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The effects of pollution on reproduction in fish

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Introduction

During the last decade there has been increasing concern at the levels of industrial and agricultural contaminants in the aquatic environment and the possible effects of such pollution on both human health and the well-being of animal populations. Since the seas and lakes, via rivers and other water courses, become the final resting point for pollutants emanating from factory effluents or agricultural applications, it is in such environments that we might expect to find the first warnings of environmental catastrophe. It is not only the populations of fish in these environments that are at risk. Fish are part of the natural diet of both aquatic mammals and birds and, increasingly, many humans are dependent upon fish as a protein source, both directly and indirectly as a feedstock for their domestic animals. The obvious sign of gross pollution, dead fish, has long been recognized, but there is increasing evidence that low-level pollution may decrease the fecundity of fish populations, leading to a long-term decline and eventual extinction of important natural resources. This, together with overfishing, may account for a decline in

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major fisheries, such as those of the North Sea, in the industrialized West. It is also of importance in peasant communities in Asia where a decline in yield of fish in the paddy fields can result from excessive application of pesticides to rice and other crops.

There have been very few reviews on such sublethal effects of pollutants on reproduction and these have been limited to a few pesticides with special emphasis on species of commercial importance in India (Singh and Singh, 1982a; Singh *et al.*, 1989). This review therefore aims to present the effects of sublethal pollution, both industrial and agricultural, on all aspects of fish reproduction, from gonadal development through to spawning, together with a discussion of how some of these effects may be a result of disturbance of the reproductive endocrine system. Although the postspawning effects of pollution on embryos and larvae are outside the scope of this review, references to embryonic survival and hatch rate have been included where this has been affected by either maternal exposure or exposure of gametes prior to fertilization. Behavioural effects are also included where these are induced by pollutants acting via the reproductive system rather than by direct influence on the brain itself.

The information used in this review may be of value in considering long-term population changes in polluted environments and in framing legislation limiting aquatic pollutants. Since there is such a tremendous variety in the pollutants, species, doses and exposure times involved, the effects of pollutants are summarised in tabular form under the general categories of heavy metals (Table 1), pesticides, including organophosphorus, organochlorine and carbamates (Tables 2–4) and other pollutants, including poly-aromatic hydrocarbons, acid waters and factory effluents (Table 5). These tables therefore serve as a source of reference for the possible deleterious effects of specific pollutants on fish reproduction, and together with the references cited in the papers cover most of the literature up to 1993. The exposure times and doses cited can therefore be used in conjunction with those determined for a particular polluted area to predict possible harmful effects on the reproductive development or spawning of fish in the waterway.

Whilst the tables are intended as an information source, the review itself is organized in terms of particular target effects so that comparison may be made of the effects of different pollutants on specific organs or enzyme systems. The review itself is therefore divided into separate sections to cover effects on the ovary and on the testis which include gross morphological changes, effects on gametogenesis and gamete viability. Since many of these effects may have their origin in perturbations of the reproductive endocrine system, this is discussed in a separate section which deals with primary effects on the hypothalamus, pituitary and gonads and their hormones. The liver has a dual role in both producing vitellogenin for the ovary and in catabolizing gonadal hormones as well as the xenobiotics themselves. Although the review covers the primary organs and hormones involved in reproduction, these may be modulated by a myriad of other factors which are discussed only briefly since they are strictly beyond the scope of this review. In the final section, comment is made on the present status of work on the effects of pollution on fish reproduction and suggestions are made for the direction of further studies.

Effects of pollution on the teleost ovary

The teleost ovary undergoes a seasonal reproductive cycle which may, for convenience, be divided into four main phases: (1) vitellogenesis, involving the major growth phase of

the ovary during which ovarian secretion of oestradiol stimulates hepatic synthesis of vitellogenin which, in turn, is incorporated into the developing oocyte, (2) oocyte maturation, during which the germinal vesicle migrates to the periphery of the oocyte and breaks down under control of pituitary gonadotrophins and ovarian progestogens, (3) ovulation and spawning and (4) postspawning in which the gonads regress in preparation for the next reproductive cycle. Clearly the stage at which fish are exposed to the pollutant and the duration of such exposure will determine to a large extent the effect on the ovary. Long-term exposure, which generally begins early in the reproductive cycle and finishes towards oocyte maturation, has often used gonadosomatic index (GSI), oocyte stage, histological examination, or number and viability of ovulated eggs as measures of pollutant effect. Shorter-term exposures, or *in vitro* studies, are better for the examination of the specific mechanisms involved, such as vitellogenesis, steroidogenesis and pituitary activity. In this section only the physical changes in the ovary will be considered, but we must also bear in mind that ovarian recrudescence and maturation is determined largely by endocrine mechanisms which will be discussed in a later section.

OVARIAN MORPHOLOGY

Long-term exposure to pollutants almost invariably leads to a decrease in GSI, more smaller, less-developed oocytes and fewer large, mature oocytes, and an increase in the numbers of atretic follicles (Tables 1–5). Oocytes frequently contained less yolky granules (Sukumar and Karpagaganapathy, 1992), ruptured oocyte walls, damaged yolk vesicles (Kulshrestha and Arora, 1984) and nucleoli and cytoplasm which had undergone major changes (Kirubagaran and Joy, 1988a; Murugesan and Haniffa, 1992). Nucleic acids, protein content and the activity of alkaline phosphatase were all decreased after exposure to malathion* or textile mill effluents (Ansari and Kumar, 1987; Murugesan and Haniffa, 1992). Intra- and extranuclear inclusions staining for protein and carbohydrate, suggesting the presence of metal–glycoprotein complexes, have been found after heavy metal exposure (Ram and Sathyanesan, 1986, 1987; Katti and Sathyanesan, 1987). Cadmium and mercury exposure resulted in extensive vacuolation in the oocortex, necrosis of oolemma and hypertrophy of the follicular cells and, while mercury had little effect on the incorporation of vitellogenin, cadmium appeared to inhibit the transfer of nutrients across the oolemma (Victor *et al.*, 1986). Arsenic exposure resulted in prominent follicular spaces, reduced development of stage II and III oocytes and reduced number and diameter of nucleoli and increased atretic follicles in *Colisa fasciatus* (Belontidae) (Shukla and Pandey, 1984a). β-HCH caused abnormal yolk formation as evidenced by non-fusion of yolk droplets, abnormal staining properties of yolk and the occurrence of exogenous yolk droplets in immature perinucleolus stage-I oocytes along with an increase in cortical alveoli (Wester *et al.*, 1985). Ovaries from cadmium, malathion and 3-methylcholanthrene-exposed fish showed disorganization of gonadal lamellae, inhibition of oogenesis, necrosis, fibrosis of lamellar walls and in some cases, total disintegration of the elements in many gonadal cavities formed by folding of lamellae (Singh, 1989). Hepatic and ovarian ascorbic acid and protein content were also decreased by an organophosphorus pesticide (Ram and Sathyanesan, 1987).

Different stages of oocytes may be differentially affected by pesticide, in *Puntius conchonius* (Cyprinidae) for example, stage V were most susceptible to aldrin,

*Full names of pesticides etc. are given in footnotes to the tables.

methoxychlor and monocrotophos and stage 0–II oocytes were depleted only by aldrin while stage III were unaffected by any of these pesticides. Similarly zinc affected primarily young stage 0–II oocytes while copper and lead caused atresia in the older stage III–IV oocytes (Kumar and Pant, 1984, 1988).

The morphological damage caused by pollutants is not necessarily permanent. Shukla and Pandey (1985) have shown that the morphological damage and inhibition of steroidogenic enzymes caused by exposure of tilapia to 0.001 mg l^{-1} DDT for 20 days is reversed 30 days after return to clean water.

An exception to the general inhibitory effect of pollutants appears to be cadmium, which in one report was shown to have a stimulatory effect on ovarian growth, probably resulting from a stimulation of gonadotrophic hormones (GtH), steroidogenesis and vitellogenesis (Thomas, 1990). It is not clear why this study contradicts the general inhibitory action of this metal as discussed above.

LIPID PRODUCTION AND VITELLOGENESIS

Lipids

The liver plays an important role in ovarian recrudescence in its production of the yolk protein, vitellogenin, under the stimulation of ovarian oestradiol. Vitellogenin is a glycolipophosphoprotein derived from hepatic lipids. Pollutant effects on liver lipid synthesis may, therefore, affect ovarian development and cholesterol is, of course, also a precursor of all of the steroid hormones. Pollutants generally have an inhibitory effect on hepatic and ovarian lipid levels, but the precise effect may be very much determined by the stage of maturity of the fish and the duration of exposure. In *Clarias batrachus* (Clariidae), for example, γ-BHC and malathion decreased hepatic free fatty acids in the previtellogenic and regressed phases, but increased it during the vitellogenic and postvitellogenic phases compared with control fish (Lal and Singh, 1987), and similar differences in effect during gonadal recrudescence have been found in *Heteropneustes fossilis* (Heteropneustidae) (Singh, 1992). Changes in hepatic, ovarian and plasma content of lipids may be a reflection of pollutant inhibition of the mobilization of hepatic lipids into the plasma and its incorporation into developing oocytes of the ovary (Lal and Singh, 1987; Singh, 1992). Since such mobilization is maximal during the vitellogenic phase, it is at this period that pollutants might have maximal effect on lipid distribution.

In addition to total lipid, the balance of the different lipid components, free fatty acids, mono-, di-, and triglycerides, free and esterified cholesterol and of the phospholipids (phosphatidylcholine, phosphatidyl serine, phosphatidyl inositol and phosphatidylethanolamine) can be affected by γ-BHC and malathion pesticides (Lal and Singh, 1987; Singh, 1992; Singh and Singh, 1992a,b; Singh and Kime, 1994). Such a change may affect the nature of the yolk protein incorporated into the oocyte and thus affect the developing embryo. The conversion of esterified to free cholesterol can be affected by γ-BHC and may affect steroidogenesis (Singh, 1992). It is not clear whether these are primary effects on the liver and ovary or whether they are, as suggested by Singh and Singh (1992a), modulated via pituitary gonadotrophin or ovarian oestrogens which regulate the synthesis of vitellogenin and its incorporation into the oocyte.

Pesticides and many industrial hydrocarbons in particular are highly lipid soluble and may concentrate in lipid-rich tissues such as ovary and liver. The hepato–ovarian axis may therefore be particularly sensitive to such pollutants during ovarian growth and lead to high levels in the egg. Even within the gonads there may be a preferential uptake of

such compounds into the eggs and sperm rather than the gonadal somatic tissue (Hose *et al.*, 1981).

Vitellogenesis

Since the incorporation of vitellogenin into the developing oocyte accounts for the major part of the growth during ovarian recrudescence, the lower GSI in fish exposed to pollutants is probably due to inhibition of vitellogenesis. A more direct measure is obtained from plasma, hepatic and ovarian vitellogenin concentrations. In cyanide-exposed rainbow trout, during late vitellogenesis, plasma vitellogenin increased while gonadal levels fell and hepatic concentrations were unchanged compared with control fish, suggesting that the pollutant inhibited uptake of the protein into the oocyte (Ruby *et al.*, 1987). During early vitellogenesis, however, cyanide inhibited the hepatic synthesis of vitellogenin, possibly by inhibiting oestrogen synthesis (Ruby *et al.*, 1986). Cyanide may act, in part, by decreasing serum calcium below basal levels, during the critical phase of early vitellogenesis, and thereby blocking exogenous yolk production (Da Costa and Ruby, 1984).

The reduction in plasma vitellogenin in winter flounder (*Pleuronectes americanus*, Pleuronectidae) was related to the concentration of cadmium in the liver although it is not clear whether this is the actual site of action of the metal or simply reflects body uptake (Pereira *et al.*, 1993). Plasma vitellogenin in oestradiol-injected flounder is also inhibited by cadmium, which preferentially accumulates in the liver and may act by interfering with the protein-synthesizing apparatus at the transcriptional level, since it reduced the RNA:DNA ratio (Povlsen *et al.*, 1990). In the rainbow trout, cadmium has a hypocalcaemic affect resulting from a decrease in free plasma calcium, and a reduction in bound calcium as a result of both decreased plasma vitellogenin and reduced binding of calcium to vitellogenin (Haux *et al.*, 1988). Cadmium, malathion and 3-methylcholanthrene reduced plasma vitellogenin in *Monopterus albus* (Synbranchidae), probably as a result of decreased plasma oestradiol levels (Singh, 1989). Although one possible cause of decreased vitellogenesis is such an inhibition of oestrogen synthesis, it has also been suggested that one common pesticide, γ-BHC, is itself oestrogenic since it will increase vitellogenin production and hermaphroditism in juvenile guppies and medaka (Wester *et al.*, 1985; Wester and Canton, 1986; Wester, 1991). Recent studies (Purdom *et al.*, 1994) have shown that sewage effluent contains oestrogenic substances which induce vitellogenesis in male trout. This activity was attributed to alkyl phenols which are the final products of biodegradation of alkylphenol-polyethoxylates, a major group of industrial surfactants, and are known to be present in sewage effluent (Jobling and Sumpter, 1994). In salmonids, acid waters both decrease plasma vitellogenin levels and inhibit the oestrogen stimulation of plasma vitellogenin, possibly due to stress effects (Tam *et al.*, 1987; Mount *et al.*, 1988; Roy *et al.*, 1990).

OOCYTE MATURATION, OVULATION AND SPAWNING

Oocyte final maturation in teleost fish is a necessary condition for successful ovulation. This phase of oocyte development is initiated by gonadotrophin, which induces both migration of the germinal vesicle to the periphery of the oocyte and the follicular synthesis of a maturation-inducing steroid (which is often considered to be 17,20β-dihydroxy-4-pregnen-3-one (17,20βP)). The maturation-inducing steroid then causes germinal vesicle breakdown (GVBD) which is usually followed by ovulation. Unlike the

prolonged period of vitellogenesis, oocyte maturation is very short, less than 1 day in cyprinids and up to 7 days in salmonids, and is potentially very susceptible to pollution, especially in river and pond systems where it might correspond with a surge in pollutant concentration from applied pesticide or industrial waste.

Compared with vitellogenesis there have been relatively few studies on the effect of pollutants on oocyte maturation, probably due to the difficulty in obtaining fish at a precisely defined stage when changes are taking place extremely rapidly. The *in vitro* induction of GVBD by luteinizing hormone (LH) was inhibited by both organophosphorus (malathion, phosdrin, birlane and gardona) and organochlorine (endosulfan) insecticides (Haider and Upadhyaya, 1986; Haider and Inbaraj, 1988), but it is not clear whether this is due to inhibition of LH-induced GV migration or to inhibition of the synthesis of the maturation-inducing steroid. High water temperatures in Lake Erie may decrease plasma concentrations of testosterone and 17,20βP (but not GtH) in coho salmon (*Oncorhynchus kisutch*, Salmonidae), resulting in a breakdown in timing of oocyte final maturation and ovulation which may be responsible for the decreased fertility attributed to over-ripe eggs (Flett *et al.*, 1991). Such effects of thermal pollution might also be of particular importance in rivers and lakes affected by cooling water effluents from power stations (Luksienė, 1978, 1981, 1982).

The few reports describing delayed or inhibited ovulation or oviposition after exposure to Kepone, 2,4-D, DDT, and acid pH (Cope *et al.*, 1970; Curtis and Beyers, 1978; Tam and Payson, 1986; Hose *et al.*, 1989) may reflect more the retardation of oocyte development than direct effects on the physiological processes of maturation and ovulation. The author is aware of only one case in which delayed spawning has been reported in fish exposed to a pollutant (zinc) only during the period of gamete final maturation (Speranza *et al.*, 1977). Pollutant exposure at this critical phase of reproduction can have a major impact on population dynamics. Recent extinctions of a number of species in acidic lakes in proximity to metal smelting complexes in Canada have been shown to be related to the failure of ovulation in fish with apparently normal mature ovaries and is apparently linked with an abnormally low serum calcium level. There was considerable species variation in the pH at which fish stopped reproduction, with smallmouth bass (*Micropterus dolomieui*, Centrarchidae), walleye (*Stizostedion vitreum*, Percidae) and burbot (*Lota lota*, Gadidae) affected at pH 6–5.5, lake trout (*Salvelinus namaycush*, Salmonidae) and trout perch (*Percopsis omiscomaycus*, Percopsidae) at pH 5.5 to 5.2, brown bullhead (*Ictalurus nebulosus*, Ictaluridae), white sucker (*Catostomus commersoni*, Catastomidae) and rock bass (*Ambloplites rupestris*, Centrarchidae) at 5.2 to 4.7, with lake herring (*Coregonus artedii*, Salmonidae), yellow perch (*Perca flavescens*, Percidae) and lake chub (*Couesius plumbeus*, Cyprinidae) being the most tolerant and reproducing down to pH 4.7–4.5 (Beamish, 1976). A number of insecticides caused abortions in pregnant mosquitofish (*Gambusia affinis*, Poeciliidae) (Boyd, 1964) and guppies (*Poecilia reticulata*, Poeciliidae) (Yasuno *et al.*, 1980). In *Anabas testudineus* (Anabantidae), lead increased the spawning activity, resulting in release of immature unviable eggs, possibly as a result of either accumulation of this pollutant in the brain and its consequent effect on hypothalamic and pituitary activity, or to the stimulation of corticosteroids (Tulasi *et al.*, 1989).

EGG NUMBERS AND VIABILITY

Together with GSI, the numbers of spawned eggs and their survival are the most common

indicators of the long-term impact of pollutants on reproduction. Measuring these indicators uses simple techniques, but gives no information as to the mechanism by which the pollutant acts. All of these studies indicate that pollution has a harmful effect on the numbers of eggs produced, possibly resulting from decreased availability of vitellogenin for yolk production. The numbers of these eggs which hatch, and the survival of larvae from exposed parents, may reflect both pollutant-induced changes in egg structure which decrease fertilization rate, and teratological effects resulting in malformed embryos. In this review the survival of larvae which have themselves been exposed to pollutant is not considered, although this might be expected to be a particularly sensitive period in the life of the fish and to make a significant contribution to any decrease in fish stocks.

Fecundity, as indicated by the numbers of eggs spawned, was decreased by the plasticizer di-n-butyl phthalate in *Rivulus marmoratus* (Aplocheilidae) (Davis, 1988), phenylmercuric acetate in zebrafish (Kihlström *et al.*, 1971) and by low pH in both the flagfish *Jordanella floridae* (Cyprinodontidae) (Craig and Bakshi, 1977) and the desert pupfish *Cyprinodon n. nevadensis* (Cyprinodontidae) (Lee and Gerking, 1980). Fertility, as indicated by the percentage of spawned eggs that hatched, was decreased by DDT and PCB in Lake Geneva charr (*Salvelinus alpinus*, Salmonidae) and in white croaker *Genyonemus lineatus* (Sciaenidae) (Monod, 1985; Cross and Hose, 1988; Hose *et al.*, 1989), cadmium in the guppy *Poecilia reticulata* (Hatakeyama and Yasuno, 1987), DDT in salmonids (Burdick *et al.*, 1972), low pH in desert pupfish (Lee and Gerking, 1980) and chlorinated phenolics from bleach plant effluent in zebrafish *Brachydanio rerio* (Cyprinidae) (Landner *et al.*, 1985). One explanation for the decreased hatch rate could be a lower rate of fertilization which can result from interactions between the pollutant and the micropyle and prevent entry of sperm (Khan and Weis, 1993). The decreased number of eggs in acid-exposed brook trout *Salvelinus fontinalis* (Salmonidae) was correlated with the lower body weight resulting from exposure (Tam and Payson, 1986).

Of particular environmental concern is the demonstration of a correlation between ovarian concentrations of PCBs and hatching success in starry flounder *Platichthys stellatus* (Pleuronectidae) (Spies and Rice, 1988), flounder *Platichthys flesus* (Pleuronectidae), herring *Clupea harengus* (Clupeidae), and whiting *Merlangus merlangus* (Gadidae) (von Westernhagen *et al.*, 1987) taken from polluted marine waters. DDE has a similar effect in herring (Hansen *et al.*, 1985) and whiting, as has dieldrin in cod (*Gadus morhua*, Gadidae) (von Westernhagen *et al.*, 1987). Ova from minnows (*Phoxinus phoxinus*, Cyprinidae) fed a diet containing the PCB Clophen A50 also had decreased hatchability, probably resulting from the reduced hatching time (in degree days) in treated fish (Bengtsson, 1980). Elevated water temperature has also been implicated in the low survival rate of eggs from Lake Erie coho salmon, *Oncorhynchus kisutch* (Flett *et al.*, 1991).

Both fecundity and fertility were decreased by Kepone in sheepshead minnow (*Cyprinodon variegatus*, Cyprinodontidae) (Goodman *et al.*, 1982), DDT and copper in *Salvelinus fontinalis* (Macek, 1968; McKim and Benoit, 1971), bleached kraft mill effluent in brown trout *Salmo trutta* (Salmonidae) (Vuorinen and Vuorinen, 1985), zinc in zebrafish *Brachydanio rerio* (Speranza *et al.*, 1977), and anthracene in fathead minnows *Pimephales promelas* (Cyprinidae) (Hall and Oris, 1991). Although bleached kraft mill effluent exposure decreased sex steroid levels, gonad and egg size and sperm motility in white suckers, exposed fish had equal or greater fertilization potential than

control fish and there was no difference in hatchability of eggs or larval size at hatch (McMaster *et al.*, 1992).

Deformities of offspring from exposed parents have been found for plasticizer in *Rivulus marmoratus* (Davis, 1988), anthracene in fathead minnows (Hall and Oris, 1991), and DDT in the winter flounder *Pseudopleuronectes americanus* (Pleuronectidae) (Smith and Cole, 1973). It is likely that these effects are due to the presence of pollutant in the yolk, because exposure of eggs, produced by unexposed parents, to pollutants can have similar effects, presumably by absorption into the yolk (Khan and Weis, 1987a,b,c; Munkittrick and Dixon, 1989). As already indicated, the high lipid solubility of these hydrocarbons provides a ready route to their incorporation into the developing oocyte.

Since sex determination in fish occurs during the embryonic stage, possibly by secretion of steroids from the embryonic gonads, it is possible that exposure to pollutants at this stage could inhibit the sex-determining mechanism and lead to a change in sex ratio of the fry. The high oestrogenic activity of a number of pesticides (Thomas *et al.*, 1985; Wester *et al.*, 1985; Wester, 1991) might also be expected to result in a skewed sex ratio.

Effects of pollution on the teleost testis

TESTICULAR MORPHOLOGY AND SPERMATOGENESIS

As with the female, one of the most common measures of the effects of pollutants in male fish is the GSI, although the increase in gonadal weight during recrudescence is much smaller in the male than in the female. The male has less-well-defined stages of maturation and the hormonal relationship is less clear than in females, but histological studies of the stage of spermatogenesis can provide useful information.

GSI is decreased by mercury, fenitrothion and carbofuran in *Channa punctatus* (Channidae) (Ram and Sathyanesan, 1986; Saxena and Mani, 1985, 1987), cadmium in *Puntius ticto* (Cyprinidae) and *Lebistes reticularis* (Poeciliidae) (Sehgal and Pandey, 1984; Pundir and Saxena, 1990), and Carbaryl in *Channa striatus* (Channidae) (Arora and Kulshrestha, 1984). Inhibition of spermatogenesis, with large numbers of spermatogonia and spermatocytes and few spermatids and mature sperm, low activity, atrophy or necrosis of the interstitial cells and changes in Sertoli cell structure are common effects of heavy metal (cadmium, copper, arsenic, mercury and lead) poisoning (Ahsan and Ahsan, 1974; Sehgal and Pandey, 1984; Sehgal *et al.*, 1984; Shukla and Pandey, 1984b,d; Ram and Sathyanesan, 1983; 1986; Srivastava, 1987; Pundir and Saxena, 1990). While copper caused only a transient arrest in spermatogenesis, with the disappearance of all intermediate stages between spermatogonia and sperms within 2 months of exposure and their reappearance in increasing numbers after 3 months, zinc and lead induced more permanent and severe damage, including necrosis and degeneration of seminiferous tubules (Kumar and Pant, 1984). Sperm also exhibited varying degrees of malformation (Sehgal and Pandey, 1984). Other structural changes to the male reproductive system caused by heavy metals include damage to the sperm duct, and misshapen and damaged lobular walls and blood vessels (Sangalang and O'Halloran, 1973; Sehgal and Pandey, 1984; Sehgal *et al.*, 1984; Shukla and Pandey, 1984b,c; Srivastava, 1987; Pundir and Saxena, 1990).

Similar effects also result from exposure to pesticides (Carbamide, urea, Endosulfan, malathion, parathion, Arochlor 1254, fenitrothion, carbofuran and TEPA) (Stock and

Cope, 1969; Billard and de Kinkeln, 1970; Sangalang *et al.*, 1981; Pandey and Shukla, 1982; Arora and Kulshrestha, 1984; Shukla and Pandey, 1984d; Sadhu and Mukhopadhyay, 1985; Saxena and Mani, 1985, 1987), although Hirose (1975) found no effect of γ-BHC on medaka testes in contrast to deleterious effects in *Oreochromis mossambicus* (Cichlidae) (Pandey and Shukla, 1980). There was a significant uptake of malathion, carbofuran and Arochlor 1254 into the testis after pesticide exposure (Sangalang *et al.*, 1981; Sadhu and Mukhopadhyay, 1985).

SPERM MOTILITY AND SURVIVAL

As noted above, exposure of males to pollutants may result in malformed sperm. Direct exposure of sperm may have similar effects and has been used as a toxicological measure. Such tests, using the fertilization rate of eggs from unexposed females (McIntyre, 1973; Billard and Roubaud, 1985; Khan and Weis, 1987a,b; Anderson *et al.*, 1991) have demonstrated the inhibitory effects of chromium, iron, cyanide, zinc, copper and mercury on fertilization success. Milt volume, spermatocrit levels and seminal plasma constituents were not affected by bleached kraft mill effluent (McMaster *et al.*, 1992) and methyl mercury had no effect on sperm morphology (Khan and Weis, 1987a,b). Sperm motility, however, is decreased by both mercury (Khan and Weis, 1987a,b), and bleached kraft mill effluent (McMaster *et al.*, 1992) although acid pH had relatively little effect on sperm of the white sucker, *Catostomus commersoni* (Mohr and Chalanchuk, 1985). The disappearance of fish populations resulting from acid rain is of increasing concern and could result from decreased motility of sperm at low pH. Of particular interest is the study of Duplinsky (1982) in which sperm from two species of pike, northern pike (*Esox lucius*, Esocidae) and chain pickerel (*Esox niger*, Esocidae), had very different sensitivities to acid medium, suggesting that the latter species would be better suited to acidic fresh waters, and therefore better for restocking waters in which pike stocks had fallen as a result of acidification. In northern latitudes or mountain areas subjected to acid rain, where meltwater run-off corresponds to the spawning season, such effects may be of major importance resulting in the selective survival of species in which sperm were more acid tolerant. Such differential effects could have major ecological consequences.

Sperm may be a particularly good measure of toxicity because they are more susceptible to pollutants than the ova in females (Anderson *et al.*, 1991), they require only a short exposure time, and their motility is rapidly measured. The ready availability of cryopreserved sperm suggests that this might be very useful in toxicity testing since it is available throughout the year and can be readily prepared from the species to be tested.

Effects of pollution on the reproductive hormones

Seasonal reproductive cycles in teleosts, often regulated by photoperiod and temperature, are mediated by the hormones of the hypothalamus (gonadotrophin releasing hormone; GnRH) and of the pituitary (gonadotrophin; GtH) which in turn act on the gonads to stimulate synthesis of steroid hormones. Many of the effects discussed above can be attributed to the disruptive effects of pollutants on the endocrine system, the inhibition of hormone production leading to developmental disruption of gonads and gametes.

HYPOTHALAMIC AND PITUITARY HORMONES

Two approaches have been used to examine the effects of pollutants on the hypothal-

amic–pituitary system, histological and hormone assay. In *Channa punctatus* exposed to mercury or the organophosphorus pesticide Cythion, the pituitary gonadotrophs are small, inactive and few in number, similar in fact to fish in the resting phase, while in control fish they are hypertrophied and actively secreting (Ram and Sathyanesan, 1983, 1986, 1987). Deformed cells, vacuolization and exhaustion of cytoplasm and severe damage to the acidophils and cyanophils of the proximal pars distalis with decreased neurosecretory material in the pars intermedia was found in DDT-, BHC- and malathion-treated tilapia (Shukla and Pandey, 1984e). Endosulphan treatment of this species resulted in degeneration of the pituitary acidophils and basophils, vacuolation of gonadotrophs and thyrotrophs and deformed hypothalamic nuclei, nucleus preopticus and nucleus lateralis tuberalis (Shukla and Pandey, 1986). Exposure of *Puntius ticto* to cadmium resulted in deformed pituitary cells and decreased diameter of thyrotrophs and gonadotrophs, but these gradually returned to normal after the fish were returned to fresh water (Pundir and Saxena, 1992). Lead treatment of *Clarias batrachus* gave a marked accumulation of neurosecretory material in the anterior neurohypophysis and degenerative changes in the neurones of both the nucleus preopticus and nucleus lateralis tuberis, resulting in inhibition of gonadal maturation (Katti and Sathyanesan, 1986).

Paramar M50, Cythion, hexadrin and aldrin decreased hypothalamic GnRH-like substance in *Heteropneustes fossilis* as measured by ovarian bioassay (Singh and Singh, 1982b). Plasma GtH was decreased by Metacid-50 and carbaryl in the murrel *Channa punctatus* (Ghosh *et al.*, 1990), and γ-HCH in goldfish (Singh *et al.*, 1994), but was unaffected in coho salmon by the polluted waters of Lake Erie in which reproduction was inhibited (Flett *et al.*, 1991). Exposure of Atlantic croaker to Arochlor 1254 inhibited, while cadmium stimulated, the *in vitro* production of GtH (Thomas, 1989). Pituitary and plasma gonadotrophin, as measured by the activity of pituitary extracts in stimulating gonadal ^{32}P uptake, was inhibited by Cythion, hexadrin, aldrin and parathion (Paramar) in *Heteropneustes fossilis* (Singh and Singh, 1980a,b,c, 1981, 1982b). Gonads of exposed fish remained responsive to luteinizing hormone (LH) and catfish pituitary extracts, which suggests that the pesticides do not affect the gonadal GtH receptors (Singh and Singh, 1980b). GnRH stimulated plasma GtH less in fish which had been exposed to bleached kraft pulp mill effluent than controls (Van der Kraak *et al.*, 1992). Hypothalamic GnRH, as determined by bioassay, may also be affected by pesticides (Singh and Singh, 1982b; Ghosh *et al.*, 1990) and this in turn could be responsible for some of the observed effects on GtH.

OVARIAN STEROIDS

Histological studies have shown the inhibition of 5-ene-3βHSD after exposure of *Mystus vittatus* (Bagridae) to the organophosphorus insecticides birlane, gardona, phosdrin and malathion (Haider and Upadhyaya, 1985), and in carp exposed to fenitrothion (Kapur *et al.*, 1978). Both phenol and sulphide inhibited the uptake of ^{14}C cholesterol into carp ovary from the peripheral circulation and its ovarian conversion to progesterone and pregnenolone (Mukherjee *et al.*, 1991).

The early stage of vitellogenesis is regulated by oestradiol secreted by the ovarian follicle and many of the effects discussed above, such as reduced GSI and inhibition of ovarian growth, may be attributable to pollutant-induced inhibition of oestrogen synthesis. Decreased plasma oestrogen has been shown to result from exposure to Arochlor 1254, lead and benzo[a]pyrene in Atlantic croaker (Thomas, 1988, 1989,

1990), cadmium, malathion and 3-methylcholanthrene in *Monopterus albus* (Singh, 1989), γ-BHC in *Heteropneustes fossilis* (Singh and Singh, 1991; Singh *et al.*, 1993a), γ-BHC and malathion in *Clarias batrachus* (Singh and Singh, 1987a), γ-BHC and Cythion in *Clarias batrachus* (Singh and Singh, 1987b), sediment contaminated with aromatic and chlorinated hydrocarbons in sole, *Parophrys vetulus* (Pleuronectidae) (Johnson *et al.*, 1988; Stein *et al.*, 1991), low environmental pH in brook trout (Tam *et al.*, 1990), bleached kraft mill effluent in white sucker (Munkittrick *et al.*, 1991) and Arochlor in trout and carp (Sivarajah *et al.*, 1978a). There may be different effects from short and long-term exposures since in the freshwater Indian perch, *Anabas testudineus*, exposed to carbaryl or metacid-50, plasma and ovarian oestradiol initially increased more rapidly than in control fish, but after 15 days there was a dramatic fall in the hormone level together with a decrease in GSI presumably reflecting the decreased vitellogenin production (Choudhury *et al.*, 1993).

The effect may be very different at different stages of ovarian recrudescence. Arochlor 1254, for example, decreased plasma oestradiol and gave a statistically non-significant decrease in *in vitro* steroid production after 17 days exposure, but after 30 days had no effect on plasma oestrogen level while increasing *in vitro* production (Thomas, 1988, 1989). It is possible that such contradictory effects might be attributable to differences in the period at which the gonad was exposed or to the exact stage at which the fish were killed. Such differential effects at different stages of ovarian recrudescence have also been found for γ-BHC in *Clarias batrachus* (Singh and Singh, 1987a). In *Heteropneustes fossilis*, γ-BHC also decreased ovarian content of oestradiol and inhibited the conversion of cholesterol ester to free cholesterol by the ovary (Singh and Singh, 1991; Singh *et al.*, 1993). Curiously, cadmium has a stimulatory effect on vitellogenesis and both plasma concentration and *in vitro* synthesis of oestradiol in the Atlantic croaker (Thomas, 1989).

Plasma androgens, which in females increase towards the end of vitellogenesis, are also depressed by Cythion and γ-BHC in *Clarias batrachus* (Singh and Singh, 1987a,b), malathion and γ-BHC in *Heteropneustes fossilis* and γBHC in goldfish (Singh and Singh, 1991, 1992c; Singh *et al.*, 1993; Singh, Kime and Singh, unpublished data), lead, benzol[a]pyrene and Arochlor 1254 in Atlantic croaker (Thomas, 1988), bleached kraft mill effluent in white sucker *Catostomus commersoni* and lake whitefish *Coregonus clupeaformis* (Salmonidae) (McMaster *et al.*, 1991; Munkittrick *et al.*, 1992) and cadmium, malathion and 3-methylcholanthrene in *Monopterus albus* (Singh, 1989).

The nature of the maturation-inducing steroid, often assumed to be 17,20β-dihydroxy-4-pregnen-3-one (17,20βP), is not yet clear for all teleosts (Kime, 1993) and very few studies have been made on the possible effects of pollution on its secretion. This is probably due both to difficulties in obtaining antisera for their measurement and to the fact that it is secreted for only a very short period – a few days in salmonids, but only a few hours in most other species. It is, therefore, difficult to obtain fish at exactly comparable stages of maturation for such experiments, but pollutant spills coinciding with oocyte final maturation could have potentially disastrous effects by disruption of the hormonal sequence and might result in total failure of spawning for the season. In the white sucker, plasma levels of 17,20βP and its *in vitro* production from endogenous precursors were depressed after exposure to pulp mill effluent and were not stimulated by GnRHa which also failed to induce ovulation (McMaster *et al.*, 1991; Van der Kraak, *et al.*, 1992). It is, however, not possible to tell whether the oocytes from the two populations were at exactly the same stage of maturation prior to treatment, since their respon-

siveness to GtH in the production of progestogen can change very rapidly over only a few hours in the period preceding GVBD when the germinal vesicle migrates to the periphery of the oocyte (Kime *et al.*, 1987). Pulp mill effluent also decreased oestradiol in both *Coregonus clupeaformis* and *Catostomus commersoni* (McMaster *et al.*, 1992; Munkittrick *et al.*, 1992). Unlike heavy metals and pesticides, exposure of trout to low pH had no significant effect on plasma concentrations of oestradiol, androgens or 17,20βP (Weiner *et al.*, 1986).

Measures of plasma concentrations will indicate overall effects on circulating steroid hormones but do not give information as to the site or sites at which pollutants exert their effect. Decreased plasma steroids could result from inhibition of hypothalamic GnRH or pituitary GtH, decreased ovarian cholesterol, decreased activity of any of the enzymes in the biosynthetic sequence from cholesterol to the hormone, or increased rate of hepatic catabolism and excretion. Nor is it always clear whether the gonadal damage described above is the cause or effect of altered steroid synthesis. In this respect *in vitro* incubations of tissue using endogenous or exogenous precursors might be expected to yield valuable information.

In goldfish, *in vitro* endogenous production of 17,20βP was very low in all fish, but both testosterone and 11-deoxycortisol, which is the major *in vitro* metabolite of 17-hydroxyprogesterone in this species (Kime *et al.*, 1992), were higher in incubations of ovaries from unexposed fish to which the pesticide had been added than in control incubations (Kime, Singh and Singh, unpublished data). In contrast, the ovarian metabolism of ^{3}H-17-hydroxyprogesterone was virtually unaffected by the pesticide which suggests that it exerts its action on stages prior to the synthesis of 17-hydroxyprogesterone. By contrast, long-term exposure of fish to the pesticide decreased endogenous production of 11-deoxycortisol, possibly by increasing its conjugation, although production of both testosterone and its glucuronide were also suppressed (Singh *et al.*, 1994). In the Atlantic croaker exposed to lead or Arochlor 1254, oestradiol production by ovarian tissue was increased, while testosterone was decreased both by these pollutants and by benzo[a]pyrene, when expressed as ng steroid per gram ovary (Thomas, 1988), but since the ovaries of exposed fish were only 25–66% of control weights the overall steroid output was smaller. During vitellogenesis it is not entirely clear whether decreased oestradiol production is a cause (by reducing vitellogenesis) or effect of lower ovarian size, since the proportion of follicular steroidogenic sites to oocyte may alter.

TESTICULAR STEROIDS

Histological evidence shows that testicular 3βhydroxysteroid dehydrogenase (3βHSD) is decreased by fenitrothion in carp (Kapur *et al.*, 1978), mercurials in *Clarias batrachus* (Kirubagaran and Joy, 1988b), and both 3βHSD and 17βhydroxysteroid dehydrogenase (17βHSD) are inhibited by DDT in *Oreochromis mossambicus* (Bhattacharya and Pandey, 1989) and organophosphorus insecticide quinalphos in *Clarias batrachus* (Bagchi *et al.*, 1990).

The major androgens in male teleosts, testosterone and 11-ketotestosterone, are, like the steroids in females, generally inhibited by pollutants. Acid waters decreased androgens in the Atlantic salmon (Freeman *et al.*, 1983), but had no effect in another salmonid, the rainbow trout (Weiner *et al.*, 1986). Plasma androgens were also decreased after exposure of salmon and flounder to crude petroleum (Truscott *et al.*, 1983). By

contrast, exposure of fish to cadmium had a stimulatory effect on plasma androgens in brook trout (Sangalang and Freeman, 1974) which could be due to impaired clearance. Cadmium exposure also inhibited the production of 11-ketotestosterone from ^{14}C-pregnenolone, probably by decreasing conversion from its immediate precursor 11-hydroxytestosterone (Sangalang and O'Halloran, 1973). A similar effect was found in rainbow trout testes in which addition of cadmium to the incubation medium stimulated endogenous androgen production by the testis, especially of testosterone and 11β-hydroxytestosterone, while there was only a small increase in 11-ketotestosterone (Kime, 1984). PCBs have similar effects to cadmium in that testes of exposed fish gave higher yields of 11β-hydroxytestosterone *in vitro* from ^{14}C pregnenolone while not affecting 11-ketotestosterone which again suggests inhibition of 11βHSD activity (Freeman and Idler, 1975). Plasma 11-ketotestosterone was unaffected by exposure to PCB, but neither testosterone nor 11β-hydroxytestosterone were measured. The concentration of pollutant may also be important in such experiments, since in the cod, plasma 11-ketotestosterone and testosterone levels were stimulated at low PCB (Arochlor 1254) concentrations but inhibited at higher levels (Freeman and Sangalang, 1977a; Freeman *et al.*, 1980, 1984). A similar concentration effect was found in the conversion of ^{14}C-progesterone to 11-ketotestosterone, but in this case could not be attributed to decreased 11βHSD.

Bleached kraft pulp mill effluent (BKME) also has pronounced effects on testicular steroidogenesis. Plasma levels of testosterone, 11-ketotestosterone and 17,20βP were lower in BKME-exposed fish, and except for testosterone, which showed a transitory increase, did not respond to GnRH injection, whereas fish from a reference site responded by increased plasma steroids. As well as depressed plasma testosterone, BKME-exposed fish showed a complete absence of plasma testosterone glucuronide in most fish, compared with 4 ng/ml in controls, suggesting a preferential suppression of conjugation (McMaster *et al.*, 1991; Munkittrick *et al.*, 1992; Van der Kraak *et al.*, 1992), although it is not clear whether the plasma glucuronides were of testicular or hepatic origin.

In vitro studies with goldfish and roach testes (Singh *et al.*, 1994; Kime, Singh and Singh, unpublished data) suggest that γ-BHC has a more pronounced effect on steroidogenesis from endogenous precursors than on conversion of exogenous ^{3}H-17-hydroxyprogesterone to androgens and progestogens. Endogenous androgen synthesis was depressed after 4 weeks exposure in goldfish, but both testosterone and 11-deoxycortisol were stimulated while 11-ketotestosterone remained unchanged in incubations to which γ-BHC had been added. In roach, however, γ-BHC increased testosterone glucuronide, but decreased 17-hydroxyprogesterone and 11-ketotestosterone, and at high concentrations also testosterone, while conversion of exogenous 17-hydroxyprogesterone remained unaffected.

HEPATIC CATABOLISM

Decreased plasma concentrations of steroid hormones may result not only from decreased gonadal synthesis or decreased pituitary gonadotrophin release, but by increased hepatic catabolism. Conversely, increased plasma hormones could be a result of decreased hepatic catabolism rather than increased synthesis. PCB, for example, stimulated the metabolism of steroids to polar metabolites by the carp hepatopancreas which could explain its effect on decreasing plasma concentrations (Yano and Matsuyama, 1986). Chlorinated paraffins decreased hepatic 6β-hydroxylase but

increased 5α/β-reductase activity in flounder (Haux *et al.*, 1982), and even treated municipal wastewater decreased 6β-hydroxylase while increasing 17βHSD activity, effects that have been associated with polyaromatic hydrocarbon contamination (Förlin and Hansson, 1982). In contrast, hepatic mixed function oxygenases were elevated by pulp mill effluents which also elevated the hepatosomatic index (McMaster *et al.*, 1991; Munkittrick *et al.*, 1992). Some enzymes, such as glucuronyl transferase, are common to both liver and gonads in teleosts, and such effects as its increased activity in Arochlor 1254-treated channel catfish liver (Ankley *et al.*, 1986) might suggest the presence of similar effects in the gonads.

Other effects of pollution on reproduction

In the foregoing review the recent literature on the effects of pollution on reproduction has been covered insofar as it concerns the main sites of reproductive action, the gonadal production of gametes and reproductive steroids and the pituitary in its secretion of gonadotrophin. It is, however, clear that reproduction may also be influenced by pollutants affecting a number of other regulatory compounds which are outside the scope of this review, but which nevertheless may affect reproductive activity. Extensive studies have been made on the effects of pollutants on thyroid activity (Singh *et al.*, 1989), while prostaglandins, prolactin, inhibins, enkephalins and other such general regulatory substances might also be sensitive to pollution and could account for some of their effects on reproduction. Seasonal cyclicity in fish is regulated via the pineal and its secretion of melatonin which might also be disrupted by pollutants.

Pollutants are obvious stressors, and measures used to determine safe and sublethal concentrations of pollutant only consider obvious visible signs of discomfort to the fish and may miss other indicators of stress which could have an inhibitory effect on reproduction. Stress, as mediated by the adrenal, has well-known effects on the reproductive system (Kime *et al.*, 1980; Pickering, 1981; Pickering *et al.*, 1987; Carragher *et al.*, 1989) and might account for significant indirect effects leading to decreased levels of reproductive hormones.

In addition to such effects on the direct functions of the gonads, we must also consider that in many fish, courtship involves elaborate behavioural patterns which, while they may be regulated by gonadal hormones, also involve complex brain patterns which could be affected directly by pollutants. Tilapia for example, which show very advanced behavioural patterns in the breeding season, experience pathological lesions in brain tissue during endosulfan spraying against tsetse fly. Plasma androgens in this species were not affected by endosulfan and there were no pathological lesions in the gonads, but fish exposed as juveniles showed a significant delay in onset of breeding behaviour compared with controls, although exposed adults were unaffected (Matthiesssen and Logan, 1984). Such neural effects on reproduction, which may be quite widespread, are, however, beyond the scope of this review.

Comments and future directions

During the preparation of this review, it became apparent that studies on the effects of pollutants and reproduction in fish contained a number of repetitive themes and several important areas of omission. A large amount of the literature cited is rather repetitive and

in strictly scientific terms falls into the 'me too' category – just another species and pollutant. This is particularly true of many studies of the effects of pollutants on GSI or gonadal histology. It may be for this reason that such work often appears in less-well-known journals, and because of the simplicity of technique often emantes from the less fortunately endowed laboratories. Although such criticism may well be made on purely scientific grounds, the studies nevertheless show that the effects observed are widespread amongst teleost fish and that similar effects are found for a wide variety of pollutants. For this reason they have been included in the tables as sources of information, although only brief mention, if any, is made in the text. Probably as a result of current 'market orientation' of funding bodies, most of the studies relate to particular species of local commercial interest and to pollutants perceived to be most hazardous in the locality of the research laboratory and its potential funding agencies, and there are very few comparative studies.

The literature covered by this review clearly demonstrates that almost all pollutants may adversely affect the reproductive potential of species, covering a wide range of families, at concentrations below that at which significant mortality occurs. The concentrations to which fish have been exposed are usually classed as 'sublethal' or 'safe', although in many cases it is only the observer's view that the fish are not subject to stress and the fish themselves may feel very differently. What is frequently lacking is use of a wide enough range of concentrations to determine the maximum safe level at which the pollutant has no effect on reproduction. Such levels are likely to lie at the lower end of the range cited in the tables for a particular pollutant. The tables suggest that exposure to 0.001 mg l^{-1} (1 ppb) of pollutant is generally sufficient to produce harmful effects for long-term exposure, although some organochlorines show harmful effects even at one-thousandth of this level. Some studies have used much higher levels than this over shorter periods, but this may be equally relevant for exposure resulting from localized spraying, in paddy fields for example, although it is unlikely that fish would survive long-term exposure to such concentrations. Pollutant levels, by their very nature, are not predictable. Baseline levels are likely to be very low, but may well exceed that found harmful for organochlorines, particularly after exposure for several years during which significant accumulation may have occurred in the lipid-rich tissues of the gonads. Pollution resulting from local spraying or industrial spills may reach far higher levels over a short period before further dilution in the waterways, and in extreme cases will cause significant mortality of the fish population. Frequently such mortality is the first indication that a spillage or unauthorized discharge has taken place, but the data presented in this review show that it is levels well below this, which often go unnoticed, that present the major threat to fish populations. The exact stage of gonadal maturity at which such short-term exposure occurs may be critical in determining whether successful spawning takes place during the year, but few studies have compared exposure at different phases of the reproductive cycle.

In vitro studies have used a wide range of concentrations since it is difficult to determine what is a realistic value to use. Gonadal and hepatic tissues in particular have a high lipid content and accumulate both pesticides and heavy metals, but even tissue concentration gives little guidance as to how much pollutant to add to the incubation medium, since it may be localized within specific areas of the tissue. The major advantage of the *in vitro* technique is that large numbers of tests may be carried out using tissue from only a few fish. Results obtained may be of little value in determining harmful river

concentrations, but have great potential in the study of relative toxicities of different pollutants and in clarifying their mechanism and site of action. It is in this area that the greatest omissions are found. No doubt the literature will continue to expand the cases of harmful effects of pollutants on reproduction, but it is perhaps more important now to provide more information on relative toxicities and mechanisms of action so that real choices may be made for the use of the least harmful pesticide and for setting limitations on the levels of industrial effluents. *In vitro* techniques, possibly using partly purified enzyme systems that may be stored frozen for extended periods, may provide valuable methods for comparing such toxicities. They may also be well suited to studies on the cellular mechanisms of action of pollutants. Do pollutants affect the enzyme activity itself, or the synthesis of the enzyme at the level of gene translation or transcription? Is steroidogenesis depressed due to such effects on the enzymes, or is the second messenger system involved in gonadotrophin stimulation affected, or GtH synthesis or its release by GnRH? The evidence suggests that steroidogenic enzymes are affected, but few investigations have examined these other possible sites of action. *In vitro* experiments clearly show that the depression of reproductive activity is not solely the result of stress.

The nature of pollutants is so diverse that even if hazardous levels of all of those cited in this review (over 60) are known, it is quite impossible to assay water for all of them. Possible synergistic action of pollutants arising from industrial and agricultural sources have been largely ignored, yet most rivers contain a cocktail of chemicals which pose particular problems for analysis. An urgent need is therefore development of a non-specific method for monitoring watercourses for concentrations of chemicals that are potentially harmful to reproduction of fish. Such methods must be available at all times of the year, not just during the reproductive season, and cannot therefore depend on *in vivo* use of fish. It has been suggested in this review that the motility of cryopreserved sperm might afford such a bioassay and this possibility is currently under investigation in the author's laboratory.

With the increasing pollution of aquatic ecosystems, and the potential harm to the human food chain and water supplies, such monitoring systems are urgently needed. Such systems if properly designed will not only be valuable for monitoring the health of our waterways but may also be able to give measures of relative toxicities of different pollutants and their synergistic effects, and by use of tissue from different species determine interspecific differences in toxicities of the pollutants.

Although the evidence points to decreased fecundity of fish populations resulting from pollution, hard evidence of this as a cause of decreasing fish stocks is lacking. In the marine environment overfishing and pollution probably both contribute to such a decrease but the relative contribution of each is not clear. In rivers where pollution incidents occur at irregular intervals the lack of particular year cohorts may point to reproductive failure resulting from such incidents. The correlation of population dynamics with seasonal pollutant levels may be a rewarding area of investigation.

Conclusions

The general conclusion which we can draw is the obvious one that pollutants have an inhibitory effect on reproduction. There is, surprisingly, little difference between the different classes of pollutant delineated in the tables. Evidence has been presented to show that these effects may occur at multiple sites of the reproductive system. They may

cause lesions, haemorrhage, or malformations in the gonads, pituitary, liver and the brain. Production and secretion of hormones of the hypothalamus, pituitary, and gonads is usually inhibited and their metabolism by the liver can be altered. Little work has been reported on their effects on the binding of hormones to their cellular receptor sites, to plasma binding proteins, or on the production and activity of such receptors. Effects might also be expected at the level of gene transcription or translation though these are not, perhaps, likely to be specific to reproduction. Gametes have been shown to be particularly sensitive to pollutants, both in their development, particularly the production and growth of oocytes involving vitellogenin synthesis, and in their fertility. Sperm motility, in particular, has special potential as a rapid and sensitive indicator of pollutant activity.

There is also a considerable literature on the survival of eggs, larvae and fry, which are particularly susceptible to pollutants, and may have a major impact on population dynamics. The scope of the review has, however, been limited to reproductive events prior to fertilization, although the boundary is not clear cut since absorption of pollutant into the yolk during vitellogenesis may result in death or malformation of the embryos or larvae at much later stages of development.

Pollutant effects on reproduction are often, of necessity, considered in isolation from other effects on the whole fish. We must, however, realize that its lifespan may be considerably shortened as a result of such exposure and that it will therefore experience significantly fewer reproductive cycles which will also affect the population dynamics. The overall impact of long-term environmental pollution can, therefore, decrease population by decreasing fecundity, decreasing the numbers of reproductive cycles in the lifetime of each fish, and decreasing the survival of the offspring at early stages of their life cycle. Where heavy pollution is combined with intensive fishing, as in the North Sea for example (Dethlefsen, 1989), the resulting decline in fisheries catch might be expected to be catastrophic.

In cases where pollutants completely suppress reproduction, rapid extinction will occur as found in acid-polluted Canadian lakes (Beamish, 1976). In other cases the levels of pollutant may be such that fecundity or fertility is significantly depressed. This leads to the possibility that those fish which are, or whose gametes are, more resistant to the pollutant will produce more offspring, leading eventually to a more pollutant-resistant strain of the species. There is evidence that this might occur in the killifish (*Fundulus heteroclitus*, Cyprinodontidae) in which eggs from a polluted creek showed a higher fertilization rate when mixed with sperm in the presence of methyl mercury than eggs taken from a clean area (Khan and Weis, 1987c). Such adaptation to pollution may be much more widespread and could be useful in providing resistant stocks for sport fishing in recently polluted areas. Adaptation of this type, however, poses considerable dangers since such species no longer act as biological indicators of dangers to human health and their flesh may well contain unacceptable levels of the pollutant. Stocks in which such selection has occurred, and the extent of such an occurrence, might be recognizable by the lower genetic diversity in the surviving fish population than in comparable areas. In addition to such adaptation, we know little about whether fish sense low-level pollution and avoid contaminated areas or whether the toxicity of the pollutant is affected by other factors, such as pH, calcium or oxygen content etc.

No attempt has been made to set the results described into the context of pollutant effects in mammals and birds for which there is a very extensive literature, but the reader

who is so interested will find ample discussion of this aspect in many of the references cited. Fish are a prime food source for both humans and their domestic animals, as well as for birds and marine mammals, and the effect of pollutants described in this review might be expected to have similar disturbances on these consumers. Pollutants can thus act both indirectly to decrease the food supply, and directly by concentration in the food chain itself on the ultimate predator, ourselves. Our understanding of their effects on fish may enable us to limit such harmful effects by monitoring and limiting their release into the aquatic environment.

The literature covered in this review leaves no doubt that all types of pollutants have a serious inhibitory effect on fish reproduction, even when present in minute quantities. Fish thus make excellent bioindicators of the harmful effects that might be expected in mammals in general, and human populations in particular. The longer period of exposure of human populations to pollutants before completion of their reproductive activity compared with fish, and their position at the top of the food chain, together with their concentration in high population densities in close proximity to the major sources of pollution, suggests that humans may be particularly susceptible to the effects observed in fish. Recent studies (Sharpe and Skakkebak, 1993) suggest that such a predicted decrease in human fecundity is already occurring and, as a species, we ignore such early warnings at our peril.

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References

Ahsan, S.N. and Ahsan, J. (1974) Degenerative changes in the testis of *Clarias batrachus* (Linn.) caused by cadmium chloride. *Ind. J. Zool.* **15**, 39–43.

Anderson, B.S., Middaugh, D.P., Hunt, J.W. and Turpen, S.L. (1991) Copper toxicity to sperm, embryos and larvae of topsmelt *Atherinops affinis*, with notes on induced spawning. *Mar. env. Res.* **31**, 17–35.

Ankley, G.T., Blazer, V.S., Reinert, R.E. and Agosin, M. (1986) Effects of Arochlor 1254 on cytochrome P-450-dependent monoxygenase, glutathione-S-transferase, and UDP-glucuronyl-transferase activities in Channel catfish liver. *Aquat. Toxicol.* **9**, 91–103.

Ansari, B.A. and Kumar, K. (1987) Malathion toxicity: effect on the ovary of the zebra fish *Brachydanio rerio* (Cyprinidae). *Int. Rev. ges. Hydrobiol.* **72**, 517–28.

Arora, N. and Kulshrestha, S.K. (1984) Comparison of the toxic effects of two pesticides on the testes of a fresh water teleost *Channa striatus* Bl. *Acta Hydrochim. Hydrobiol.* **12**, 435–41.

Bagchi, P., Chatterjee, S., Ray, A. and Deb, C. (1990) Effect of quinalphos, organophosphorus insecticide, on testicular steroidogenesis in fish, *Clarias batrachus. Bull. env. Contam. Toxicol.* **44**, 871–5.

Beamish, R.J. (1976) Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. *Water, Air, Soil Poll.* **6**, 501–14.

Bengtsson, B.E. (1980) Long-term effects of PCB (Clophen A50) on growth, reproduction and swimming performance in the minnow, *Phoxinus phoxinus. Water Res.* **14**, 681–7.

Bhattacharya, L. and Pandey, A.K. (1989) Inhibition of steroidogenesis and pattern of recovery in the testes of DDT exposed cichlid – *Oreochromis mossambicus. Bangladesh J. Zool.* **17**, 1–14.

Bhattacharya, T., Ray, A.K. and Bhattacharya, S. (1984) Response of *Channa punctatus* (Bloch)

Table 1. Effects of heavy metal pollution on fish reproduction.

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Arsenic	*Colisa fasciatus*	2–14	15–30	Degeneration of testis and ovary.	Shukla and Pandey (1984a,b,d)
Cadmium	*Oncorhynchus mykiss*	0.1	28	Decreased calcium and plasma vitellogenin.	Haux *et al.* (1988)
	Monopterus albus	3	7	Reduced testosterone, oestradiol and vitellogenin. Reduced GSI, histological change in gonads.	Singh (1989)
	Micropogonias undulatus (Sciaenidae)	1	30	Increased ovarian growth, steroidogenesis, vitellogenesis and GtH secretion.	Thomas (1990)
		1	40	Increased ovarian growth, plasma oestradiol and *in vitro* GtH.	Thomas (1989)
Cadmium acetate	*Puntius ticto*	26	4	Decreased testis size and abnormalities.	Pundir and Saxena (1990)
		8	30	Damage to pituitary cells, reversed by 30 days in clean water.	Pundir and Saxena (1992)
Cadmium chloride	*Lepidocephalichthys thermalis* (Cyprinidae)	0.2	20	Degenerative lesion in ovary impaired vitellogenesis.	Victor *et al.* (1986)
	Puntius sarana (Cyprinidae)	20	10	Testicular and pituitary damage.	Kumari and Dutt (1991)
	Clarias batrachus	0.005 mg per kg bw[a]	× 8	Testicular degeneration, inhibited spermatogenesis.	Ahsan and Ahsan (1974)
	Salvelinus fontinalis	0.010–0.025	1	Testicular damage, inhibition of androgen synthesis.	Sangalang and O'Halloran (1973)
		0.010–1.0	0.2 *in vitro*	Inhibition of KT[b] synthesis from pregnenolone.	Sangalang and O'Halloran (1973), Freeman and Sangalang (1977b)
		0.001	10–90	Higher plasma T[c] and KT.	Sangalang and Freeman (1974)

	Oncorhynchus mykiss	10–1000	0.08 *in vitro*	Increased *in vitro* synthesis of T and 11βT by testis.	Kime (1984)
	Poecilia reticulata	270 mg per kg diet	30–210	Decreased number of fry.	Hatakeyama and Yasuno (1987)
		0.01–0.02	182	Decreased number of fry.	Hatakeyama and Yasuno (1987)
	Lebistes reticularis	225	30	Histophysiological abnormality of testis, arrested spermatogenesis.	Sehgal *et al.* (1984); Sehgal and Pandey (1984)
	Pleuronectes americanus	25–50	71	Decreased plasma vitellogenin, decreased HSI and GSI.	Pereira *et al.* (1993)
	Platichthys flesus	2 mg per kg bw	10	Depressed plasma vitellogenin.	Povlsen *et al.* (1990)
Chromium	*Oncorhynchus mykiss*	0.005–1	0.03	Decreased fertilization rate of exposed gametes.	Billard and Roubaud (1985)
Copper	*Puntius conchonius*	0.2	60–120	Decreased spermatogenesis, ovarian atresia.	Kumar and Pant (1984)
	Catostomus commersoni	0.012–0.015	Life	Decreased egg size, increased larval deformity.	Munkittrick and Dixon (1989)
	Salvelinus fontinalis	0.002–0.032	240	Reduced number of viable eggs and hatchability.	McKim and Benoit (1971)
	Lebistes reticularis	0.5	30	Histophysiological abnormality of testis, arrested spermatogenesis.	Sehgal *et al.* (1984)
	Atherinops affinis (Atherinidae)	0.018–0.1	2	Exposed egg and sperm gave reduced fertilization and larval abnormalities.	Anderson *et al.* (1991)
Iron	*Oncorhynchus mykiss*	0.005–1	0.03	Decreased fertilization rate of exposed gamates.	Billard and Roubaud (1985)
Lead	*Micropogonias undulatus*	0.2 mg per kg bw	30	Impaired ovarian growth, steroidogenesis and GtH secretion.	Thomas (1990)
		68 mg per kg bw	30	Decreased GSI, plasma testosterone and oestradiol.	Thomas (1988)
Lead nitrate	*Puntius conchonius*	0.12	60–120	Decreased spermatogenesis, ovarian atresia.	Kumar and Pant (1984)
	Clarias batrachus	5	275	Retarded ovarian growth, induction of nuclear inclusions.	Katti and Sathyanesan (1987)

Table 1. *continued*

Pollutant	Species	Dose (mg l^{-1})	Time (days)	Effect	Reference
		5	150	Accumulation of neurosecretory material in neurohypophysis, degeneration of nucleus preopticus and nucleus lateralis tuberis.	Katti and Sathyanesan (1986)
	Anabas testudineus	1–20	30	Reduced GSI, reduced number of eggs, increased spawning.	Tulasi *et al.* (1989)
	Colisa fasciatus	15	4	Reduced spermatogenic activity, haemorrhage in testis.	Srivastava (1987)
Mercuric chloride	*Notopterus notopterus* (Notopteridae)	0.4–0.9	30	Hepatic lipid decreased, ovarian lipid increased.	Verma and Tonk (1983)
	Lepidocephalichthys thermalis	0.1	20	Degenerative lesion in ovary, lytic changes in oolemma.	Victor *et al.* (1986)
	Oncorhynchus mykiss	0.001–1	0.028	Exposed gametes had lower fertilization rate.	Billard and Roubaud (1985)
	Fundulus heteroclitus	0.01–0.05	0.001–0.017	Exposure of sperm reduced fertilization rate.	Khan and Weis (1987a)
	Channa punctatus	0.017	7–91	Decreased GSI, oocyte diameter and oocyte maturity.	Dey and Bhattacharya (1989)
		0.01	182	Reduced GSI, inhibited development of ovary and testis, smaller gonadotrophs.	Ram and Sathyanesan (1983)
		0.01	180	Protein and lipid decreased in liver and ovary, increased hepatic and decreased ovarian cholesterol.	Ram and Sathyanesan (1984)
		0.05	90–180	Decreased GSI, impaired vitellogenesis, oocyte degeneration.	Kirubagaran and Joy (1988a)
				Impaired testicular 3βHSD[d].	Kirubagaran and Joy (1988b)
Methylmercuric chloride	*Clarias batrachus*	0.04	90–180	Decreased GSI, impaired vitellogenesis, oocyte degeneration.	Kirubagaran and Joy (1988a)
				Impaired testicular 3βHSD.	Kirubagaran and Joy (1988b)

	Oncorhynchus mykiss	1–10	0.02	Exposed sperm had decreased viability.	McIntyre (1973)
	Oryzias latipes (Adrianichthyidae)	0.001–0.01	30–90	Induced vitellogenesis and hermaphroditism.	Wester (1991)
		1	0.014	Blockage of the micropyle of eggs.	Khan and Weis (1993)
	Fundulus heteroclitus	0.01–0.05	0.003	Exposed sperm had decreased motility and fertilization rate.	Khan and Weis (1987b)
		0.5–5	0.014	Exposed eggs gave malformed embryos.	Khan and Weis (1987c)
	Poecilia reticulata	0.001–0.01	30–90	Induced vitellogenesis and hermaphroditism.	Wester (1991)
Phenyl mercuric acetate	*Brachydanio rerio*	0.0002–0.02	50	Fewer eggs laid, lower spawning frequency.	Kihlström *et al.* (1971)
Emisan[e]	*Clarias batrachus*	0.5	90–180	Decreased GSI, impaired vitellogenesis, oocyte degeneration.	Kirubagaran and Joy (1988a)
				Impaired testicular 3βHSD.	Kirubagaran and Joy (1988b)
	Channa punctatus	0.2	182	Reduced GSI, gonadal degeneration, reduced pituitary gonadotrophs.	Ram and Sathyanesan (1986)
Zinc	*Brachydanio rerio*	5	9	Delayed spawning, decreased egg viability.	Speranza *et al.* (1977)
	Puntius conchonius	11	60–120	Decreased spermatogenesis, ovarian atresia.	Kumar and Pant (1984)
	Catostomus commersoni	0.21–0.25	Life	Decreased egg size, increased larval deformity.	Munkittrick and Dixon (1989)

[a]Body weight.
[b]KT = 11-ketotestosterone.
[c]T = testosterone.
[d]3βHSD = 3β-hydroxysteroid dehydrogenase.
[e]Emisan = 6% methoxyethylmercuric chloride + 94% inert ingredients.

Table 2. Effects of organophosphorus pesticides on fish reproduction.

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Cythion[a]	*Clarias batrachus*	1–4	4–28	Decreased plasma sex steroids.	Singh and Singh (1987b)
	Heteropneustes fossilis	9–35	28	Decreased liver lipid, no effect on liver cholesterol, no effect on ovarian lipid, increased ovarian cholesterol.	Singh and Singh (1980d)
		9	4	Increased ovarian cholesterol, no effect on liver and ovarian lipid.	Singh and Singh (1980e)
		9–35	28	Reduced ^{32}P uptake by testis, lower pituitary and serum GtH, increased testis lipid.	Singh and Singh (1980a)
		35	28	Reduced pituitary activity and ovarian ^{32}P uptake.	Singh and Singh (1980b,c, 1982b)
		35	28	Reduced GnRH-like factor in hypothalamus.	Singh and Singh (1982b)
	Channa punctatus	2	182	Inhibited gonadal development and GSI, reduced pituitary gonadotrophs, no effect on hepatic lipid or cholesterol.	Ram and Sathyanesan (1987)
Elsan[b]	*Channa punctatus*	0.017	7–91	Decreased GSI, oocyte diameter.	Dey and Bhattacharya (1989)
Fenitrothion[c]	*Cyprinus carpio* (Cyprinidae)	0.3–1.5	180	Reduced gonadal 3βHSD.	Kapur *et al.* (1978)
	Garra mullya (Cyprinidae)	1	30	Decreased ovarian weight, oocyte atresia.	Pawar and Katdare (1983)
	Poecilia reticulata	0.1–1.5	60	Abortion, reduced egg production.	Yasuno *et al.* (1980)
	Channa punctatus	1.5	120	Decreased GSI, inhibition of oocyte growth, oocyte atresia.	Mani and Saxena (1985)
		1.5	150	Decreased GSI, inhibition of oocyte growth, oocyte atresia.	Saxena and Garg (1978)
		1.5	120	Decreased ovarian RNA, protein and total lipid.	Saxena *et al.* (1986)

		1.5	120	Inhibited spermatogenesis, decreased testis weight, necrosis of spermatids.	Saxena and Mani (1985, 1987)
Lebaycid[d]	*Tilapia leucosticta* (Cichlidae)	7	14	Ovarian atresia.	Kling (1981)
Malathion[e]	*Brachydanio rerio*	0.5–1.1	7	Decreased ovarian DNA, RNA; increased amino acids, enzyme changes.	Ansari and Kumar (1987)
	Cyprinus carpio	0.05–1	1.5 *in vitro*	Inhibition of LH-induced germinal vesicle breakdown (GVBD).	Haider and Inbaraj (1988)
	Mystus vittatus	2.5	84	Decreased ovarian GSI and vitellogenesis, loss of 3βHSD.	Haider and Upadhyaya (1985)
		0.01–1	1 *in vitro*	Inhibition of LH-induced GVBD.	Haider and Upadhyaya (1986)
	Clarias batrachus	0.002–0.008	28	Changes in hepatic and ovarian lipids.	Lal and Singh (1987)
		1–4	28	Decreased plasma sex steroids.	Singh and Singh (1987a)
		0.5	30	No effect on testis morphology.	Sadhu and Mukhopadhyay (1985)
	Heteropneustes fossilis	0.005–0.02	28	Reduced hepatic, ovarian and plasma lipids.	Singh (1992)
		0.005–0.02	28	Reduced plasma steroid levels.	Singh and Singh (1992c)
	Monopterus albus	9	7	Reduced testosterone, oestradiol and vitellogenin; reduced GSI, histological change in gonads.	Singh (1989)
	Oreochromis mossambicus	2–4	20	Reduced ovarian activity, oocyte diameter, GSI; increased atresia.	Shukla *et al.* (1984)
		2–4	10	Histophysiological abnormalities of testis, decreased GSI.	Pandey and Shukla (1982)
		4	20	Damage to pituitary cells.	Shukla and Pandey (1984e)
Parathion[f]	*Poecilia reticulata*	0.01–1	40	Degeneration of all germinal cells of testis.	Billard and de Kinkeln (1970)
Methylparathion[g]	*Rasbora daniconius* (Cyprinidae)	0.1	5–75	Decreased oocyte diameter, oocyte rupture, reduced GSI.	Rastogi and Kulshrestha (1990)
Paramar M50[g]	*Heteropneustes fossilis*	32	28	Reduced pituitary activity.	Singh and Singh (1981, 1982b)

Table 2. *continued*

Pollutant	Species	Dose (mg l^{-1})	Time (days)	Effect	Reference
		32	28	Reduced GnRH-like factor in hypothalamus.	Singh and Singh (1982b)
Metacid-50[g]	*Anabas testudineus*	0.0001	90	Decreased oestrogen and GSI after 15 days.	Choudhury *et al.* (1993)
	Channa punctatus	0.0001	2–30	Decreased serum and pituitary GtH.	Ghosh *et al.* (1990)
Temephos[h]	*Poecilia reticulata*	0.25–1.5	60	Reduced normal birth.	Yasuno *et al.* (1980)
Birlane[i]	*Mystus vittatus*	0.003	84	Inhibition of vitellogenesis and loss of 3βHSD.	Haider and Upadhyaya (1985)
		0.00001–0.001	1 *in vitro*	Inhibition of LH-induced GVBD.	Haider and Upadhyaya (1986)
Gardona[j]	*Mystus vittatus*	0.146	84	Inhibition of vitellogenesis and loss of 3βHSD.	Haider and Upadhyaya (1985)
		0.001–0.1	1 *in vitro*	Inhibition of LH-induced GVBD.	Haider and Upadhyaya (1986)
Monocrotophos[k]	*Puntius conchonius*	0.053	60–120	Increased oocyte atresia.	Kumar and Pant (1988)
Phosdrin[l]	*Mystus vittatus*	0.0001	84	Inhibition of vitellogenesis and loss of 3βHSD.	Haider and Upadhyaya (1985)
		0.0001–0.000001	1 *in vitro*	Inhibition of LH-induced GVBD.	Haider and Upadhyaya (1986)
Quinalphos[m]	*Clarias batrachus*	0.025	15	Decreased testis 3βHSD, 17βHSD and seminiferous tubule diameter, increased testis cholesterol.	Bagchi *et al.* (1990)
TEPA[n]	*Poecilia reticulata*	1–25	6	Testicular atrophy, decreased male fertility.	Stock and Cope (1969)

[a]Cythion = O,O-dimethyl phosphorodithioate of diethylmercapto succinate. [b]Elsan = Phenthoate = Ethoxycarbonylbenzyldimethyl phosphorodithioate. [c]Fenitrothion = Sumithion = 50% emulsifiable concentrate of *O,O*-dimethyl-*O*-(4-nitro-*m*-tolyl)phosphorothioate. [d]Lebaycid = Fenthion = *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl]phosphorothioate. [e]Malathion = S-1,2-di(ethoxycarbonyl)ethyldimethyl phosphorothiolothionate. [f]Parathion = *O,O*-diethyl-*O*-*p*-nitrophenyl phosphorothionate. [g]Methylparathion = Paracid = Paramar M50 = Metacid-50 = *O,O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate. [h]Temephos = *O,O'*-(thiodi-p-phenylene)*O,O,O',O'*-tetramethyl phosphothioate. [i]Birlane = chlorfenvinphos = 2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate. [j]Gardona = tetrachlorvinphos = 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate. [k]Monocrotophos = 1-methyl-2-methylcarbamoylvinyldimethyl phosphate. [l]Phosdrin = Mevinphos = 2-methoxycarbonyl-1-methylvinyldimethyl phosphate. [m]Quinalphos = *O,O*-diethyl-*O*-quinoxalinyl (2)-thionophosphate. [n]TEPA = tris(1-aziridinyl) phosphine oxide.

Table 3. Effects of organochlorine pollutants on fish reproduction.

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Aldrin[a]	*Puntius conchonius*	0.00005	60–120	Increased oocyte atresia.	Kumar and Pant (1988)
	Heteropneustes fossilis	0.85	28	Reduced pituitary activity.	Singh and Singh (1981, 1982b)
		0.85	28	Reduced level of GnRH-like factor in hypothalamus.	Singh and Singh (1982b)
Endosulfan[b]	*Cyprinus carpio*	0.050–1	1.5 *in vitro*	Inhibition of LH-induced GVBD.	Haider and Inbaraj (1988)
	Rasbora daniconius	0.001	5–75	Decreased oocyte diameter, oocyte rupture, reduced GSI.	Rastogi and Kulshrestha (1990)
	Oreochromis mossambicus	0.0002–0.0015	28–63	Inhibited male reproductive behaviour.	Matthiessen and Logan (1984)
		0.001	20	Damage to pituitary and hypothalamus, decreased HSD.	Shukla and Pandey (1986)
	Channa striatus	0.00075–0.001	2–30	Inhibition of oocyte development, reduced GSI.	Kulshrestha and Arora (1984)
		0.00075–0.001	2–30	Testicular damage, failure of spermatogenesis.	Arora and Kulshrestha (1984)
Hexadrin[c]	*Heteropneustes fossilis*	0.0006–0.008	28	Decreased liver lipid, no effect on liver cholesterol. No effect on ovarian lipid, increased ovarian cholesterol.	Singh and Singh (1980d)
		0.0006	4	Increased ovarian cholesterol, no effect on liver and ovarian lipid.	Singh and Singh (1980e)
		0.0006–0.008	28	Reduced ^{32}P uptake by testis, lower pituitary and serum GtH, increased testis lipid.	Singh and Singh (1980a)
		0.008	28	Reduced pituitary activity and ovarian ^{32}P uptake.	Singh and Singh (1980b,c, 1982b)
		0.008	28	Reduced GnRH-like factor in hypothalamus.	Singh and Singh (1982b)

Table 3. *continued*

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Kepone[d]	*Oryzias latipes*	0.001–0.002	4–9	Inhibition of oviposition.	Curtis and Beyers (1978)
	Cyprinodon variegatus	0.00007–	141	Reduced egg production, decreased hatch success, shorter progeny.	Goodman *et al.* (1982)
Methoxychlor[e]	*Puntius conchonius*	0.000007	60–120	Increased oocyte atresia.	Kumar and Pant (1988)
Mirex[f]	*Oncorhynchus mykiss*	3–300 mg per kg diet	180	Inhibited oestradiol-induced plasma vitellogenin.	Chen *et al.* (1986)
β-HCH[g]	*Oryzias latipes*	0.003–1	30–90	Induced vitellogenesis and hermaphroditism.	Wester (1991)
	Poecilia reticulata	0.003–1	30–90	Excessive vitellogenin production.	Wester *et al.* (1985)
		0.003–1	30–90	Induced vitellogenesis and hermaphroditism.	Wester (1991)
γ-BHC[h]	*Carassius auratus* (Cyprinidae)	0.01–0.1	28	Changes in hepatic lipid synthesis.	Singh and Kime (1994)
		0.01–0.1	28	Decreased GSI and GtH, altered *in vitro* steroid synthesis.	Singh *et al.* (1994)
		1–20	0.12 *in vitro*	Inhibited endogenous steroidogenesis.	Kime, Singh and Singh (unpublished data)
	Rutilus rutilus (Cyprinidae)	1–20	0.12 *in vitro*	Changes in *in vitro* steroid production.	Singh and Kime (unpublished data)
	Clarias batrachus	0.002–0.008	28	Changes in hepatic and ovarian lipids.	Lal and Singh (1987)
		2–8	4–28	Decreased plasma sex steroids.	Singh and Singh (1987a,b)
	Heteropneustes fossilis	0.004–0.016	28	Reduced hepatic, ovarian and plasma lipids.	Singh (1992), Singh and Singh (1992b)
		0.005–0.02	28	Reduced plasma steroid levels.	Singh and Singh (1992c)
		16	28	Suppressed GnRH, GtH and plasma steroids and inhibited ovarian growth.	Singh and Singh (1991, 1992a), Singh *et al.* (1993)
	Oryzias latipes	0.005–0.020	28	Oocyte atresia, inhibited LH-induced *in vitro* ovulation, retarded embryonic development.	Hirose (1975)

	Oreochromis mossambicus	2–4	10	Testicular damage, lowered GSI.	Pandey and Shukla (1980)
		4	20	Damage to pituitary cells.	Shukla and Pandey (1984e)
Pentachloro-phenol	*Oncorhynchus mykiss*	0.022–0.049	18	Oocyte atresia.	Nagler *et al.* (1986)
PCB[i]	*Clupea harengus*			Viable hatch decreased with egg pollution.	von Westernhagen (1987)
		0.12 mg per kg ovary	Life	Reduction in viable hatch.	Hansen *et al.* (1985)
	Oncorhynchus mykiss	3–300 mg per kg diet	180	Inhibited oestradiol-induced plasma vitellogenin.	Chen *et al.* (1986)
	Salvelinus fontinalis	0.2	21	Increased *in vitro* 11β-hydroxylation of testosterone Decreased hatch rate.	Freeman and Idler (1975)
	Gadus morhua			Viable hatch decreased with egg pollution.	von Westernhagen (1987)
	Merlangius merlangus			Viable hatch decreased with egg pollution.	von Westernhagen (1987)
	Platichthys flesus			Viable hatch decreased with egg pollution.	von Westernhagen (1987)
	Platichthys stellatus			Embryological success decreased by egg PCB content.	Spies and Rice (1988)
Arochlor 1254[i] (PCB)	*Cyprinus carpio*	250 mg per kg ip	1	Lower plasma progesterone, testosterone and oestradiol, increased steroid catabolism.	Yano and Matsuyama (1986)
		25 mg per kg ip	28	Damage to liver, oocytes and spermatozoa. Reduced androgens and oestrogens.	Sivarajah *et al.* (1978a,b)
	Ictalurus punctatus (Ictaluridae)	1–100 mg per kg bw	1×	Changes in hepatic enzymes.	Ankley *et al.* (1986)
	Oncorhynchus mykiss	25 mg per kg ip	28	Damage to liver, oocytes and spermatozoa; reduced androgens, oestrogens. Increased P_{450}.	Sivarajah *et al.* (1978a,b)
		3–300 mg per kg diet	180	Inhibited oestradiol-induced plasma vitellogenin.	Chen *et al.* (1986)
	Gadus morhua	1–50 mg per kg diet	165	Testicular abnormalities, inhibited spermatogenesis.	Sangalang *et al.* (1981)

Table 3. *continued*

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
		1–50 mg per kg diet	92	Changes in *in vitro* steroid synthesis, testis damage.	Freeman *et al.* (1980), Freeman and Sangalang (1977a)
	Micropogonias undulatus	0.05 mg per kg bw	30	Impaired ovarian growth, vitellogenesis, steroidogenesis and GtH.	Thomas (1990)
		5 mg per kg bw	17	Impaired ovarian growth, decreased plasma oestradiol and *in vitro* GtH.	Thomas (1989)
		3 mg per kg bw	30	Decreased GSI and plasma testosterone.	Thomas (1988)
PCB (Clophen)[i]	*Phoxinus phoxinus*	20–2000 mg per kg diet	45	Reduced hatching time, low hatchability.	Bengtsson (1980)
DDT[j]	*Salmo trutta* and *Salvelinus fontinalis*	1.1–3.4 kg bw per week	98–308	Decreased fry survival.	Burdick *et al.* (1972)
	Salvelinus fontinalis	0.5–2 mg kg^{-1} $week^{-1}$	156	Mortality of fry from treated fish.	Macek (1968)
	Salvelinus namaycush	Polluted lake	Life	Fry mortality.	Burdick *et al.* (1964)
	Oreochromis mossambicus	0.001	20	Inhibited testis 3βHSD and 17βHSD.	Bhattacharya and Pandey (1989)
		0.001	20	Ovarian damage, inhibited HSD – reversed in clean water.	Shukla and Pandey (1985)
		4	20	Damage to pituitary cells.	Shukla and Pandey (1984e)
DDE[k]	*Clupea harengus*	0.018 mg per kg ovary	Life	Reduction in viable hatch.	Hansen *et al.* (1985)
2,4-D[l]	*Lepomis macrochirus* (Centrarchidae)	5	150	Delayed spawning.	Cope *et al.* (1970)
DDT, DDD,[m] methoxychlor, aldrin, dieldrin,[n]	*Gambusia affinis*	Sublethal	?	Caused abortion.	Boyd (1964)

endrin[c], toxaphene,[o] heptachlor,[p] and lindane[h]					
DDT/dieldrin	*Pseudopleuronectes americanus*	0.001–0.002	?	Decreased fertilization, deformed embryos.	Smith and Cole (1973)
DDT/PCB[q]	*Genyonemus lineatus*	4 mg/kg ovary	Life	Failure to induce spawning, decreased fecundity.	Hose *et al.* (1989)
				Decreased fertility and early oocyte loss.	Cross and Hose (1988)
DDT/PCB	*Salvelinus alpinus*	Lake Geneva	Life	Egg mortality.	Monod (1985)

[a]Aldrin = 1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-*exo*-1,4-*endo*-5,8-dimethanonaphthalene.
[b]Endosulfan = Thiodan = 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzadioxathiepin-3-oxide.
[c]Hexadrin = 20% Endrin; Endrin = 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-endo*-5,8-dimethanonaphthalene.
[d]Kepone = 1,1a,3,3a,4,5,5a,5b,6-decachloroctahydro-1,3,4-metheno-2*H*-cyclobuta (*cd*)pentalen-2-one.
[e]Methoxychlor = 2,2-bis(4-methoxyphenyl)-1,1,1-trichloroethane.
[f]Mirex = dodecachlorooctahydro-1,3,4-methano-2*H*-cyclobuta[c,d]pentalene.
[g]β-HCH = β-isomer of hexachlorocyclohexane.
[h]γ-BHC = BHC = γ = HCH = Lindane = 1α,2α,3β,4α,5α,6β-hexachlorocyclohexane.
[i]PCB = Chlophen = Arochlor 1254 = polychlorinated biphenyls.
[j]DDT = 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane.
[k]DDE = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene.
[l]2,4-D = 2,4-dichlorophenoxyacetic acid.
[m]DDD = 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane.
[n]Dieldrin = 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene.
[o]Toxaphene = 2,2,5-*endo*,6-*exo*,8,9,10-heptachlorobornane + related isomers.
[p]Heptachlor = 1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene.
[q]California coastal waters containing chlorinated hydrocarbons, notably DDT and PCBs.

Table 4. Effects of carbamate pesticides on fish reproduction.

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Carbofuran[a]	*Rasbora daniconius*	0.1	5–75	Decreased oocyte diameter, oocyte rupture, reduced GSI.	Rastogi and Kulshrestha (1990)
	Clarias batrachus	0.5	30	Morphological damage to testes.	Sadhu and Mukhopadhyay (1985)
	Colisa ialia (Belontidae)	0.7	20	Inhibited ovarian development, increased atresia.	Sukumar and Karpagaganapathy (1992)
	Channa punctatus	5	120	Decreased GSI, inhibition of oocyte growth, oocyte atresia.	Mani and Saxena (1985)
		5	120	Inhibited spermatogenesis, decreased testis weight.	Saxena and Mani (1985)
		5	120	Decreased ovarian RNA, protein and total lipid.	Saxena *et al.* (1986)
		5	120	Decreased testis weight, delayed sperm formation.	Saxena and Mani (1987)
Carbaryl[b]	*Pimephales promelas*	0.008–0.68	270	Decreased hatchability and number of eggs.	Carlson (1971)
	Anabas testudineus	1.66	90	Decreased oestrogen and GSI after 15 days.	Choudhury *et al.* (1993)
	Channa punctatus	2	150	Decreased GSI, inhibition of oocyte growth, oocyte atresia.	Saxena and Garg (1978)
		1.7	2–30	Decreased serum and pituitary GtH; higher larval mortality.	Ghosh *et al.* (1990)
	Channa striatus	10–20	2–30	Inhibition of oocyte development, reduced GSI.	Kulshrestha and Arora (1984)
		10–20	2–30	Testicular damage, failure of spermatogenesis.	Arora and Kulshrestha (1984)

[a]Carbofuran = Furadon = 2,3-dihydro-2,2-dimethylbenzofuran-7-ylmethyl carbonate (3% granules, 50% active ingredient).
[b]Carbaryl = Sevin = 50% n-methyl naphthyl-1-carbamate.

Table 5. Effects of other industrial pollutants on fish reproduction.

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Alkylphenols	*Oncorhynchus mykiss*	0.02–22	2–4	Oestrogenic, stimulates vitellogenin from male hepatocytes.	Jobling and Sumpter (1994)
Ammonia	*Channa punctatus*	0.017	7–91	Decreased GSI, oocyte diameter and oocyte maturity.	Dey and Bhattacharya (1989)
		36	1–90	Changes in hepatic and plasma cholesterol.	Bhattacharya *et al.* (1984)
Anthracene	*Pimephales promelas*	0.006–0.020	42–63	Decreased egg number, reduced survival of eggs and fry, deformed fry.	Hall and Oris (1991)
Benzo[a]pyrene	*Micropogonias undulatus*	4 mg per kg bw	30	Impaired ovarian growth and steroidogenesis.	Thomas (1990)
		979 mg per kg bw	30	Decreased GSI, plasma testosterone and oestradiol.	Thomas (1988)
Chlorinated paraffins[a]	*Platichthys flesus* (Pleuronectidae)	1 g per kg bw diet	2 ×	Changes in steroid catabolism.	Haux *et al.* (1982)
4-Chloroaniline	*Brachydanio rerio*	0.04–1	119	Decreased fertilization rate.	Bresch *et al.* (1990)
Cyanide	*Oncorhynchus mykiss*	0.01–0.03	7	Reduced serum calcium.	Da Costa and Ruby (1984)
		0.001	12	Reduced plasma vitellogenin and GSI.	Ruby *et al.* (1986)
		0.005–1	0.03	Decreased fertilization rate of exposed gametes.	Billard and Roubaud (1985)
	Salmo salar (Salmonidae)	0.005	12	Inhibition of ovarian uptake of vitellogenin.	Ruby *et al.* (1987)
Di-n-butyl-phthalate	*Rivulus marmoratus*	1–2	147	Decreased number of eggs, decreased hatchability, deformed offspring.	Davis (1988)
3-Methyl-cholanthrene	*Monopterus albus*	4	7	Reduced testosterone, oestradiol and vitellogenin; reduced GSI, histological change in gonads.	Singh (1989)

Table 5. *continued*

Pollutant	Species	Dose ($mg\ l^{-1}$)	Time (days)	Effect	Reference
Petroleum (crude)	*Salmo salar*	Oil slick	7–28	Decreased plasma androgens.	Truscott *et al.* (1983)
	Pseudopleuronectes americanus	Oil slick	12–34	Decreased plasma androgens.	Truscott *et al.* (1983)
Phenol	*Cyprinus carpio*	12	7–30	Increased HSI, decreased GSI, increased ovarian and hepatic cholesterol content.	Kumar and Mukherjee (1988), Mukherjee *et al.* (1991)
	Channa punctatus	10	1–90	Changes in hepatic and plasma cholesterol.	Bhattacharya *et al.* (1984)
Sulphide	*Cyprinus carpio*	9	7–30	Increased HSI, decreased GSI, increased ovarian and hepatic cholesterol content.	Kumar and Mukherjee (1988), Mukherjee *et al.* (1991)
Urea	*Colisa fasciatus*	2500	15–30	Morphological damage to testis.	Shukla and Pandey (1984c)
Acid pH	*Couesius plumbeus*	pH 4.7–4.5	Life	Spawning failure.	Beamish (1976)
	Catostomus commersoni	pH 3–7	Sperm	Slight decrease in motility.	Mohr and Chalanchuk (1985)
	Catostomus commersoni	pH 5.2–4.7	Life	Spawning failure.	Beamish (1976)
	Ictalurus nebulosus	pH 5.2–4.7	Life	Spawning failure.	Beamish (1976)
	Esox lucius, E. niger	pH 3.9–7.9	Sperm	Decreased sperm motility.	Duplinsky (1982)
	Coregonus artedii	pH 4.7–4.5	Life	Spawning failure.	Beamish (1976)
	Oncorhynchus mykiss	pH 4.5–7.6	20	Inhibition of vitellogenin synthesis.	Roy *et al.* (1990)
		pH 4.5–5.5	42	Reduced survival of progeny in first 7 days.	Weiner *et al.* (1986)
	Salmo salar	pH 4.7–5.6	Life	Decreased T and KT.	Freeman *et al.* (1983)
	Salvelinus fontinalis	pH 4.48	21	Inhibition of vitellogenin synthesis.	Tam *et al.* (1987)
		pH 4.5–7.3	300	Reduced number of eggs, delayed ovulation.	Tam and Payson (1986)
		pH 4.5	30–73	Increased atresia of vitellogenic oocyte, suppression of gonadotropes.	Tam *et al.* (1990)
	Salvelinus namaycush	pH 5.5–5.2	Life	Spawning failure.	Beamish (1976)
	Percopsis omiscomaycus	pH 5.5–5.2	Life	Spawning failure.	Beamish (1976)
	Lota lota	pH 6.0–5.5	Life	Spawning failure.	Beamish (1976)

	Cyprinodon n. nevadensis	pH 5–8.3	42	Decreased egg production, spawning and egg viability.	Lee and Gerking (1980)
	Jordanella floridae	pH 4.5–6.5	20	Impaired egg production and fertility.	Craig and Bakshi (1977)
				Decreased yolk deposition, retarded oocyte growth.	Ruby *et al.* (1977)
	Micropterus dolomieui	pH 6.0–5.5	Life	Spawning failure.	Beamish (1976)
	Perca flavescens	pH 4.7–4.5	Life	Spawning failure.	Beamish (1976)
	Ambloplites rupestris	pH 5.2–4.7	Life	Spawning failure.	Beamish (1976)
	Stizostedion vitreum	pH 6.0–5.5	Life	Spawning failure.	Beamish (1976)
Lake Erie	*Oncorhynchus kisutch*	Polluted	Life	Over-ripe eggs, fry deformity, low fertilization rate.	Flett *et al.* (1991)
		Polluted	Life	Lower KT and female T.	Morrison *et al.* (1985)
		Polluted	Life	Pituitary and serum GtH and plasma KT suppressed.	Leatherland *et al.* (1982)
Industrial pollutant[b]	*Channa punctatus*		30	Changes in hepatic and plasma cholesterol.	Bhattacharya *et al.* (1984)
Textile-mill effluent	*Heteropneustes fossilis*	3–7%	7–120	Oocyte atresia.	Murugesan and Haniffa (1992)
Power station effluent	*Abramis brama* (Cyprinidae)	Warm effluent	Life	Changes in gonadal maturation and spawning time.	Luksienè (1978)
	Rutilus rutilus	Warm effluent	Life	Changes in gonadal maturation and spawning time.	Luksienè (1981)
	Esox lucius	Warm effluent	Life	Changes in gonadal maturation and spawning time.	Luksienè (1982)
	Perca fluviatilis (Percidae)	Warm effluent	Life	Changes in gonadal maturation and spawning time.	Luksienè (1982)
Pulp mill[c]	*Brachydanio rerio*	0.03–0.09	7–28	Decreased hatchability and survival of larvae.	Landner *et al.* (1985)
	Rutilus rutilus	3% effluent	Life	Reduced gonad growth.	Sandström *et al.* (1988)
	Catostomus commersoni	Effluent	Life	Reduced plasma sex steroid, decreased egg size and GSI, reduced sperm motility.	McMaster *et al.* (1992)
		Effluent	Life	Lowered response to GtH and GnRH, decreased steroid production, increased liver size and mixed	Van der Kraak *et al.* (1992), McMaster *et al.* (1991)

				function oxygenase activity.	
	Salmo trutta	0.2–0.5% effluent	90	Decreased egg numbers, lower egg fertilization, fry mortality.	Vuorinen and Vuorinen (1985)
	Coregonus clupeaformis (Salmonidae)	Effluent	Life	Decreased steroid levels, increased liver size and mixed function oxygenase activity.	Munkittrick *et al.* (1992)
	Perca fluviatilis	3% effluent	Life	Reduced gonad growth.	Sandström *et al.* (1988)
Vegetable oil factory effluent	*Heteropneustes fossilis*	5–15%	150	Decreased gonadal lipid, increased fatty acid.	Kondal *et al.* (1989)
	Channa punctatus	5%	120	Retarded ovarian growth, atresia.	Saxena and Bhatia (1983)
Contaminated sediment[d]	*Parophrys vetulus*	Sediment extract	Injected	Decreased plasma oestradiol.	Stein *et al.* (1991)
		Polluted site	Life	Decreased plasma oestradiol and ovarian growth.	Johnson *et al.* (1988)
Sewage lagoon[e]	*Cyprinus carpio*		180	No effect on plasma steroids or cytochrome P_{450}.	Sivarajah *et al.* (1979)
Sewage effluent	*Oncorhynchus mykiss*	Effluent	21	Oestrogenic, stimulated vitellogenesis.	Purdom *et al.* (1994)
	Cyprinus carpio	Effluent	21	Oestrogenic, stimulated vitellogenesis.	Purdom *et al.* (1994)
Municipal wastewater	*Oncorhynchus mykiss*	1:1–1:5	14	Altered hepatic steroid catabolism.	Förlin and Hansson (1982)
North Sea	*Merlangius merlangus*, *Limanda limanda* (Pleuronectidae), *Platichthys flesus*, *Gadus morhua*, *Pleuronectes platessa* (Pleuronectidae)		life	Decreased hatch, malformed embryos.	Dethlefsen (1989)

[a]Witachlor 149 (n-paraffin, 12 carbons, 40% chlorine w/w) and Hülz 70C (n-paraffin, 12 carbons, 70% chlorine w/w).
[b]Industrial pollutant = 0.033 mg l^{-1} mercuric chloride + 33.3 mg l^{-1} cadmium chloride + 3.3 mg l^{-1} phenol + 9 mg l^{-1} ammonia or factory effluent.
[c]Kraft pulp mill effluent = 0.03 mg l^{-1} 2,4-dichlorophenol + 0.05 mg l^{-1} 2,4,6-trichlorophenol + 0.09 mg l^{-1} 3,4,5-trichloroguaiacol + 0.04 mg l^{-1} 4,5,6-trichloroguaiacol + 0.050 mg l^{-1} tetrachloroguaiacol.
[d]Contaminated sediment extract containing aromatic hydrocarbons and PCBs.
[e]Containing $1–2 \times 10^{-6}$ mg l^{-1} PCB; Carbamide = urea.

under short and long term exposures to industrial pollutants: hepatic and blood cholesterol changes. *Comp. Physiol. Ecol.* **9**, (Suppl.) 435–8.

Billard, R. and de Kinkeln, P. (1970) Stérilisation des testicules de guppies par des doses non létales de parathion. *Annls Hydrobiol.* **1**, 91–9.

Billard, R. and Roubaud, P. (1985) The effect of metals and cyanide on fertilisation in rainbow trout (*Salmo gairdneri*). *Water Res.* **19**, 209–14.

Boyd, C.E. (1964) Insecticides cause mosquitofish to abort. *Progve Fish-Cult.* **26**, 138.

Bresch, H., Beck, H., Ehlermann, D., Schlaszus, H. and Urbanek, M. (1990) A long-term toxicity test comprising reproduction and growth of zebrafish with 4 – chloroaniline. *Arch. env. Contam. Toxicol.* **19**, 419–27.

Burdick, G.E., Harris, E.J., Dean, H.J., Walker, T.M., Skea, J. and Colby, D. (1964) The accumulation of DDT in lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.* **93**, 127–36.

Burdick, G.E., Dean, H.J., Harris, E.J., Skea, J., Karcher, R. and Frisa, C. (1972) Effect of rate and duration of feeding DDT on the reproduction of salmonid fishes reared and held under controlled conditions. *NY Fish Game J.* **19**, 97–115.

Carlson, A.R. (1971) Effects of long-term exposure to carbaryl (Sevin) on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*). *J. Fish. Res. Bd Can.* **29**, 583–7.

Carragher, J.F., Sumpter, J.P., Pottinger, T.G. and Pickering, A.D. (1989) The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L., and *Salmo gairdneri* Richardson. *Gen. comp. Endocrinol.* **76**, 310–21.

Chen, T.T., Reid, P.C., Van Beneden, R. and Sonstegard, R.A. (1986) Effect of Arochlor 1254 and Mirex on estradiol-induced vitellogenin production in juvenile rainbow trout (*Salmo gairdneri*). *Can. J. Fish. aquat. Sci.* **43**, 169–73.

Choudhury, C., Ray, A.K., Bhattacharya, S. and Bhattacharya, S. (1993) Non lethal concentrations of pesticide impair ovarian function in the freshwater perch, *Anabas testudineus. Env. Biol. Fishes* **36**, 319–24.

Cope, O.B., Wood, E.M. and Wallen, G.H. (1970) Some chronic efects of 2,4-D on the bluegill (*Lepomis macrochirus*). *Trans. Am. Fish. Soc.* **99**, 1–12.

Craig, G.R. and Bakshi, W.F. (1977) The effects of depressed pH on flagfish reproduction, growth and survival. *Water Res.* **11**, 621–6.

Cross, J.N. and Hose, J.E. (1988) Evidence for impaired reproduction in white croaker (*Genyonemus lineatus*) from contaminated areas off southern California. *Mar. env. Res.* **24**, 185–8.

Curtis, L.R. and Beyers, R.J. (1978) Inhibition of oviposition in the teleost *Oryzias latipes*, induced by subacute kepone exposure. *Comp. Biochem. Physiol.* **61C**, 15–16.

Da Costa, H. and Ruby, S.M. (1984) The effect of sublethal cyanide on vitellogenic parameters in rainbow trout *Salmo gairdneri. Arch. env. Contam. Toxicol.* **13**, 101–4.

Davis, W.P. (1988) Reproductive and developmental responses in the self-fertilising fish, *Rivulus marmoratus*, induced by the plasticizer, di-n-butylphthalate. *Env. Biol. Fishes* **21**, 81–90.

Dethlefsen, V. (1989) Fish in the polluted North Sea. *Dana* **8**, 109–29.

Dey, S. and Bhattacharya, S. (1989) Ovarian damage to *Channa punctatus* after chronic exposure to low concentrations of elsan, mercury and ammonia. *Ecotoxicol. env. Safety* **17**, 247–57.

Duplinsky, P.D. (1982) Sperm motility of nothern pike and chain pickerel at various pH values. *Trans. Am. Fish. Soc.* **111**, 768–71.

Flett, P.A., Munkittrick, K.R. and Leatherland, J.F. (1991) Reproductive problems in Lake Erie coho salmon. In Scott, A.P., Sumpter, J.P., Kime, D.E. and Rolfe, M.S., eds. *Proc. 4th Int. Symp. Reprod. Physiol. Fish.* Sheffield: FishSymp91, pp. 151–3.

Förlin, L. and Hansson, T. (1982) Effects of treated municipal wastewater on the hepatic, xenobiotic, and steroid metabolism in trout. *Ecotoxicol. env. Safety.* **6**, 41–8.

Freeman, H.C. and Idler, D.R. (1975) The effect of polychlorinated biphenyl on steroidogenesis

and reproduction in the brook trout (*Salvelinus fontinalis*). *Can. J. Biochem.* **53**, 666–70.

Freeman, H.C. and Sangalang, G. (1977a) The effects of a polychlorinated biphenyl (Arochlor 1254) contaminated diet on steroidogenesis and reproduction in the Atlantic cod (*Gadus morhua*). *Int. Council Explor. Sea, Reykjavik, Iceland 1977 CM1977/E67 (Fisheries Improvement Committee)*, 12 pp.

Freeman, H.C. and Sangalang, G. (1977b) Changes in steroid hormone metabolism as a sensitive method of monitoring pollutants and contaminants. *Proc. 3rd Aquat. Toxicity Workshop, Halifax, NS, Nov. 1976. Environ. Prot. Service Tech. Rep. No. EPS-5-77-1, Halifax, Canada.* pp. 123–32.

Freeman, H.C., Uthe, J.F. and Sangalang, G. (1980) The use of steroid hormone metabolism studies in assessing the sublethal effects of marine pollution. *Rapp. P.-v. Réun. Cons. int. Explor. Mer,* **179**, 16–22.

Freeman, H.C., Sangalang, G.B., Burns, G. and McMenemy, M. (1983) The blood sex hormone levels in sexually mature male Atlantic salmon (*Salmo salar*) in the Westfield River (pH 4.7) and the Medway River (pH 5.6) Nova Scotia. *Sci. Total Environ.* **32**, 87–91.

Freeman, H.C., Sangalang, G.B. and Uthe, J.F. (1984) The effects of pollutants and contaminants on steroidogenesis in fish and marine mammals. In Cairns, V.W., Hodson, P.V. and Nriagu, J.R., eds. *Contamination Effects on Fisheries.* New York: Wiley, pp. 197–211.

Ghosh, P., Bhattacharya, S. and Bhattacharya, S. (1990) Impairment of the regulation of gonadal function in *Channa punctatus* by Metacid-50 and Carbaryl under laboratory and field conditions. *Biomed. env. Sci.* **3**, 106–12.

Goodman, L.R., Hansen, D.J., Manning, C.S. and Faas, L.F. (1982) Effects of Kepone on the sheepshead minnow in an entire life-cycle toxicity test. *Arch. env. Contam. Toxicol.* **11**, 335–42.

Haider, S. and Inbaraj, R.M. (1988) *In vitro* effect of malathion and endosulfan on the LH-induced oocyte maturation in the common carp, *Cyprinus carpio* (L.). *Water, Air Soil Poll.* **39**, 27–31.

Haider, S. and Upadhyaya, N. (1985) Effect of commercial formulation of four organophosphorus insecticides on the ovaries of a freshwater teleost, *Mystus vittatus* (Bloch) – a histological and histochemical study. *J. env. Sci. Health.* **B20**, 321–40.

Haider, S. and Upadhyaya, N. (1986) Effect of commerical formulation of four organophosphorus insecticides on the LH-induced germinal vesicle breakdown in the oocytes of a freshwater teleost, *Mystus vittatus* (Bloch) – a preliminary *in vitro* study. *Ecotoxicol. env. Safety* **12**, 161–5.

Hall, A.T. and Oris, J.T. (1991) Anthracene reduces reproductive potential and is maternally transferred during long-term exposure in fathead minnows. *Aquat. Toxicol.* **19**, 249–64.

Hansen, P.D., Westernhagen, H. von and Rosenthal, H. (1985) Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners. *Mar. env. Res.* **15**, 59–76.

Hatakeyama, S. and Yasuno, M. (1987) Chronic effects of Cd on the reproduction of the guppy (*Poecilia reticulata*) through Cd-accumulated midge larvae (*Chironomus yoshimatsui*). *Ecotoxicol. env. Safety* **14**, 191–207.

Haux, C., Larsson, Å., Lidman, U., Förlin, L., Hansson, T. and Johansson-Sjöbeck, M.-L.(1982) Sublethal physiological effects of chlorinated paraffins on the flounder, *Platichthys flesus* L. *Ecotoxicol. env. Safety* **6**, 49–59.

Haux, C., Björnsson, B. Th., Förlin, L., Larsson, Å. and Deftos, L.J. (1988) Influence of cadmium exposure on plasma calcium, vitellogenin and calcitonin in vitellogenic rainbow trout. *Mar. env. Res.* **24**, 199–202.

Hirose, K. (1975) Reproduction in medaka, *Oryzias latipes*, exposed to sublethal concentrations of γ-benzenehexachloride (BHC). *Bull. Tokai Reg. Fish. Res. Lab.* **81**, 139–49.

Hose, J.E., Hannah, J.B., Landolt, M.L., Miller, B.S., Felton, S.P. and Iwaoka, W.T. (1981) Uptake of benzo[α]pyrene by gonadal tissue of flatfish (family Pleuronectidae) and its effects on subsequent egg development. *J. Toxicol. env. Health* **7**, 991–1000.

Hose, J.E., Cross, J.N., Smith, S.G. and Diehl, D. (1989) Reproductive impairment in a fish inhabiting a contaminated coastal environment off southern California. *Env. Poll.* **57**, 139–48.

Jobling, S. and Sumpter, J.P. (1994) Detergent components in sewage effuent are weakly oestrogenic to fish: an *in vitro* study using rainbow trout (*Oncorhynchus mykiss*) hepatocytes. *Aquat. Toxicol.* (in press)

Johnson, L.L., Casillas, E., Collier, T.K., McCain, B.B. and Varanasi, U. (1988) Contaminant effects on ovarian development in English sole (*Parophrys vetulus*) from Puge Sound, Washington. *Can. J. Fish. aquat. Sci.* **45**, 2133–46.

Kapur, K., Kamaldeep, K. and Toor, H.S. (1978) The effect of fenitrothion on reproduction of a teleost fish. *Cyprinus carpio communis* Linn.: a biochemical study. *Bull. env. Contam. Toxicol.* **20**, 438–42.

Katti, S.R. and Sathyanesan, A.G. (1986) Changes in the hypothalamo–neurohypophysial complex of lead treated teleostean fish *Clarias batrachus* (L.). *Z. mikrosk.-anat. forsch.* **100**, 347–52.

Katti, S.R. and Sathyanesan, A.G. (1987) Lead nitrate-induced nuclear inclusions in the oocytes of the catfish *Clarias batrachus* (L). *Env. Res.* **44**, 238–40.

Khan, A.T. and Weis, J.S. (1987a) Toxic effects of mercuric chloride on sperm and egg viability of two populations of mummichog, *Fundulus heteroclitus. Env. Poll.* **48**, 263–73.

Khan, A.T. and Weis, J.S. (1987b) Effects of methylmercury on sperm and egg viability of two populations of killifish (*Fundulus heteroclitus*). *Arch. env. Contam. Toxicol.* **16**, 499–505.

Khan, A.T. and Weis, J.S. (1987c) Effect of methylmercury on egg and juvenile viability in two populations of killifish *Fundulus heteroclitus. Env. Res.* **44**, 272–8.

Khan, A.T. and Weis, J.S. (1993) Differential effects of organic and inorganic mercury on the micropyle of the eggs of *Fundulus heteroclitus. Env. Biol. Fishes* **37**, 323–7.

Kihlström, J.E., Lundberg, C. and Hulth, L. (1971) Number of eggs and young produced by zebrafishes (*Brachydanio rerio*, Ham.–Buch.) spawning in water containing small amounts of phenylmercuric acetate. *Env. Res.* **4**, 355–9.

Kime, D.E. (1984) The effect of cadmium on steroidogenesis by testes of the rainbow trout, *Salmo gairdneri. Toxicol. Lett.* **22**, 83–8.

Kime, D.E. (1993) Classical and non-classical steroids in teleost fish. *Rev. Fish Biol. Fisheries* **3**, 160–80.

Kime, D.E., Vinson, G.P., Major, P.W. and Kilpatrick, R. (1980) Adrenal–gonad relationships. In Chester Jones, I. and Henderson, I.W., eds. *General, Comparative and Clinical Endocrinology of the Adrenal Cortex*, Vol. 3. New York and London: Academic Press, pp. 183–264.

Kime, D.E., Epler, P., Bieniarz, K., Sokolowska, M., Motyka, K. and Mikolajczyk, T. (1987) The temporal sequence of changes in oocyte maturation and ovarian steroid hormone production during induced ovulation in the common carp, *Cyprinus carpio. Gen. comp. Endocrinol.* **68**, 313–21.

Kime, D.E., Scott, A.P. and Canario, A.V.M. (1992) *In vitro* biosynthesis of steroids, including 11-deoxycortisol and 5α-pregnane-3β, 7α, 17,20β-tetrol, by ovaries of the goldfish *Carassius auratus* during the stage of oocyte final maturation. *Gen. comp. Endocrinol.* **87**, 375–84.

Kirubagaran, R. and Joy, K.P. (1988a) Toxic effects of mercuric chloride, methylmercuric chloride, and emisan 6 (an organic mercurial fungicide) on ovarian recrudescence in the catfish *Clarias batrachus* (L.). *Bull env. Contam. Toxicol.* **41**, 902–9.

Kirubagaran, R. and Joy, K.P. (1988b) Inhibition of testicular 3β-hydroxy-Δ_5-steroid dehydrogenase (3β-HSD) activity in catfish *Clarias batrachus* (L.) by mercurials. *Ind. J. exp. Biol.* **26**, 907–8.

Kling, D. (1981) Total atresia of the ovaries of *Tilapia leucosticta* (Cichlidae) after intoxication with the insecticide Lebaycid. *Experientia.* **37**, 73–4.

Klondal, J.K., Saxena, P.K. and Soni, G.L. (1989) Effect of vegetable oil factory effluent on the gonads of a freshwater teleost, *Heteropneustes fossilis* (Bl.) – a biochemical study. *Env. Poll.* **57**, 117–25.

Kulshrestha, S.K. and Arora, N. (1984) Impairments induced by sublethal doses of two pesticides in the ovaries of a freshwater teleost *Channa striatus* Bloch. *Toxicol. Lett.* **20**, 93–8.

Kumar, S. and Pant, S.C. (1984) Comparative effects of the sublethal poisoning of zinc, copper and lead on the gonads of the teleost *Puntius conchonius* Ham. *Toxicol. Lett.* **23** 189–94.

Kumar, S. and Pant, S.C. (1988) Comparative sublethal ovarian pathology of some pesticides in the teleost, *Puntius conchonius* Hamilton. *Bull. env. Contam. Toxicol.* **41**, 227–32.

Kumar, V. and Mukherjee, D. (1988) Phenol and sulfide induced changes in the ovary and liver of sexually maturing common carp, *Cyprinus carpio. Aquat. Toxicol.* **13**, 53–60.

Kumari, M.and Dutt, N.H.G. (1991) Cadmium induced histomorphological changes in the testis and pituitary gonadotrophic hormone secreting cells of the cyprinid *Puntius sarana. Boll. Zool.* **58**, 71–6.

Lal, B. and Singh, T.P. (1987) The effect of malathion and γ-BHC on the lipid metabolism in relation to reproduction in the tropical teleost, *Clarias batrachus. Env. Poll.* **48**, 37–47.

Landner, L., Neilsson, A.H., Sörensen, L., Tärnholm, A. and Viktor, T. (1985) Short-term test for predicting the potential of xenobiotics to impair reproductive success in fish. *Ecotoxicol. env. Safety* **9**, 282–93.

Leatherland, J.F., Copeland, P., Sumpter, J.P. and Sonstegard, R.A. (1982) Hormonal control of gonad maturation and development of secondary sexual charactersitics in coho salmon. *Oncorhynchus kisutch*, from Lakes Ontario, Erie and Michigan. *Gen comp. Endocrinol.* **48**, 196–204.

Lee, R.M. and Gerking, S.D. (1980) Survival and reproductive performance of the desert pupfish. *Cyprinodon n. nevadensis* (Eigenmann and Eigenmann) in acid waters. *J. Fish Biol.* **17**, 507–15.

Luksienė, D. (1978) Effect of thermic regimen of the basin-condenser of the Lithuanian State Regional Electric Power Plant on fish reproduction: 1. Characteristics of the sexual cycle of the bream. *Lithuanian SSR Acad. Sci. Ser. C*, **4(84)**, 81–91 (in Russian). (*Biol. Abstr.* **68** 59654 (1979)).

Luksienė, D. (1981) Effect of thermic regimen of the basin-condenser of the Lithuanian State Regional Electric Power Plant on fish reproduction: 2. Features of oogenesis in the roach *Rutilus rutilus. Lithuanian SSR Acad. Sci. Ser. B*, **1(73)**, 61–73 (in Russian). (*Biol. Abstr.* **74**, 42665 (1982)).

Luksienė, D. (1982) Effect of thermic regimen of the basin-condenser of the Lithuanian State Regional Electric Power Plant on fish reproduction: 3. Characteristics of oogenesis of the perch *Perca fluviatilis* and the pike *Esox lucius. Lithuanian SSR Acad. Sci. Ser. B*, **2(78)**, 32–46 (in Russian). (*Biol. Abstr.* **76**, 40368 (1983)).

Macek, K.J. (1968) Reproduction in brook trout (*Salvelinus fontinalis*) fed sublethal concentrations of DDT. *J. Fish. Res. Bd Can.* **25**, 1787–96.

McIntyre, J.D. (1973) Toxicity of methyl mercury for steelhead trout sperm. *Bull. env. Contam. Toxicol.* **9**, 98–9.

McKim, J.M. and Benoit, D.A. (1971) Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Bd Can.* **28**, 655–62.

McMaster, M.E., Van der Kraak, G.J., Portt, C.B., Munkittrick, K.R., Sibley, P.K., Smith, I.R. and Dixon, D.G. (1991) Changes in hepatic mixed-function oxygenase (MFO) activity, plasma steroid levels and age at maturity of a white sucker (*Catostomus commersoni*) population exposed to bleached kraft pulp mill effluent. *Aquat. Toxicol.* **21**, 199–218.

McMaster, M.E., Portt, C.B., Munkittrick, K.R. and Dixon, D.G. (1992) Milt characteristics, reproductive performance, and larval survival and development of white sucker exposed to bleached kraft mill effluent. *Ecotoxicol. env. Safety* **23**, 103–17.

Mani, K. and Saxena, P.K. (1985) Effect of safe concentrations of some pesticides on ovarian recrudescence in the freshwater murrel, *Channa punctatus* (Bl.): a quantitative study. *Ecotoxicol. env. Safety* **9**, 241–9.

Matthiesssen, P. and Logazn, J.W.M. (1984) The effects of low concentrations of endosulfan insecticide on reproductive behaviour in the tropical cichlid fish *Sarotherodon mossambicus. Bull. env. Contam. Toxicol.* **33**, 575–83.

Mohr, L.C. and Chalanchuk, S.M. (1985) The effect of pH on sperm motility of white suckers, *Catostomus commersoni*, in the Great Lakes Area. *Env. Biol. Fishes* **14**, 309–14.

Monod, G. (1985) Egg mortality of Lake Geneva Charr (*Salvelinus alpinus* L.) contaminated by PCB and DDT derivatives. *Bull env. Contam. Toxicol.* **35**, 531–6.

Morrison, P.F., Leatherland, J.F. and Sonstegard, R.A. (1985) Plasma cortisol and sex steroid levels in Great Lakes coho salmon (*Oncorhynchus kisutch* Walbaum) in relation to fecundity and egg survival. *Comp. Biochem. Physiol.* **80A**, 61–8.

Mount, D.R., Hockett, J.R. and Gern, W.A. (1988) Effect of long-term exposure to acid, aluminium, and low calcium on adult brook trout (*Salvelinus fontinalis*). 2. Vitellogenesis and osmoregulation. *Can. J. Fish. aquat. Sci.* **45**, 1633–42.

Mukherjee, D., Guha, D., Kumar, V. and Chakrabarty, S. (1991) Impairment of steroidogenesis and reproduction in sexually mature *Cyprinus carpio* by phenol and sulfide under laboratory conditions. *Aquat. Toxicol.* **21**, 29–40.

Munkittrick, K.R. and Dixon, D.G. (1989) Effects of natural exposure to copper and zinc on egg size and larval copper tolerance in white sucker (*Catostomus commersoni*) *Ecotoxicol env. Safety* **18**, 15–26.

Munkittrick, K.R., Portt, C.B., Van der Kraak, G.J., Smith, I.R. and Rokosh, D.A. (1991) Impact of bleached kraft mill effluent on population characteristics, liver MFO activity, and serum steroid levels of a Lake Superior white sucker (*Catostomus commersoni*) population. *Can. J. Fish. aquat. Sci.* **48**, 1371–80.

Munkittrick, K.R., McMaster, M.E., Portt, C.B., Smith, I.R. and Dixon, D.G. (1992) Changes in maturity, plasma sex steroid levels, hepatic mixed-function oxygenase activity, and the presence of external lesions in Lake Whitefish (*Coregonus clupeaformis*) exposed to bleached kraft mill effluent. *Can. J. Fish. aquat. Sci.* **49**, 1560–69.

Murugesan, A.G. and Haniffa, M.A. (1992) Histopathological and histochemical changes in the oocytes of the air-breathing fish *Heteropneustes fossilis* (Bloch) exposed to textile-mill effluent. *Bull. env. Contam. Toxicol.* **48**, 929–36.

Nagler, J.J., Aysola, P. and Ruby, S.M. (1986) Effect of sublethal pentachlorophenol on early oogenesis in maturing female rainbow trout (*Salmo gairdneri*). *Arch. env. Contam. Toxicol.* **15**, 549–55.

Pandey, A.K. and Shukla, L. (1980) Effect of benzene hexachloride – an insecticide on the testicular histology in *Tilapia mossambica. Geobios* **7**, 251–3.

Pandey, A.K. and Shukla, L. (1982) Effect of an organophosphorus insecticide malathion on the testicular histophysiology in *Sarotherodon mossambicus. Nat. Acad.Sci. Lett.* **5**, 141–3.

Pawar, K.R. and Katdare, M. (1983) Effect of sumithion on the ovaries of freshwater fish *Garra mullya* (Sykes). *Curr. Sci.* **16**, 784–5.

Pereira, J.J., Mercaldo-Allen, R., Kuropat, C., Luedke, D. and Sennefelder, G. (1993) Effect of cadmium accumulation on serum vitellogenin levels and hepatosomatic and gonadosomatic indices of winter flounder (*Pleuronectes americanus*). *Arch. env. Contam. Toxicol.* **24**, 427–31.

Pickering, A.D. (1981) *Stress and Fish.* London: Academic Press. 368 pp.

Pickering, A.D., Pottinger, T.G., Carragher, J. and Sumpter, J.P. (1987) The effect of acute and chronic stress on the levels of reproductive hormones in the plasma of mature male brown trout *Gen. comp. Endocrinol.* **68**, 249–59.

Povlsen, A.F., Korsgaard, B. and Bjerregaard, P. (1990) The effect of cadmium on vitellogenin metabolism in estradiol-induced flounder (*Platichthys flesus* (L.)) males and females. *Aquat. Toxicol.* **17**, 253–62.

Pundir, R. and Saxena, A.B. (1990) Seasonal changes in the testes of fish *Puntius ticto*, and their relation to heavy metal toxicity. *Bull. Env. Contam. Toxicol.* **45**, 288–93.

Pundir, R. and Saxena, A.B. (1992) Chronic toxic exposure of cadmium on the pituitary gland of fish *Puntius ticto* and pattern of recoupment. *J. env. Biol.* **13**, 69–74.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R. and Sumpter, J.P. (1994) Estrogenic effects of effluents from sewage treatment works. *Chem. Ecol.* **8**, 275–85.

Ram, R.N. and Sathyanesan, A.G. (1983) Effect of mercuric chloride on the reproductive cycle of the teleostean fish *Channa punctatus*. *Bull. env. Contam. Toxicol.* **30**, 24–7.

Ram, R.N. and Sathyanesan, A.G. (1984) Mercuric chloride induced changes in the protein, lipid and cholesterol levels of the liver and ovary of the fish *Channa punctatus*. *Env. Ecol.* **2**, 113–17.

Ram, R.N. and Sathyanesan, A.G. (1986) Effect of a mercurial fungicide on the gonadal development of the teleostean fish *Channa punctatus* (Bloch). *Ecotoxicol. Env. Safety* **11**, 352–60.

Ram, R.N. and Sathyanesan, A.G. (1987) Effects of long-term exposure to Cythion on the reproduction of the teleost fish, *Channa punctatus* (Bloch). *Env. Poll.* **44**, 49–60.

Rastogi, A. and Kulshrestha, S.K. (1990) Effect of sublethal doses of three pesticides on the ovary of a carp minnow *Rasbora daniconius*. *Bull. env. Contam. Toxicol.* **45**, 742–7.

Roy, R.L., Ruby, S.M., Idler, D.R. and So, Y. (1990) Plasma vitellogenin levels in pre-spawning rainbow trout, *Oncorhynchus mykiss*, during acid exposure. *Arch. env. Contam. Toxicol.* **19**, 803–6.

Ruby, S.M., Aczel, J. and Craig, G.R. (1977) The effects of depressed pH on oogenesis in flagfish *Jordanella floridae*. *Water Res.* **11**, 757–62.

Ruby, S.M., Idler, D.R. and So, Y.P. (1986) The effect of sublethal cyanide exposure on plasma vitellogenin levels in rainbow trout (*Salmo gairdneri*) during early vitellogenesis. *Arch. env. Contam. Toxicol.* **15**, 603–7.

Ruby, S.M., Idler, D.R. and So, Y.P. (1987) Changes in plasma, liver, and ovary vitellogenin in landlocked Atlantic salmon following exposure to sublethal cyanide. *Arch. env. Contam. Toxicol.* **16**, 507–10.

Sadhu, A.R. and Mukhopadhyay, P.K. (1985) Comparative effect of two pesticides malathion and carbofuran on testes of *Clarias batrachus* (Linn.). *J. Env. Biol.* **6**, 217–22.

Sandström, O., Neuman, E. and Karås, P. (1988) Effects of a bleached pulp mill effluent on growth and gonad function in Baltic coastal fish. *Water. Sci. Tech.* **20**, 107–18.

Sangalang, G.B. and Freeman, H.C. (1974) Effects of sublethal cadmium on maturation and testosterone and 11-ketotestosterone production *in vivo* in brook trout. *Biol. Reprod.* **11**, 429–35.

Sangalang, G.B. and O'Halloran, M.J. (1973) Adverse effects of cadmium on brook trout testis and on *in vitro* testicular androgen synthesis. *Biol. Reprod.* **9**, 394–403.

Sangalang, G.B., Freeman, H.C. and Corwell, R. (1981) Testicular abnormalities in cod (*Cadus morhua*) fed Arochlor 1254. *Arch. env. Contam. Toxicol.* **10**, 617–26.

Saxena, P.K. and Bhatia, R. (1983) Effect of vegetable oil factory effluent on ovarian recrudescence in the freshwater teleost, *Channa punctatus* (Bl.). *Water Air Soil Poll.* **20**, 55–61.

Saxena, P.K. and Garg, M. (1978) Effect of insecticidal pollution on ovarian recrudescence in the freshwater teleost, *Channa punctatus* Bl. *Ind. J. exp. Biol.* **16**, 690–91.

Saxena, P.K. and Mani, K. (1985) Quantitative study of testicular recrudescence in the fresh water teleost, *Channa punctatus* (Bl.) exposed to pesticides. *Bull. env. Contam. Toxicol.* **34**, 597–607.

Saxena, P.K. and Mani, K. (1987) Effect of safe concentrations of some pesticides on testicular recrudescence in the freshwater murrel, *Channa punctatus* (Bl.): a morphological study. *Ecotoxicol. env. Safety* **14**, 56–63.

Saxena, P.K., Mani, K. and Kondal, J.K. (1986) Effect of safe application rate (SAR) concentrations of some biocides on the gonads of the fresh water murrel, *Channa punctatus* (Bl.) – a biochemical study. *Ecotoxicol. env. Safety* **12**, 1–14.

Sehgal, R. and Pandey, A.K. (1984) Effect of cadmium chloride on testicular activities in guppy

Lebistes reticulatus. Comp. Physiol. Ecol. **9**, 225–30.

Sehgal, R., Tomar, V. and Pandey, A.K. (1984) Comparative effects of two heavy metallic salts on the testis of viviparous teleost, *Lebistes reticularis* (Peters). *J. env. Biol.* **5**, 185–92.

Sharpe, R.M. and Skakkebak, N.E. (1993) Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract. *Lancet* **B41**, 1392–5.

Shukla, J.P. and Pandey, K. (1984a) Arsenic induced structural changes during the ovarian cycle of a freshwater perch, *Colisa fasciatus* (Bloch and Schneider). *Bull. Inst. Zool. Acad. Sinica* **23**, 69–74.

Shukla, J.P. and Pandey, K. (1984b) Impaired spermatogenesis in arsenic treated freshwater fish, *Colisa fasciatus* (B1. and Sch.). *Toxicol. Lett.* **21**, 191–5.

Shukla, J.P. and Pandey, K. (1984c) Arsenic induced cellular and biochemical changes during the testicular cycle of a freshwater perch, *Colisa fasciatus* (B1. & Sch.). *Cell. mol. Biol.* **30**, 227–31.

Shukla, J.P. and Pandey, K. (1984d) Carbamide induced histological alterations during different phases of the testicular cycle of a freshwater perch, *Colisa fasciatus* (B1. & Sch.) *Bull. Inst. Zool., Acad. Sinica* **23**, 181–6.

Shukla, J.P. and Pandey, K. (1984e) Effects of three insecticides on the histoarchitecture of pituitary gland in *Sarotherodon mossambicus. Uttar Pradesh J. Zool.* **4**, 201–4.

Shukla, J.P. and Pandey, K. (1985) Ovarian recrudescence in a freshwater teleost, *Sarotherodon mossambicus* in DDT treatment and pattern of recoupment. *J. env. Biol.* **6**, 195–204.

Shukla, J.P. and Pandey, K. (1986) Effects of endosulphan on the hypothalamo–hypophysial complex and fish reproductive physiology. *Bull. env. Contam. Toxicol.* **36**, 122–31.

Shukla, L., Shrivastava, A., Merwani, D. and Pandey, A.K. (1984) Effect of sublethal malathion on ovarian histophysiology in *Sarotherodon mossambicus. Comp. Physiol. Ecol.* **9**, 13–17.

Singh, H. (1989) Interaction of xenobiotics with reproductive endocrine functions in a protogynous teleost, *Monopterus albus. Mar. env. Res.* **28**, 285–9.

Singh, H. and Singh, T.P. (1980a) Effects of two pesticides on testicular ^{32}P uptake, gonadotrophic potency, lipid and cholesterol content of testis, liver and blood serum during spawning phase in *Heteropneustes fossilis* (Bloch). *Endokrinologie* **76**, 288–96.

Singh, H. and Singh, T.P. (1980b) Short-term effect of two pesticides on the survival, ovarian ^{32}P uptake and gonadotrophic potency in a freshwater catfish, *Heteropneustes fossilis* (Bloch). *J. Endocrinol.* **85**, 193–9.

Singh, H. and Singh. T.P. (1980c) Effect of two pesticides on ovarian ^{32}P uptake and gonadotrophin concentration during different phases of annual reproductive cycle in the freshwater catfish, *Heteropneustes fossilis* (Bloch). *Env. Res.* **22**, 190–200.

Singh, H. and Singh, T.P. (1980d) Effect of two pesticides on total lipid and cholesterol contents of ovary, liver and blood serum during different phases of the annual reproductive cycle in the freshwater teleost *Heteropneustes fossilis* (Bloch). *Env. Poll.* **23**, 9–17.

Singh, H. and Singh, T.P. (1980e) Short-term effect of two pesticides on lipid and cholesterol content of liver, ovary and blood serum during the pre-spawning phase in the freshwater teleost, *Heteropneustes fossilis* (Bloch). *Env. Poll.* **22**, 85–90.

Singh, H. and Singh, T.P. (1981) Effect of parathion and aldrin on survival, ovarian ^{32}P-uptake and gonadotrophic potency in a freshwater catfish, *Heteropneustes fossilis* (Bloch). *Endokrinologie* **77**, 173–8.

Singh, H. and Singh, T.P. (1982a) Effects of pesticides on fish reproduction. *Ichthyologia* **15**, 71–81.

Singh, H. and Singh, T.P. (1982b) Effect of some pesticides on hypothalamo–hypophyseal–ovarian axis in the freshwater catfish *Heteropneustes fossilis* (Bloch). *Env. Poll.* **27A**, 283–8.

Singh, P.B. (1992) Impact of malathion and γ-BHC on lipid metabolism in the freshwater female catfish, *Heteropneustes fossilis. Ecotoxicol. env. Safety* **23**, 22–32.

Singh, P.B. and Kime, D.E. (1994) *In vivo* incorporation of [1-^{14}C]acetic acid into liver lipids of goldfish, *Carassius auratus*, during γ-hexachlorocyclohexane exposure. *Aquat. Toxicol.* (in press)

Singh, P.B. and Singh, T.P. (1991) Impact of γ-BHC on sex steroid levels and their modulation by ovine luteinizing hormone-releasing hormone and *Mystus* gonadotropin in the freshwater catfish, *Heteropneustes fossilis. Aquat. Toxicol.* **21**, 93–102.

Singh, P.B. and Singh, T.P. (1992a) Modulatory actions of ovine luteinizing hormone-releasing hormone and *Mystus* gonadotropin on γ-BHC-induced changes in lipid levels in the freshwater catfish, *Heteropneustes fossilis. Ecotoxicol. env. Safety* **24**, 192–202.

Singh, P.B. and Singh, T.P. (1992b) Impact of γ-BHC on lipid class levels and their modulation by reproductive hormones in the freshwater catfish, *Heteropneustes fossilis. Bull. env. Contam. Toxicol.* **48**, 23–30.

Singh, P.B. and Singh, T.P. (1992c) Impact of malathion and γ-BHC on steroidogenesis in the freshwater catfish, *Heteropneustes fossilis. Aquat. Toxicol.* **22**, 69–80.

Singh, P.B., Kime, D.E. and Singh, T.P. (1993) Modulatory actions of *Mystus* gonadotropin on γ-BHC-induced histological changes, cholesterol, and sex steroid levels in *Heteropneustes fossilis. Ecotoxicol. env. Safety* **25**, 141–53.

Singh, P.B., Kime, D.E., Epler, P. and Chyb, J. (1994) Impact of γ-hexachlorocyclohexane exposure on plasma gonadotropin levels and *in vitro* stimulation of gonadal steroid production by carp hypophyseal homogenate in *Carassius auratus. J. Fish Biol.* **44**, 195–204.

Singh, S. and Singh, T.P. (1987a) Impact of malathion and hexachlorocyclohexane on plasma profiles of three sex hormones during different phases of the reproductive cycle in *Clarias batrachus. Pesticide Biochem. Physiol.* **27**, 301–8.

Singh, S. and Singh, T.P. (1987b) Evaluation of toxicity limit and sex hormone production in response to Cythion and BHC in the vitellogenic catfish *Clarias batrachus. Env. Res.* **42**, 482–8.

Singh, T.P., Yadav, A.K., Lal, B. and Singh, S. (1989) Impact of pesticide on fish reproduction: a review. *Sci. Res. J.* **1**, 9–20.

Sivarajah, K., Franklin, C.S. and Williams, W.P. (1978a) Some histopathological effects of Arochlor 1254 on the liver and gonads of rainbow trout, *Salmo gairdneri* and carp, *Cyprinus carpio. J. Fish Biol.* **13**, 411–14.

Sivarajah, K., Franklin, C.S. and Williams, W.P. (1978b) The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. *J. Fish Biol.* **13**, 401–9.

Sivarajah, K., Franklin, C.S. and Williams, W.P. (1979) Some studies on the hepatic microsomal enzyme activities and steroid hormone levels in carp, *Cyprinus carpio* exposed for six months. *J. Fish Biol.* **15**, 249–53.

Smith, R.M. and Cole, C.F. (1973) Effects of egg concentrations of DDT and dieldrin on development in winter flounder (*Pseudopleuronectes americanus*). *J. Fish. Res. Bd Can.* **30**, 1894–8.

Speranza, A.W., Seeley, R.J., Seeley, V.A. and Perlmutter, A. (1977) The effect of sublethal concentrations of zinc on reproduction in the zebrafish, *Brachydanio rerio* Hamilton–Buchanan. *Env. Poll.* **12**, 217–22.

Spies, R.B. and Rice, D.W., jun. (1988) Effects of organic contaminants on reproduction of the starry flounder *Platichthys stellatus* in San Francisco Bay. II. Reproductive success of fish captured in San Francisco Bay and spawned in the laboratory. *Mar. Biol.* **98**, 191–200.

Srivastava, A.K. (1987) Changes induced by lead in fish testis. *J. env. Biol.* **8**, 329–32.

Stein, J.E., Hom, T., Sanborn, H.R. and Varanasi, U. (1991) Effects of exposure to a contaminated-sediment extract on the metabolism and disposition of 17β-estradiol in English sole (*Parophrys vetulus*), *Comp. Biochem. Physiol.* **99C**, 231–40.

Stock, J.N. and Cope, O.B. (1969) Some effects of TEPA, an insect chemosterilant, on the guppy, *Poecilia reticulata. Trans. Am. Fish. Soc.* **2**, 280–87.

Sukumar, A. and Karpagaganapathy, P.R. (1992) Pesticide-induced atresia in ovary of a freshwater fish, *Colisa ialia* (Hamilton–Buchanan). *Bull. env. Contam. Toxicol.* **48**, 457–62.

Tam, W.H. and Payson, P.D. (1986) Effects of chronic exposure to sublethal pH on growth, egg

production, and ovulation in brook trout, *Salvelinus fontinalis*. *Can. J. Fish. aquat. Sci.* **43**, 275–80.

Tam, W.H., Birkett, L., Makaran, R., Payson, P.D., Whitney, D.K. and Yu, C.K.-C. (1987) Modification of carbohydrate metabolism and liver vitellogenic function in brook trout (*Salvelinus fontinalis*) by exposure to low pH. *Can. J. Fish. aquat. Sci.* **44**, 630–35.

Tam, W.H., Fryer, J.N., Valentine, B. and Roy, R.J.J. (1990) Reduction in oocyte production and gonadotrope activity, and plasma levels of estrogens and vitellogenin, in brook trout exposed to low environmental pH. *Can. J. Zool.* **68**, 2468–76.

Thomas, J.A., Korach, K.S. and McLachlan, J.A. (eds) (1985) *Endocrine Toxicology*. New York: Raven Press. 404 pp.

Thomas, P. (1988) Reproductive endocrine function in female Atlantic croaker exposed to pollutants. *Mar. env. Res.* **24**, 179–83.

Thomas, P. (1989) Effects of Arochlor 1254 and cadmium on reproductive endocrine function and ovarian growth in Atlantic croaker. *Mar. env. Res.* **28**, 499–503.

Thomas, P. (1990) Teleost model for studying the effects of chemicals on female reproductive endocrine function. *J. exp. Zool.* **Suppl. 4**, 126–8.

Truscott, B., Walsh, J.M., Burton, M.P., Payne, J.F. and Idler, D.R. (1983) Effect of acute exposure to crude petroleum on some reproductive hormones in salmon and flounder. *Comp. Biochem. Physiol.* **75C**, 121–30.

Tulasi, S.J., Reddy, P.U.M. and Rao, J.V.R. (1989) Effects of lead on the spawning potential of the fresh water fish, *Anabas testudineus*. *Bull. Env. Contam. Toxicol.* **43**, 858–63.

Van der Kraak, G.J., Munkittrick, K.R., McMaster, M.E., Portt, C.B. and Chang, J.P. (1992) Exposure to bleached kraft pulp mill effluent disrupts the pituitary–gonadal axis of white sucker at multiple sites. *Toxicol. appl. Pharmacol.* **115**, 224–33.

Verma, S.R. and Tonk, I.P. (1983) Effect of sublethal concentrations of mercury on the composition of liver, muscles and ovary of *Notopterus notopterus*. *Water, Air, Soil Poll.* **20**, 287–92.

Victor, B., Mahalingam, S. and Sarojini, R. (1986) Toxicity of mercury and cadmium on oocyte differentiation and vitellogenesis of the teleost, *Lepidocephalichthys thermalis* (Bleeker). *J. Env. Biol.* **7**, 209–14.

Von Westernhagen, H., Dethlefsen, V., Cameron, P. and Janssen, D. (1987) Chlorinated hydrocarbon residues in gonads of marine fish and effects on reproduction. *Sarsia* **72**, 419–22.

Vuorinen, P.J. and Vuorinen, M. (1985) Effects of bleached kraft mill effluent on reproduction of brown trout (*Salmo trutta* L.) on a restricted diet. *Finnish Fish. Res.* **6**, 92–105.

Weiner, G.S., Schreck, C.B. and Li, H.W. (1986) Effects of low pH on reproduction of rainbow trout. *Trans. Am. Fish. Soc.* **115**, 75–82.

Wester, P.W. (1991) Histopathological effects of environmental pollutants β-HCH and methyl-mercury on reproductive organs in freshwater fish. *Comp. Biochem. Physiol.* **100C**, 237–9.

Wester, P.W. and Canton, J.H. (1986) Histopathological study of *Oryzias latipes* (medaka) after long-term β-hexachlorocyclohexane exposure. *Aquat. Toxicol.* **9**, 21–45.

Wester, P.W., Canton, J.H. and Bisschop, A. (1985) Histopathological study of *Poecilia reticulata* (guppy) after long-term β-hexachlorocyclohexane exposure. *Aquat. Toxicol.* **6**, 271–96.

Yano, T. and Matsuyama, H. (1986) Stimulatory effect of PCB on the metabolism of sex hormones in carp hepatopancreas. *Bull. Jap. Soc. scient. Fish.* **52**, 1847–52.

Yasuno, M., Hatakeyama, S. and Miyashita, M. (1980) Effects on reproduction in the guppy (*Poecilia reticulata*) under chronic exposure to temephos and fenitrothion. *Bull. env. Contam. Toxicol.* **25**, 29–33.

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