Environmental perturbation and fish populations:

ARE FISH IN HOT WATER?

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The theme of this issue of Fiskerikandidaten is 'The environment'; this has become a hot topic. The word environment crops up in many different contexts; in scientific literature, in the titles of meetings and conferences, in legislative and regulatory documents, in the media and popular press and on a multitude of internet sites. Discussions about the environment often revolve around ecology, although laymen that take part in these discussions may not be aware of this. We can define ecology as the study of the relationships between organisms and their physical and biological environments, or the study of the spatial and temporal patterns of the distribution and abundance of organisms, including causes and consequences.

When asked about my line of work I often reply that I am a fish ecophysiologist. What does this mean? Ecophysiology is a branch of ecology, and fish ecophysiology is concerned with the study of how the physiology of fishes is affected by environmental changes. It encompasses acute responses made to sudden change as well as the physiological mechanisms involved in adapting to long term environmental changes. In this article I will use examples from ecophysiological studies to illustrate how environmental change can have an impact on fish reproduction and development.

The sub-title of this article is 'Are fish in hot water?' This requires explanation. When an Englishman says that he is in hot water he will usually mean that he is in serious trouble, so the question is 'Do we have evidence that fish populations are in trouble?'

The status of fish stocks

Many of the world's commercial fish stocks are either fully-exploited or over-exploited, and a number of formerly productive stocks have collapsed. For some stocks there are few signs of recovery despite the imposition of reductions in fishing effort in an attempt to increase spawning biomass. The status of several Atlantic cod, *Gadus morhua*, stocks illustrates this latter point. Further, a number of fish species have become scarce in, or are now absent from, areas where they were once abundant, either as a result of over-exploitation or as a consequence of habitat degradation (Fig. 1).

Several fish species are now on the 'red list' of threatened or endangered species. Although not generally the main target of commercial fisheries, elasmobranch fishes (sharks, skates and rays) are strongly represented on the 'red list'. Elasmobranchs are usually taken as by-catch, rather than target, species but appear to be particularly vulnerable due to their combination of life history traits. Most are slow-growing, long-lived, late-maturing species that produce few offspring; many give birth to small numbers of live young after a gestation period lasting several months.

In addition to the influences of direct exploitation on fish stocks, environmentalists and conservation biologists are becoming increasingly concerned that some fish populations are at risk due to the impacts of human activities on aquatic ecosystems. A case in point is the potential for habitat degradation as a result of the release of industrial, agricultural and domestic wastes into aquatic environments. Aquatic environments are particularly susceptible to such forms of pollution because there is considerable intentional release of domestic sewage effluent and industrial wastes into rivers, lakes and the sea.

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They also receive a lot of accidental releases of chemicals from spills, run-off from land, and atmospheric deposition. The pollution pressure on aquatic habitats has increased with the increase in industrialisation and urbanisation, and greater use of water resources as a result of human population growth. This has led to the situation that over half of the flow in some rivers of densely-populated, industrialised nations may be made up of a combination of industrial and domestic effluents. When it is also considered that over 100,000 man-made chemicals are in everyday use, it is certain that many chemicals enter aquatic environments in considerable quantities.

The issue to be discussed is not if pollutants enter aquatic environments, but whether or not they are having significant effects on aquatic wild-life. If the answer is in the affirmative, there is a rider; are the effects generally positive or are they mostly negative? Negative effects could range from mass fish mortalities observed in lakes or rivers that have been subject to large chemical spills (Fig. 1), to more subtle, and difficult to detect, long-term effects arising from disturbance to the animal's physiology. The disruptive effects may not become apparent until years after exposure of the animal to the pollutant, and in some instances the changes can be so subtle that several generations may be needed before the effects can be detected at the population level.

A special category of pollutants: Endocrine disrupting chemicals

Endocrine disrupting chemicals (EDCs) are defined as substances that perturb the endocrine system by interfering with the production or action of hormones. EDCs can mimic the action of natural hormones, i.e. act as hormone agonists, they can oppose the action of the natural hormone, i.e. act as antagonists, or they can affect hormone synthesis, release, transport and metabolism. We now know that there are many EDCs found in aquatic environments; some are natural and others are man-made.

Medicines: Drugs and pharmaceutical agents

Drugs, pharmaceutical agents and their metabolites that reach aquatic environments have a high likelihood of acting as EDCs. This is implicit in the nature of these chemicals; they have been developed to exert effects on specific chemical reactions and biochemical pathways in human subjects when given at low doses. The pathways that are drug targets in humans are, however, often similar in a wide range of animals. This means that if a drug exerts an effect on a human patient it is also likely to influence the physiology of a fish.

Do drug residues reach aquatic environments in significant quantities? The answer is almost certainly yes. Many drugs and pharmaceutical agents are either not, or are only partially, degraded in sewage treatment plants. As an example, antidepressant drugs at concentrations of ca. 10-400 ng/L have been detected in sewage outlet effluents in Tromsø (Langnes, Breivika, Åsgård and Hamna). This means that drug residues are continuously being discharged into rivers, lakes and the sea, leading to chronic exposure of aquatic organisms to low concentrations of these bioactive agents.

Drug residues and pharmaceutical agents may interfere with many physiological processes in aquatic organisms, but it is the effects on fish reproduction that have been most studied. Some of the chemicals are oestrogenic and others androgenic; oestrogen is the term used to describe the female sex steroid hormones, whereas the androgens are male sex steroid hormones. One example of a pharmaceutical agent that has effects on fish reproduction is the synthetic steroid ethinyl-oestradiol (EE2). EE2 is the main active ingredient in the contraceptive pill. The steroid and its metabolites are present in the urine of women who take this pill. The steroid chemicals reach rivers and lakes in the effluents from sewage treatment plants and can have feminizing effects on fish. Male fish can display female characters, start to produce the egg-yolk precursor vitellogenin, and become infertile. Sometimes they become intersex individuals, and some male fish may even be sex-reversed. In intersex males parts of the testes (male gonads) contain oocytes (female germ cells) and the reproductive ducts may be malformed. In sex-reversed males the gonads look like ovaries (female gonads), and contain only oocytes, even though the fish is a genetic male.

There are several other pharmaceutical agents that are EDCs with effects on fish reproduction. Diethylstilbestrol (DES) was previously widely used as a growth promoting agent for cattle, and to emasculate cockerels to produce quick-growing, heavy-muscled capons. DES causes deformities to the reproductive organs of fish, and can reduce fertility or induce sterility. As a final example, trenbolone, a synthetic androgen, is a potent EDC that can affect fish reproduction.

Figure 1. Long-term degradation of aquatic habitats can give seasonal die-offs of fish as a result of oxygen shortages, and acute fish kills can occur following accidental releases of chemical pollutants into ponds, lakes and rivers. Photo: USFWS.

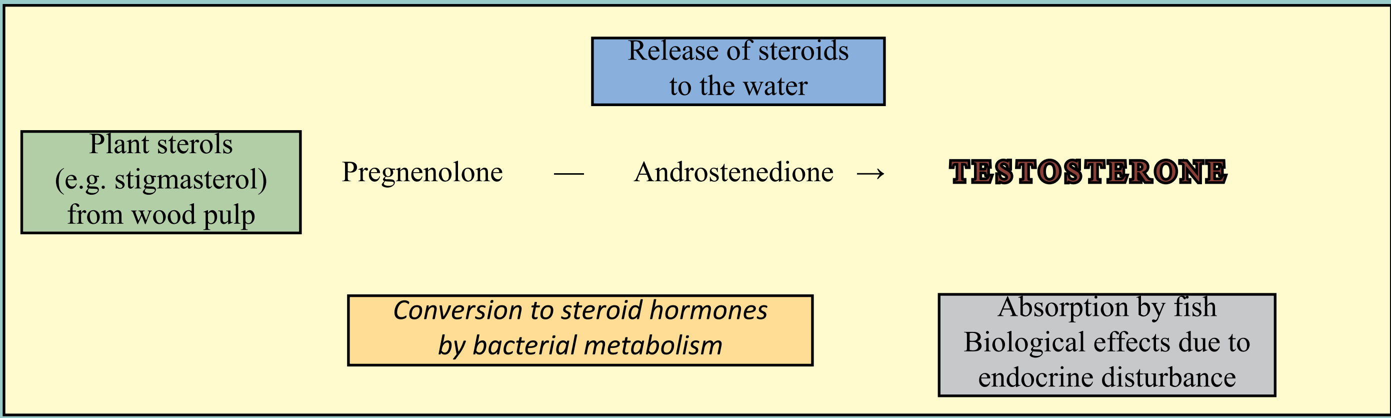


Figure 2. Aquatic bacteria and fungi can convert the plant sterols (phytosterols) present in wood extracts into bioactive steroids that may adversely influence reproductive physiology of fish. Testosterone is a potent male sex steroid hormone (androgen) that can act as an endocrine-disrupting chemical (EDC), leading to masculinisation of female fish.

Trenbolone is an anabolic steroid used as a growth-promoting agent in beef cattle production in some countries, such as USA. The steroid is administered by implantation into the ear lobe, but some of the steroid and its metabolites are excreted in the urine of the treated cattle. These residues reach ponds, lakes and streams in the effluent from beef cattle feedlot units. Trenbolone and its metabolites have masculinising effects on female fish. Fertility is often reduced, i.e. few eggs are produced and these are of poor quality, and the female fish may develop male sexual characters.

Agricultural chemicals:
Pesticides, fungicides and herbicides

Pesticides, fungicides and herbicides are likely to be potent EDCs because they kill organisms by interrupting vital metabolic processes. In other words, chemicals that interfere with the metabolic pathways of target organisms are also likely to have effects on the biochemical reactions and physiology of non-target species. Several agricultural poisons are known to be EDCs. Perhaps the best known of these are chlorobenzene derivatives, such as DDT and methoxychlor, and PCBs, such as chlordane, aldrin, dieldrin and mirex. These synthetic chemicals are soluble in fat, but not in water, and they usually remain stable for weeks or months after application. Although many of these chemicals are no longer in use several are still present in the environment at sufficiently high concentrations to act as EDCs; POPs, persistent organic pollutants, may be used as the generic term to describe them.

Tributyltin (TBT) is another man-made chemical known to act as an EDC in aquatic organisms. TBT has been added to paints used on ship hulls and other marine structures; the chemical hinders the settlement and growth of sessile organisms, so acts as an anti-fouling agent. TBT induces imposex in molluscs; imposex refers to the development of a penis and male reproductive ducts in females. These morphological changes interfere with egg-laying and often lead to reproductive failure.

Other sources of EDCs

Several industrial chemicals used as surfactants and in the manufacture of plastics and epoxy-resins are EDCs with impacts on fish reproduction. Some of these chemicals, which include alkylphenolic chemicals, phthalates and bisphenol-A, are also present in a range of household products, such as washing powders, liquid cleaning agents and packaging materials. These EDCs may, therefore, be discharged into rivers and lakes as components of both industrial and domestic sewage effluents; although most are not particularly potent they can occur at concentrations that are high enough to disrupt the reproductive physiology of the fish present in the recipient water body.

Finally, there are naturally-occurring compounds of plant, fungal and bacterial origin that can act as EDCs. One of the first recorded examples of disruption of fish reproduction involved some of these naturally-occurring compounds; it was observed that there was masculinisation of female fish living downstream from sites at which effluents from wood-pulping plants and paper mills were released into rivers and streams. It seems likely that the bioactive compounds are phytosterols or phytosterol metabolites produced by bacterial and fungal actions on wood extractives present in the effluents (Fig. 2).

Some plants, most notably legumes, such as soybean and alfalfa, produce phytoestrogens; compounds that mimic female sex steroid hormones (oestrogens). These phytoestrogenic compounds include genistein, coumesterol, daidzein and equol. Although the phytoestrogens are not very potent, there are some recorded instances of them acting as EDCs; exerting oestrogenic effects that cause perturbations to the reproductive physiology of both male and female fish. This has most frequently been observed in fish held in captivity and given feed pellets containing large amounts of soybean meal. In male fish the results of consuming such feeds may be reduced sperm production, decreased activity of the sperm and the induction of synthesis of the egg-yolk precursor vitellogenin in the liver. Females may suffer disruptions in the timing of their reproductive cycle, leading to disturbances to ovulation and the production of eggs with reduced fertility as the end result.

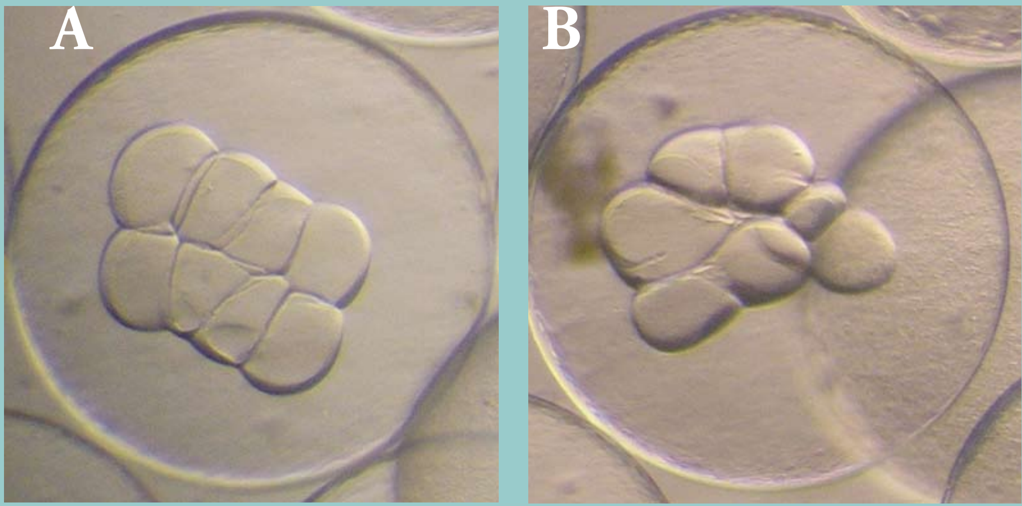


Figure 3. Incubation of fish eggs at high temperature can lead to abnormal cell division, with reduced survival to hatch as the result. (A) cod egg showing normal cell cleavage; (B) cod egg with abnormal cell division. Photos: Helge Tveiten, Nofima Marine.

PERSPECTIVES

There can be little doubt that EDCs have the potential to perturb reproduction of individual fish in a variety of ways, but the consequences for fish populations are more uncertain. For example, we have limited knowledge about the reproductive capabilities of intersex fish, although their reproductive abilities are probably impaired compared with those of normal individuals. Looking at all available evidence, it is probably wisest to say that EDCs discharged into aquatic environments could pose a real threat to the sustainability of fish populations.

Thermal physiology of fish

Environmental temperature influences many aspects of fish physiology; reproduction and growth are generally only possible within relatively narrow temperature ranges. These are much narrower than the temperature range over which short-term survival is possible. It is also usual that the temperature range for growth is wider than that for reproduction. In addition, the thermal requirements for the production of eggs and sperm and for spawning may differ from those for egg and larval development. As a generalisation it can be said that successful reproduction can be expected only over about 10-20% of the range of temperatures tolerated by adult fish. This means that environmental temperature changes are more likely to have pronounced effects upon reproduction and the recruitment of young fish to a population than upon survival and growth of older individuals within the population.

Temperature effects on reproduction and early development

Temperature may have a profound influence upon various aspects of reproduction, from the hormonal regulation of ovarian and testicular growth, to the timing of spawning, the rate of development of fertilized eggs and the timing of tissue and organ differentiation in developing embryos. Unfavourable temperatures experienced by adults may lead to ovarian and testicular disorders, abnormalities in gamete (egg and sperm) development, inhibition of the maturation and release of eggs and sperm, delays in the timing of spawning, and low fertilization rates of eggs. Temperatures experienced by eggs and embryos will affect rates of development and the differentiation of organ systems.

Rates of egg and embryo development usually increase with increasing temperature, but when eggs are incubated at high temperatures there is reduced survival to hatching (Fig. 3). It is also often observed that the incidence of abnormal or malformed offspring increases as temperature approaches the extremes at which survival is possible. Incubation temperature has significant effects on organ development and the timing of the differentiation of hard body parts, such as vertebrae, fin rays and scales. For example, when eggs and hatchlings are exposed to low temperature, the resulting juveniles often have more vertebrae than those that hatch from eggs incubated at high temperature.

Figure 4. Juvenile cod, Gadus morhua, *with osteological malformation of the head giving deformed jaws. Photos: Stefano Peruzzi, NFH.*

Unfavourable temperatures experienced during early development may give rise to osteological malformations in juvenile fish. Malformed opercula (gill covers) are commonly observed in juveniles that hatch from eggs incubated at high temperature; such fish may have problems

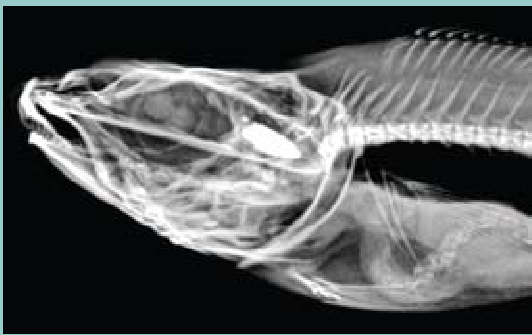
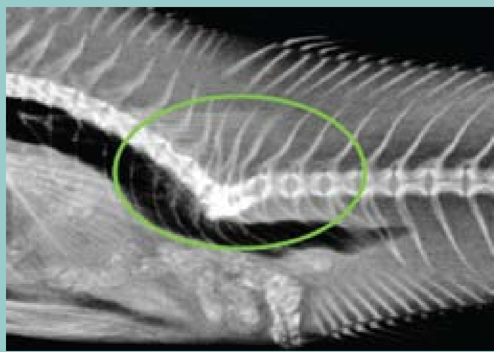


Figure 5. Juvenile cod, Gadus morhua, *with spinal deformities in the neck region and at several points along the backbone.*

Photo: Stefano Peruzzi, NFH. X-radiographs: Ingrid Lein, Nofima Marine.



with breathing correctly, and they may also be more predisposed to gill infections than normal individuals. Abnormal development of the head, involving jaw deformation, may interfere with the ability of the young fish to feed (Fig. 4). Alternatively, the development of a crooked or twisted backbone may reduce the ability of the fish to swim and capture prey (Fig. 5). All of these developmental abnormalities are likely to increase the chance that the young fish will either fall victim to a predator or suffer death from disease or starvation. All of this means that the negative influences of unfavourable temperatures upon the development of eggs, embryos and newly-hatched offspring could result in recruitment failure to the population.

Temperature-dependent sex determination

When fish are exposed to elevated temperatures during the earliest phases of their development it is sometimes seen that the sex ratio within the population differs from the expected 50% male and 50% female. In populations with sex ratios that deviate from 50:50 there are usually more males than females, but this preponderance of males does not seem to be the result of a higher mortality rate amongst females. In other words, sex determination in fish appears to be thermolabile, with the processes involved in sexual differentiation being sensitive to the effects of environmental temperature. This means that the thermal conditions experienced by a fish during a critical phase in early life can determine whether it develops as a male or a female, irrespective of its genetic make-up; genetic females that are exposed to warm water during early development may become sex-reversed (Fig. 6). They show male characters, their gonads differentiate into testes rather than ovaries, and when they mature they produce sperm rather than eggs. How might temperature act to influence sex differentiation?

The processes involved in sexual differentiation are under hormonal control, with development as a female being dependent upon exposure of tissues to oestrogens (female sex hormones) for a short critical period early in the life of the fish. Male development, on the other hand, occurs when androgens (male sex hormones) are present. Cholesterol is the compound used as the starting material for production of sex steroid hormones. The synthesis of both androgens and oestrogens occurs in a number of steps involving reactions catalysed by the P-450 enzyme system. One particular enzyme in this system, P-450 aromatase, is responsible for the conversion of testosterone (an androgen) to oestradiol, the most important female sex steroid hormone in fish:

$$\text{Cholesterol} \xrightarrow{\textit{Several enzymatic steps}} \text{Testosterone} \xrightarrow{\textit{P-450 aromatase enzyme}} \text{Oestradiol}$$

Females usually have high P-450 aromatase enzyme activity in their tissues, particularly in the brain and gonads, whereas males do not. Thus, it is thought that the main effect of temperature on sex determination, and particularly the sex-reversal of genetic females, is mediated via thermal influences on the P-450 enzyme system. High temperature inhibits the expression and action of P-450 aromatase, resulting in reduced conversion of testosterone to oestradiol. This means that the tissues of the young female fish are exposed to a low concentration of the oestrogen, oestradiol, and to a high concentration of the androgen, testosterone. As a result of this, sexual differentiation is led in the male direction even though the fish is a genetic female. Based upon this evidence we can conclude that environmental temperature can act as an endocrine-disrupting factor that exerts its influence in a similar fashion to some of the EDCs discussed earlier in this article.

How might temperature-dependent sex determination affect fish populations? The sex-reversal of some genetic females to functional males automatically reduces the numbers of females capable of producing eggs, with the consequence of a reduction in effective spawning stock size. In addition, the sex-reversed females, now functioning as males, compete with the normal males to mate with the small numbers of normal females present in the population. Over time this could lead to the demise of genetic males, leaving small self-perpetuating populations of all-female fish; a small number are female throughout their lives and provide the eggs that are fertilized by the sperm produced by the sex-reversed females. In a worst case scenario all of the females could become sex-reversed, leaving none to produce fertile eggs; the obvious end result of this is population extinction.

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Aquatic environments can experience relatively localised temperature increases, such as close to where industrial plants and power stations discharge warm-water effluents. Alternatively, the temperature changes can be far-reaching and pervasive, as envisaged to be the case with climate change and global warming. Both types of change will have impacts on individual fish and also upon fish populations. The distributions of fish are partly determined by species temperature tolerances and preferences, so an increase in environmental temperature may lead to a change in the geographical distribution of the species; the distributional range may expand or contract, there may be a general distributional shift towards higher latitudes where water will normally be cooler, and when a temperature increase is restricted to certain water bodies there may be local extinctions within a species' distributional range. The effects that temperature has upon reproduction and early development are likely to be a major contributor to these wide-scale changes.

A Parthian shot

Humans can influence fish reproduction and development in a variety of ways. Sometimes the influences are inadvertent, but interventions are also undertaken with a distinct purpose in mind. For example, in the farming of fish thermal treatments are used to increase rates of development and growth, influence the timing of maturation and control parts of the reproductive cycle. In addition, hormones may be applied to induce the maturation and release of eggs (ovulation) in species that are reluctant to breed in captivity. Hormone treatment is also used in the production of monosex (single-sex) populations of fish, and it is an integral part of the process used to produce monosex, triploid populations of fish. Triploid individuals are often sterile or infertile, so they either do not become sexually mature and develop gonads (ovaries or testes) and/or are not able to produce viable offspring if they attempt to breed (either in the captive farm environment or following inadvertent release or escape to the wild).

The inadvertent effects of human activities on fish reproduction may involve the same environmental factors. Endocrine-disrupting factors, be they EDCs or a physical factor such as high water temperature, can influence:

- sexual differentiation (i.e. whether a fish develops as a male or a female)
- sexual development (i.e. whether or not the gametes – eggs and sperm – are fertile)
- the reproductive cycle (rate at which the gametes develop and the timing of egg release and spawning)

In some cases exposure of fish to these factors leads to immediately-observable effects, but sometimes the outcomes of the endocrine

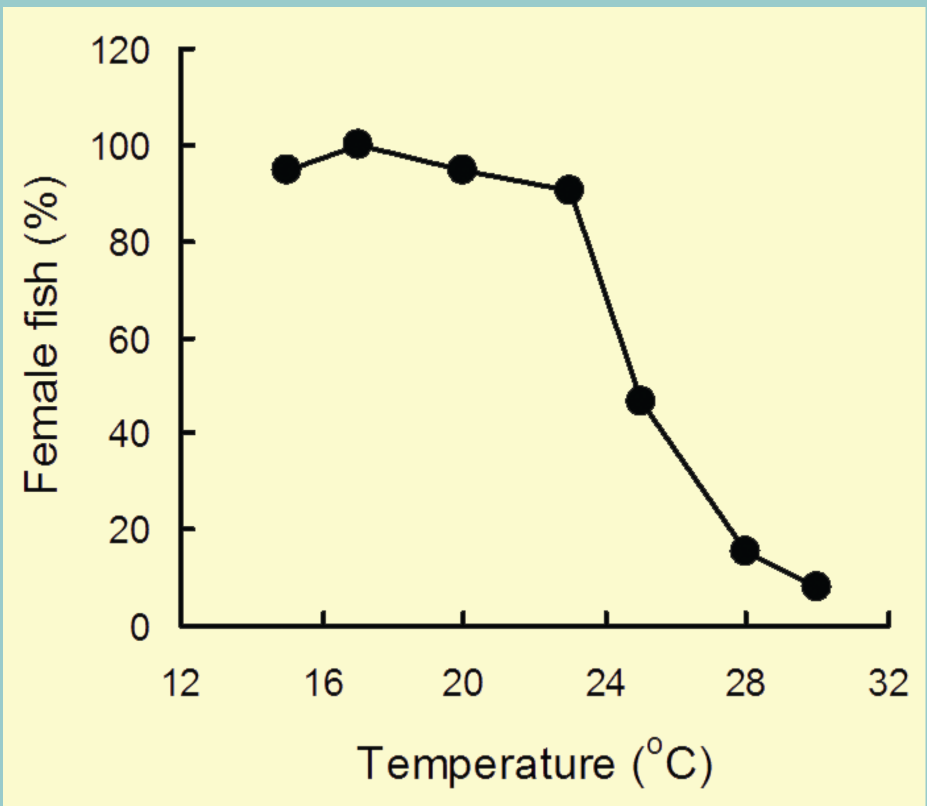

Figure 6. The exposure of genetic female goldfish, Carassius auratus, *to high temperature during early development (from day 12 after egg fertilization to 3 months of age) resulted in sex-reversal of many of the fish; the sex-reversed females displayed male characters and developed testes, rather than ovaries.*
Data from Godo-Kazeto et al. (2006).

disruptions may not become apparent until years later, and it is then most difficult to attribute observed changes to a specific cause-and-effect relationship.

It is highly unlikely that only a single endocrine-disrupting factor will be operative in a lake, river or the sea. Several EDCs are likely to be present simultaneously. In addition, there may be periodic perturbations to environmental temperature that result from the discharge of heated effluents, or more long-lasting temperature changes that result from other causes. This means that aquatic organisms will usually be exposed to chemical cocktails that can interact with the thermal environment to impact several of the steps in the reproductive endocrine cascade; from the synthesis and release of hormones, through transport and detection by target cells, to the activation of reproductive events, and the degradation of the hormones and excretion of their metabolites.

There is increasing evidence that several fish stocks are in decline. Some of these declines appear to be closely linked to the disruptive effects that industrial and agricultural effluents and domestic wastes have upon reproduction. The effects of the EDCs must not be viewed in isolation; over-exploitation by commercial fisheries, habitat degradation, the introduction of exotic species and changes relating to the thermal environment, are probably all part of the complete picture. What is certain is that there is a whole battery of subtle and pervasive environmental changes that may exert disruptive effects on fish reproduction, but the overriding question remains: Which of these are responsible for forcing populations of fish deeper into hot water and down the slippery slope of decline?

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