

## VCI research project: "Field studies on the sex ratio in indigenous fish populations"

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In the public debate on adverse effects in wild animal communities living in waters - attributable to hormonal disruptions caused by environmental chemicals - ecological field findings are of central importance. The majority of studies performed up until now were limited to laboratory testing (in vitro and in vivo). However, findings gathered in laboratory tests can be confirmed exclusively by ecological field studies, because only they provide reliable information on population-relevant adverse effects (e.g. population size, genetic variety) in the environment. Ecological field studies are highly complicated and require much input in terms of time and resources. So far, ecological field studies have been implemented in only very few cases.

The range of contradictory information on hormonally induced adverse effects on ecosystems has led to growing uncertainty, not only in the general public but also in scientific circles. For example, on the one hand the water authorities of German federal states report clearly increasing fish populations (spectrum of species, population density) in streams and rivers while, on the other hand, various parties speculate about "feminization" and a general danger to fish populations.

The German chemical industry association – Verband der Chemischen Industrie e.V. (VCI) – has contributed to this scientific question, which is interesting from both, a theoretical and an applied point of view. In a research project contracted to an external party, the primary aim was to find out if the population-relevant endpoint "**Sex ratio in wild fish**" in various unpolluted surface water types shows deviations from the theoretically expected 1:1 ratio and whether variances connected with oestrogenic disruptions are observed at polluted sites. Additionally to the sex ratio as the central endpoint, the biomarkers "vitellogenin" (yolk protein) and gonadal growth

(gonadosomatic index "GSI") as well as histopathological characteristics of genital organs were included in the study as accessory endpoints, even though views in the scientific community on their interpretation, assessment and significance are currently still highly contradictory. Parasitization and age structures of the fish populations were also determined to complete the range of available data.

In order to obtain results as representative as possible, the study was carried out in two species of freshwater fish (roach, perch) with different lifestyles and reproduction strategies. Studied waters in the German federal states of Hessen and Baden-Württemberg were selected from the aspect of a certain geographic and water-morphological breadth, applying the condition that they were not exposed to direct burdens due to anthropogenic impacts. Instead of broad measuring programmes, a pragmatic approach was followed for classification in the category "unpolluted site" by additionally using a combination of the following criteria:

- Natural and location-typical spectrum of species in the fish fauna,
- No signs of acute or chronic intoxications of fish (external morphology, liver),
- Good nutritional status of the fish,
- Age structures of the populations are typical of the species,
- Reproduction cycle is seasonally synchronized.

Simultaneously complying with these five "integral" biological criteria, the sites Stockstadter-Erfelder Altrhein (Kühkopf), Goldgrund, Taubergießen, Guckaisee and Wispersee were classified as unpolluted, in the meaning of a weight-of-evidence approach. To enable a comparison, the following waters with direct anthropogenic use were studied: Lampertheim marina (sports boat harbour), Schierstein harbour (industrial harbour use) and the Schwarzbach (in particular municipal waste water disposal).

The study stretched over two years (2001-2003). Fish were caught twice a year in spring and in autumn, using the electro-fishing method. In total, many more than 2,000 fish were included in the study.

Dr Allner was retained to implement this comprehensive field study (project management; initially by HLUG, from 2003 GOBIO GmbH). The final report by Dr Allner is available for download. He gives full information on methods, results and data from the scientific investigations.

The project revealed the following **results**:

At sites classified as **unpolluted** the sex ratio in the two fish species – aggregated over the entire study period 2001-2003 and across all sites – was well-balanced. In isolated (seasonal) fish catching, quite significant variances in the sex ratios at the individual sites were observed; this result highlights that findings derived from limited data basis can involve a strong danger of misinterpretation.

In the assessment of gonadal development the project showed for roach in general that no "intersex forms" occur while for perch, in the histological picture of the gonads, in ca. 7% of male animals also development stages of female gametes were detectable. The latter equally applied to both unpolluted and polluted sites. Obviously this is a species-typical particularity which expresses – independently of any speculation about endocrine induced effects – a certain "plasticity" in the sex development of perch. Further details about this interesting phenomenon are given in the final report.

At sites classified as **polluted** within this study (Lampertheim marina; Schierstein harbour; Schwarzbach) there were clear impairments regarding individual or several of the above-mentioned biological criteria; no real surprise – and expected in the meaning of this study - because local fish populations are exposed to a mix of different stressors throughout the year.

In more detail, the following "deviations from norm" were observed for the studied biological endpoints at the three polluted sites:

- In Lampertheim – but not at the other two sites – the sex ratio in perch diverged slightly, but statistically significantly, from an equal ratio (60% males; 40% females).
- At Lampertheim, Schierstein and in the Schwarzbach there were indications of a time delay in sex development. The increased incidence of unspecific

histopathological findings in the gonads is, according to Dr Allner, rather attributable to general pollution than to specific, endocrine induced effects.

- Vitellogenin – the characteristic yolk protein of reproductively active female fish – was also detected in some adult males of roach in concentrations > 10 µg/ml.

Frequently, at the end of even carefully planned and well-implemented field studies there are more questions than at their beginning, and often these questions cannot be answered with the chosen methodological approach. This holds true for the given case, too, where the question of the population biological relevance of the observed adverse effects remains open – especially because the numeric basis, i.e. the number of caught adult males and females, is extraordinarily low and does not enable a detailed statistical confirmation. Interesting follow-up questions arise which, however, go far beyond the conceptual approach of the present study: Do the norm deviations in biomarkers point to an impaired reproduction of roach and perch? If so, where do the evidenced adult fish originate, where do the juvenile animals originate? What about the migration ability of both species? Perhaps even the reliability of the catching method (electro-fishing) leaves somewhat to be desired at the polluted sites, due to increased electrolyte contents? Such questions become obvious in this study; questions which furthermore give rise to doubts about the validity of many field studies on similar subjects which, however, were inadequately planned and based on fewer data.

Consequently, this is also the main conclusion that VCI draws from the results of this study: In the given case the sex ratio – as a significant parameter for the reproductive health of fish populations – has proven largely stable, both under polluted and unpolluted site conditions. To make a reliable statement on a seemingly profane question – and this is part 2 of the conclusion – a series of fish catches over an extended period of time is needed, because results of isolated fish catches lack in statistical relevance.