetry, sperm with shorter heads, and fewer live eggs in their nests (Sopinka et al. 2012).

Fig. 5.5 Survival and reproduction of *F. heteroclitus* collected from New Bedford Harbor and West Island, MA, USA and held under laboratory conditions for 5 weeks. Northern Estuary site is closest to PCB hotspot. (**a**) Adult female mortality (**b**) embryo and larval survival and (**c**) spinal abnormalities in larvae. *Bars* with *different letters* are statistically different p < 0.05. Error Bars = SD (Reprinted from Black et al. 1998a: 1411), courtesy John Wiley and Sons



5.2.4 Other Taxa

Corals

Oil

Oil damages the reproductive system of corals resulting in fewer breeding colonies, fewer ovaries per polyp, fewer planula larvae, premature shedding of planulae, abnormal behavior of planulae, and lower growth rates (Loya and Rinkevich 1980). A review of effects of dispersants indicated that they enhanced the damage. Sublethal effects of oil on coral reproduction were evaluated 39 months after a spill in Panama using *Siderastrea siderea* at oiled and unoiled reefs. The number of reproductive colonies and number of gonads per polyp did not differ, but gonads were larger at unoiled than at oiled reefs during spawning periods. Years after the spill, injuries, reduced colony size, and decreased size of gonads at oiled reefs can reduce the number of reproductively viable colonies (Guzmán and Holst 1993).

Effects of the water-accommodated fraction (WAF) of a natural gas condensate on reproduction of the brooding coral *Pocillopora damicornis* were studied in laboratory experiments (Villanueva et al. 2011). Exposure during gametogenesis did not inhibit subsequent production of larvae, but exposure of gravid corals to >25 % WAF during early and late embryogenesis caused abortion and early release of larvae, with higher percentages of larvae expelled by corals in higher concentrations. Aborted larvae were small, had low metamorphic competency, and were white with a pale brown oral end (indicating low density of zooxanthellae).

Sea Anemones

Responses of the sea anemone *Actinia equina* to oil were investigated by Ormond and Caldwell (1982). Exposure to 2.5 ml l^{-1} crude oil for 7 weeks resulted in ejection of increased numbers of the young which are normally brooded within the gastric cavity. Subsequently the numbers of surviving young being produced fell to zero, and the ovaries were found to be regressed and lacking ova.

Polychaetes

Late gametogenic *Nereis virens* were incubated for up to 2.5 months in environmentally relevant concentrations of copper-spiked sediment by Watson et al. (2012). Sediments were spiked with cupric nitrate solutions to give nominal concentrations of 50, 500 and 1,000 mg kg^{-1} (dry weight) and non-spiked sediment was also included as a control. Oocytes were significantly smaller at higher concentrations. Spawning of males took place a number of days earlier in the higher concentrations. Differences in the number of embryos developing normally after *in vitro* fertilizations of oocytes fertilized with sperm from exposed males and non-exposed males showed that sperm were more susceptible to toxicity, but oocytes were also affected at the highest concentration. These results show that there are direct and indirect reproductive consequences of parental exposure to copper with implications for recruitment and colonization of polluted sediments.

5.3 Mating and Fertilization

The majority of marine animals reproduce by spawning, the release of sperm by males and eggs by females into the water column. This requires coordinated timing of gamete release, and fertilization takes place in the water column. Some groups, including decapod crustaceans, have mating, in which males release sperm into females and fertilization takes place internally. There may be elaborate behavior preceding mating, such as release of pheromones in some male crabs to attract females, or waving behavior by male fiddler crabs. Contaminants may affect these processes by interfering with the chemical or visual communication determining timing of gamete release or altering mating behaviors.

The fertilization process is initiated by the acrosome at the anterior end of the sperm contacting the egg. As the sperm approaches the egg, the acrosome reaction occurs – the membrane surrounding the acrosome fuses with the plasma membrane of the egg, releasing the contents of the acrosome. The contents include enzymes that break through the egg coat, allowing fertilization to occur. At this point the egg undergoes a cortical reaction. Cortical granules are secretory vesicles just below the egg's plasma membrane. When the fertilizing sperm contacts the egg plasma membrane, it triggers fusion of the cortical granule membranes with the egg plasma membrane, liberating the contents of the granules into the extracellular space. The granule contents modify a protein coat on the outside of the plasma membrane so that it is released from the membrane and elevates, as the fertilization membrane, which prevents further sperm from penetrating. Contaminants can affect fertilization by impairing sperm swimming or their ability to fertilize eggs. Contaminants can affect egg cells by prematurely triggering a cortical reaction, so that no sperm can fertilize them.

5.3.1 Crustaceans

Seuront (2011) examined whether the WSF of diesel oil (0.01, 0.1 and 1 %) affected male copepods' (*Temora longcornis*) ability to locate, track and mate with females. All concentrations impacted mating behavior and mating success. The ability of males to detect female pheromone trails, follow trails and track a female decreased with increasing oil concentrations, leading to decreased contact and mating (Fig. 5.6). Poulton and Pascoe (1990) devised a behavioral bioassay for pollutant stress based on disrupting the mating behavior (precopula) of amphipods.

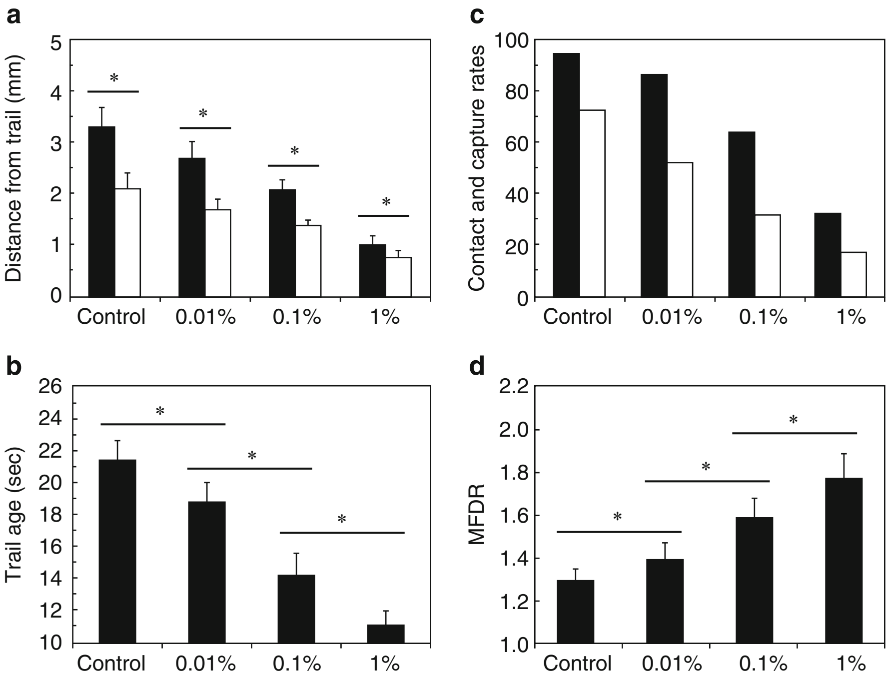


Fig. 5.6 *Temora longicornus* (**a**) Male distance from female track-line at detection of female trail (*black bars*) and during tracking (*white bars*). (**b**) Age of female's trail at detection by male. (**c**) Male–female contact rate (*black bars*) and capture rate (*white bars*). (**d**) Male-to-female displacement ratio (MFDR). Error bars = SD. * = significant differences ($p < 0.05$) (Reprinted from Seuront 2011: art. e26283, Open access)

5.3.2 Mollusks

Treatment of *Mytilus edulis* sperm with Cu or Zn (0.1–3.3 mM) decreased sperm motility; Zn was more inhibitory than Cu and produced greater mitochondrial damage, as revealed by transmission electron microscopy, than Cu (Earnshaw et al. 1986). This is a reversal of the usual relative toxicity of Cu and Zn. The reduction of sperm motility can be explained by respiratory inhibition. However, Zn had a less pronounced effect on sperm motility than on respiration.

5.3.3 Fishes

A particular characteristic of the teleost egg is the chorion, the outer protective membrane that is initially synthesized in the ovary. A canal, the micropyle, forms a pore in the membrane, through which sperm must pass in order to fertilize the eggs. Once a sperm penetrates the egg, a cortical reaction occurs, in which vesicles in the outer layer of the egg release material that blocks the micropyle to prevent polyspermy, and elevates the fertilization membrane, increasing its ability to act as a barrier. There have been relatively few studies of the impact of contaminants on gametes prior to fertilization.

Metals

Exposure of mummichog (*F. heteroclitus*) sperm to 0.01 mg l^{-1} meHg or mercuric chloride for as little as 1 min reduced fertilization success, while similar exposure of eggs had no effect on fertilization or subsequent development (Khan and Weis 1987a, b). Reductions in sperm motility were seen in treated groups. Sperm from fish from Piles Creek (PC) a polluted site in New Jersey, were unaffected by 0.01 or 0.05 mg l^{-1} meHg until they were exposed for 5 min, showing tolerance in fish from this site. Higher concentrations of either $HgCl_2$ or meHg and longer exposures of unfertilized eggs were needed to reduce fertilization and/or produce abnormalities in embryos that were subsequently raised in clean water (Khan and Weis 1987c, d). Thus Hg incorporated prior to fertilization could produce embryonic malformations in eggs that were successfully fertilized, another example of delayed effects. Eggs from PC fish were more tolerant of meHg exposure. The two forms of mercury had different mechanisms to reduce fertilization: meHg triggered a cortical reaction, preventing sperm from entering the micropyle, while $HgCl_2$ caused a swelling of the lip of the micropyle, reducing its diameter and possibly impeding sperm from swimming through (Khan and Weis 1993).

Organics

Pesticides

Exposure of mature male salmon to some currently used pesticides inhibited male olfactory detection of female pheromones that are involved in synchronization of spawning between the sexes (Moore and Waring 1996; Waring and Moore 1997). Exposure for 30 min to 1 μg l^{-1} diazinon suppressed olfactory responses. Physiological responses of males to female urine, such as increased milt volume and level of sex hormones were reduced after 120 h exposure to 0.3 μg l^{-1} diazinon or 0.04 μg l^{-1} atrazine.

Exposure to subchronic levels of the herbicide glyphosate (Roundup) caused a significant decrease in the number of copulations and mating success in male cyprinodontid fish, *Jenynsia multidentata* (Hued et al. 2012). Reproduction of Atlantic salmon was impaired by the pyrethroid insecticide Cypermethrin at 0.1 μg l^{-1}. Affected fish had reduced fertilization rates, as well as lower hormone levels in males (Moore and Waring 2001). This pesticide is used to treat salmonids in aquaculture for sea lice, so farmed fish and nearby wild fish can be exposed to high levels of this chemical.

Oil

Toxicity of dispersed weathered crude oil to early life stages of Atlantic herring (*Clupea harengus*) was tested for short exposures, from 1 to 144 h (McIntosh et al. 2010). Crude oil dispersed with Corexit® 9500 was very toxic to gametes and dramatically impaired fertilization success. For brief exposures, gametes and free-swimming embryos were the most sensitive life stages. Male American plaice (*Hippoglossoides platessoides*) exposed to sediments contaminated with PAHs and PCBs had 30–50 % reduction in hatch of eggs fertilized with their sperm (Nagler and Cyr 1997).

Contaminants of Emerging Concern

Female three-spined sticklebacks were fed with freeze-dried chironomids contaminated with low or high doses of polybrominated diphenyl ethers (PBDEs), polychlorinated naphthalenes (PCNs), or PCBs for 3.5 months (Holm et al. 1993). No significant difference in number of eggs was found, but while spawning success in the controls was 80 %, it was 20 and 25 % in the groups that received high doses of PBDE or PCB, respectively. Levels of PBDE accumulated in the low- and high-dose groups were 861 ± 271 and 1,630 ± 275 mg/kg fat, respectively, whereas the corresponding concentrations of PCN in the PCN groups were 845 ± 43 and 1,929 ± 72 mg/kg fat, respectively. Concentrations of PCB in fish from the PCB groups were 1,972 ± 158 and 3,594 ± 521 mg/kg fat, respectively. Morphological examination of the liver revealed pronounced lipid accumulation in all exposed groups.

5.3.4 Other Taxa

Sea urchin fertilization success is a commonly employed bioassay, so considerable work has been done on *Strongylocentrotus* and other sea urchin species on toxicity of metals and organic contaminants to fertilization (reviewed by Dinnel et al. 1989). A few more recent studies are reviewed here.

Metals

Fertilization in corals can impeded by some metals. Reichelt-Brushett and Harrison (2005) examined effects of Cu, Pb, Zn, Cd, and Ni on fertilization success of gametes of the corals *Goniastrea aspera, Goniastrea retiformis, Acropora tenuis,* and *Acropora longicyathus*. The EC_{50} values (concentration that reduces the fertilization rate by 50 %) for Cu was 15–40 $\mu g\ l^{-1}$, while other metals were much less toxic. Hédouin and Gates (2013) investigated how Cu alters fertilization success of the coral *Montipora capitata* over several nights of spawning and found that gametes are sensitive to Cu, with EC_{50} after 3 h ranging from 16.6 to 31.7 μg l^{-1}. In addition, the sensitivity of the gametes was affected by the night of spawning during which fertilization experiments were performed. This likely reflected changes in the quality of gametes over the spawning period.

Populations of the echinoderms *Asterias rubens* and *Echinus acutus* that occur naturally along a contamination gradient of Cd, Cu, Pb, and Zn in a Norwegian fjord were studied. Sperm motility, a measure of sperm quality, was quantified using a computer-assisted sperm analysis system. The RNA/DNA ratio, a measure of protein synthesis, was also assessed. Although both species accumulated metals at high concentrations, neither sperm motility in *A. rubens* nor the RNA/DNA ratio in either species were affected (Catarino et al. 2008).

Organics

The chlorinated pesticides methoxychlor, dieldrin, and lindane affect fertilization and early development of sea urchin, *Paracentrotus lividus*. Pesando et al. (2004) observed that fertilization decreased when sperm were incubated for various period of time with 100 μM of dieldrin or lindane. Treatment of eggs (1 h with 100 μM) did not prevent fertilization, but increased the rate of polyspermy, delayed or blocked the first mitotic divisions, and altered early embryonic development.

Oil mixed with dispersants is highly toxic to coral early life stages. Coral are extremely sensitive to the combined effects, with fertilization failure in the presence of dispersant and dispersed oil, compared with mostly successful fertilization in the presence of oil alone (Negri and Heyward 2000; Shafir et al. 2007; Epstein et al. 2000). Negri and Heyward (2000) studying *Acropora millepora* found that 20 % v/v PFW (production formation water) inhibited fertilization by 25 %. This was equivalent 0.0721 mg l^{-1} total hydrocarbon (THC). Crude oil WAF did not inhibit fertilization unless dispersant was added. Dispersed oil was slightly more toxic to fertilization than dispersant alone, suggesting toxicity may be additive. The minimum concentration of dispersed oil which inhibited fertilization was 0.0325 mg l^{-1} THC.

To study effects of chronic exposure to produced water (an oil production effluent) on gametogenesis and gamete performance of the purple sea urchin (*Strongylocentrotus purpuratus*) Krause (1994) caged urchins at varying distances from an outfall. Those living closer to the outfall produced larger gonads. Gamete performance was measured using a fertilization bioassay that held eggs constant and varied the amount of sperm added. The proportion of eggs fertilized under each sperm concentration increased with distance from the outfall, indicating that although the exposed adults had larger gonads, they showed a marked decrease in gamete performance.

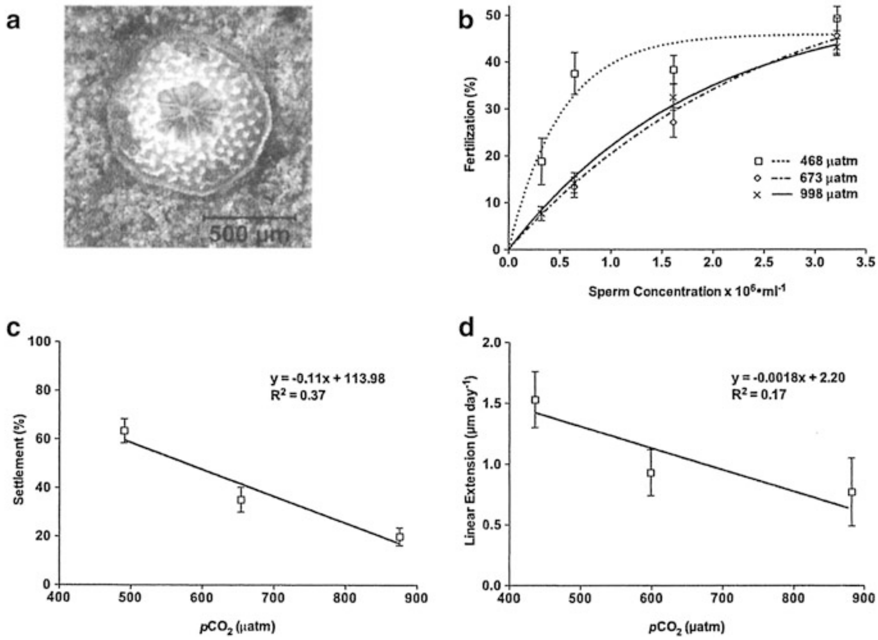


Fig. 5.7 Results of fertilization, settlement and growth experiments (mean ± SE). (**a**) 26-day old *A. palma* juveniles reared under ambient CO_2. (**b**) Nonlinear regressions of fertilization data by CO_2 treatment. (**c**) Percent settlement, (**d**) Linear growth (*u*m day^{-1}) of juveniles over 50 days (Reprinted from Albright et al. 2010: 20401), courtesy National Academy of Sciences)

Climate Change/Acidification

For many marine invertebrates, sperm flagellar motility is likely initiated when intracellular pH is elevated and suppressed when it decreases. The fertilization potential of eggs may also be influenced by changes to internal pH.

Corals

Elevated pCO_2 negatively affected fertilization success of the coral, *Acropora palmata*, but the effect was due to reduced sperm concentration. As sperm concentration declined, the effect of pCO_2 was exacerbated – higher sperm concentrations were required to achieve comparable fertilization rates to controls (Fig. 5.7) (Albright et al. 2010).

Nakamura and Morita (2012) investigated effects of different pCO_2 conditions (300, 400, and 1,000 mg l^{-1}) on sperm motility of *Acropora digitifera,* and found that acidification could suppress the flagellar motility. They calculated that sperm motility will likely decline by ~30 %, which may impact fertility.

Echinoderms

Sperm concentration effects of pCO_2 on fertilization success have also been found for the sea urchin, *Strongylocentrotus franciscanus,* in which fertilization efficiency decreased. Elevated pCO_2 reduced the ability of sea urchin eggs to block polyspermy, which inhibits successful embryo development (Reuter et al. 2011). Acidification-induced changes may be due to effects on the sperm, egg, or both.

Interactive effects of warming and acidification were observed on fertilization and embryonic development of the sea urchin *Sterechinus neumayeri* in elevated temperature (+1.5 and 3 °C) and decreased pH (−0.3 and −0.5 pH units) treatments (Ericson et al. 2012). Fertilization was resilient to acidification at ambient temperature, but at elevated temperatures, there was a negative interaction of temperature and pH on percentage fertilization (11 % reduction at +3 °C). Cleavage stage embryos, showed a significant, but small reduction (6 %) in the percentage of normal embryos at pH 7.5. For blastulae, a 10–11 % decrease in normal development occurred in the +3 °C treatments at all pHs. The results highlight the importance of considering the impacts of both temperature and pH in assessing responses to climate change. Interactive effects of near-future ocean warming and acidification on fertilization of a variety of intertidal and shallow subtidal echinoids (*Heliocidaris erythrogramma*, *H. tuberculata*, *Tripneustes gratilla*, *Centrostephanus rodgersii*), an asteroid (*Patiriella regularis*) and an abalone (*Haliotis coccoradiata*) were examined. Eggs from multiple females were fertilized by sperm from multiple males in combinations of three temperature and three pH/PCO_2 treatments (Byrne et al. 2010) based on near-future conditions for southeast Australia. No significant effects were seen, indicating that fertilization is robust to temperature and pH/PCO_2 fluctuation. This may reflect adaptation to fluctuations in temperature and pH in their shallow water habitats.

5.4 Conclusions

From endocrine effects to gametogenesis, mating, and fertilization, reproduction can be impaired in many different ways by contaminants. It can be seen that there is continuity and overlap of effects from gametes to fertilization to embryonic development which is discussed in the following chapter. Effects initiated by endocrine disruption, for example can be followed through the life cycle. Reproduction is clearly a process with obvious repercussions at the population level. However, many marine organisms normally produce enormous numbers of embryos, so it is not clear what degree of reproductive impairment would be required to lead to population level effects, which are rare. One clear example of population level effects is that of TBT on dog whelks. Nevertheless, additive effects of impairments at numerous stages of reproduction and development imply that overall reproductive success in many taxa could be severely compromised in a number of pollution scenarios.

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