

Aquat. Sci. 69 (2007) 51–70
1015-1621/07/010051-20
DOI 10.1007/s00027-006-0841-6
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Aquatic Sciences

Research Article

Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers

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Received: 1 November 2005; revised manuscript accepted: 28 June 2006

Abstract. To assess potential causes for the decline in catch of brown trout and their impaired health status in Switzerland, a 5-year multidisciplinary research project was conducted. Multiple causal hypotheses were postulated and investigated in a variety of laboratory and field studies. We present here the application of a weight-of-evidence analysis to evaluate the results of these studies and to assess the causes for decline in brown trout abundance. Based on human health epidemiological criteria, the method considers the exposure situation, the correlation between causes and effects, specificity of effects, and amelioration due to removal. For our evaluation, we concentrated on four test rivers and included data on fish health and population density, water quality, and habitat

parameters. Our results showed that proliferative kidney disease (PKD) caused by a parasite and clinical outbreak supported by other factors is a very probable single parameter for the decline of brown trout abundance at the sites of the test rivers where it occurs. Elevated levels of nitrogen compounds may also be posing a serious risk at several sites, in particular those downstream of sewage treatment plants. Several habitat parameters, such as large width, low percentage of riffles or elevated winter temperatures, were identified as factors likely contributing to impaired health, recruitment, and abundance at single sites. At most sites, more than one factor must be acting jointly to cause the observed decline in brown trout abundance.

Key words. Weight-of-evidence approach; brown trout; health; abundance; water quality; proliferative kidney disease.

Introduction

Brown trout (*Salmo trutta*) populations in Switzerland are seriously at risk. Angler catch records indicate a decrease of up to 50% since the beginning of the 1980s (Friedl, 1999). This catch decline has been observed to be geographically widely distributed and are most probably a sign for a decrease in population (Fischnetz, 2004). In parallel with the indications of decreasing catch, fish health studies have yielded evidence of an impaired

health status. Brown trout with both macroscopic lesions and histopathological alterations of liver, kidney and gills were documented in a number of rivers and streams (Bernet et al., 2000; Schmidt-Posthaus et al., 2001).

The causes of the widespread health problems and decreased catch are not readily apparent, due to the variety of human activities in the affected regions. To document the spatial and temporal patterns of catch decline and impaired fish health and to demonstrate a general population decline, identify the most important causal factors and suggest measures for improvement, the five-year, nationwide project “Fischnetz” (Netzwerk Fischrückgang Schweiz: project on declining fish catch in Switzerland) was conducted (Burkhardt-Holm et al., 2002). As a part of a systematic and structured search for

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Published Online First: March 2, 2007

possible causes, multiple causal hypotheses were postulated. These included poor water quality, altered habitat, increased fine sediment inputs, infectious diseases, increased water temperature, altered hydrological regime, as well as changed angler behaviour and fisheries management (Burkhardt-Holm et al., 2005).

A variety of field and laboratory studies were conducted to assess the importance of the various possible causal factors. With the recent completion of these studies (Fischnetz, 2004), methods are required to integrate the results in a manner useful for causal assessment and as a basis for further development of management options. Because of the historical nature of the fish catch decline problem, retrospective evaluations are also necessary.

Here we present the application of a weight-of-evidence approach (WOE) as a semi-quantitative method for identifying causal factors that are likely to explain adverse effects occurring in investigated ecosystems (Forbes and Calow, 2002; Goede and Barton, 1990; Suter et al., 2002). This method is useful because it makes the process of assessment more transparent, systematic and logical as well as facilitates the summarizing and communication of results. Nevertheless, the approach can never be absolutely decisive, as it is post-hoc. Therefore, although it is unrealistic to expect this method to be definitive in terms of ascribing causation, it does allow defining factors as being more or less likely and allows for informed management and regulatory decisions based on the preponderance of evidence. The approach is based on human health epidemiological criteria and includes the consideration of several basic questions and an assessment of the likelihood of the potential causal factors. The relevant questions deal with issues such as the exposure situation, the correlation between causes and effects, specificity of effects and amelioration due to removal of agents.

The weight of evidence approach outlined above can also be referred to as retrospective ecological risk assessment (Forbes and Calow, 2002) or ecoepidemiology (Suter and Bartell, 1993; Adams, 2003). We adopted the model of Forbes and Calow (2002), modified it to our specific conditions, and applied it to assess the influence of potential factors involved in the catch decline and impaired health of Swiss brown trout.

A judgement and assessment of the likelihood of potential causal factors is facilitated by using studies in which selected river basins were investigated with respect to identical variables over the same time period and with identical methods. Such a study was performed over two years in four river basins (further on called "test areas") with differing characteristics that represent the range of conditions found in the Swiss midlands. Here, we investigated health parameters, reproduction, recruitment, ecomorphological characteristics, physico-chemical parameters, water quality variables, and the hydrological regime. In each river, three reaches were selected and in-

vestigated in detail. As the final endpoint of the weight-of-evidence analyses, we selected the brown trout abundance. Decline in catch was observed over the last two decades, but was not selected as an endpoint in this study because spatial resolution of this data is not sufficiently detailed to differentiate between individual sites at the test areas.

Methods

Weight-of-evidence approach

The evidence available to assess the potential causes of ecological impairments is often complex and widely variable. Even well performed scientific studies can be ambiguous. Sufficient quantitative data are usually not available for all potential factors. Similarly, evidence for the involvement of putative factors may only be of a correlative nature. In addition, there are confounding factors which might be either unknown (e.g. unidentified infectious agents) or uncontrollable (e.g. weather, flood events, predators). Therefore, rarely is one line of evidence sufficient to demonstrate causation. Rather, only by assembling and evaluating all of the evidence can some factors be ruled out and others maintained for further action or analysis. What follows is a series of key questions developed by Forbes and Calow (2002) with which to challenge the available evidence. For the present work, the key questions of Forbes and Calow (2002) have been slightly modified to better reflect the situation of fish declines in Switzerland. In particular, causative agents represent the range of potential factors addressed by the hypotheses of Fischnetz, in addition to the classical physical, chemical, and biological stressors. The method is case-specific, so most questions relate to a particular fish population while others relate to the transfer of knowledge gained from other locations or from the literature. We have also reordered the questions of Forbes and Calow to better reflect their importance in ascribing causation. For example, we believe that the plausibility question should be applied first, as a screening criterion to determine the causes that should be considered for further analysis. Additionally, unlike Forbes and Calow (2002), we think that most of the criteria can be applied sequentially, rather than simultaneously as they do for five of the seven questions. A decision diagram shows the order and conclusions resulting from the seven questions we apply (Fig. 1).

1) *Does the proposed causal relationship make sense logically and scientifically?*

This question seeks to document the plausibility of the causal relationship. The intent is to preclude consideration of relations that clearly have a spurious basis. Documentation may include the description of a specific causal mechanism linking the stressor and the adverse effect, a comparison of the hypothesized

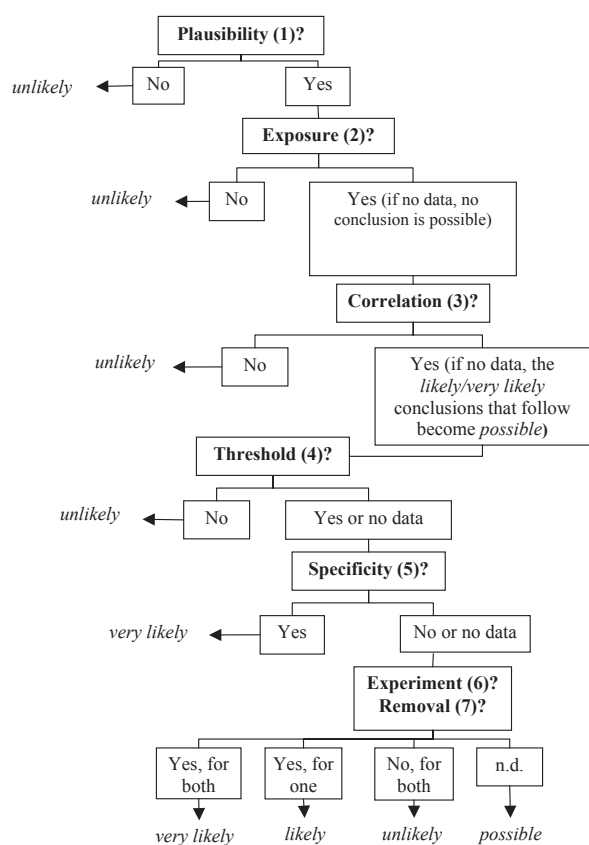


Figure 1. Flow diagram to decide on the 7 questions of the weight-of-evidence analysis (see text) and the resulting assessments.

relationship to other well-established cases, and a review of relevant studies reported in the scientific literature. The answer to this question will invariably require some scientific judgement.

2) *Is there evidence that the fish population is, or has been, exposed to the causal factor?*

This site-specific question encourages documentation of exposure levels of a population to a stressor. There may be situations where exposure is suspected but has not actually been demonstrated at the study site in question. For most potential causes, evidence of exposure will come from monitoring programs, site surveys, or historical data. The answer to this question should be as detailed and quantitative as possible. However, often not more than pure incidental observations are available.

3) *Is there evidence for association between adverse effects in the population and presence of the causal factor, either in time or space?*

The objective of this question is to provide evidence that the candidate cause and the effect are generally observed together at the same time and/or place and that when the cause is not observed, neither is the effect. In contrast to criterion 2, a biological gradient or

any kind of formal relation between adverse effect and the stressor is asked for. Ideally, the answer to this question will involve a statistical correlation or regression analysis and therefore may rely on data from a number of different sites. For some causal factors, sufficient data may not be available, and only a subjective judgement of trends is possible. In this study a correlation was stated for the downstream sites if the causal factor changed in accordance to the adverse effect when compared to the reference site in the same river. Of course, even strong correlation does not prove a cause-effect relationship, especially in the presence of confounding variables. However, this is one important line of evidence.

4) *Do the measured or predicted exposure levels exceed quality criteria or biologically meaningful thresholds?*

It is assumed that most stressors have a threshold level, below which adverse effects on a population are unlikely. Therefore, it is important to determine whether the site-specific values stated in answer to question 2 exceed critical values. For some stressors, such as chemical pollutants, environmental quality standards (EQS) and predicted no effect concentrations (PNEC) exist. We used the PNEC as a critical level and calculated risk quotients for fish according to the formula ($\text{Risk} = \text{effect concentration}/\text{PNEC fish}$) with a risk value above 1.0 still might be assumed to cause an adverse effect. However, for non-chemical factors, other indices (such as critical food benthos biomass in g/m^2) are required. It is also possible that the population may be exhibiting adverse effects resulting from past exposures at critical levels that are no longer present. This possibility should be considered, and, if possible, historical data should also be evaluated.

5) *Is there an effect in the population known to be specifically caused by exposure to the stressor?*

Some stressors are known to elicit very specific responses in the target populations. Some diseases have very specific symptoms and were diagnosed in our study (Zimmerli et al., 2007). For example, vitellogenin is a yolk precursor protein specifically synthesized in male vertebrates after exposure to (xeno)estrogens. Most responses are often referred to as “biomarkers of exposure”. Because of the possibility of non-identification of biomarkers, their presence in a population has greater weight as causal evidence than their absence. It should also be noted that specific responses are not only the result of chemical exposure or disease.

6) *Have the results from controlled experiments in the field or laboratory led to similar effects?*

This question seeks to document situations in which the stressor has been applied in a controlled way in

real field situations or in appropriately designed laboratory experiments. Because such studies are made under controlled conditions they will be given more weight than the correlative observations described in answer to question 3. Site-specific studies are most relevant, but experiments performed under similar conditions are also informative.

7) *Has removal of the stressor led to an amelioration of effects in the population?*

If possible factors are known or suspected, their intentional removal may provide an opportunity to help identifying an associated effect. Such situations may result from a controlled experimental program or the implementation of a management measure. It is important to remember, however, that recovery of a population is rarely immediate. Therefore, while amelioration following removal of a stressor provides useful evidence and confidence for its previous importance, lack of amelioration does not disprove its importance. Careful judgement must be applied in considering negative results from such manipulations. However, general rules on the rates of recovery of different ecological target following removal of various factors do not exist.

Some of the questions (2, 3, 4) are answered by the results of the site-specific studies, while others (1, 5, 6, 7) are mainly answered by data available from other investigations. Forbes and Calow (2002) suggest organizing the weight of evidence assessment as a table, with the proposed causal factors along one margin of the table and the seven questions along the other margin. Answers to each question for each factor are then given in the cells of the table. A decision diagram (Fig. 1) can then be used to apply the information in the table to an overall determination of the relative likelihood of the various causes. In this way, the potential factors can be easily classified as “very likely”, “likely”, “possible”, or “unlikely”. If data are not sufficient to assess a parameter in question, it is held for further consideration as “possible”. In contrast, when judged as “unlikely” the factor is excluded from further assessment due to the lack of exposure, correlation, exceedance of the threshold or negative results of both types of experiments.

Case study locations

Four river basins, the Emme, Liechtensteiner Binnenkanal, Necker, and Venoge were chosen to represent the range of conditions in Switzerland and Liechtenstein (Fig. 2). Selection criteria for the river basins included evidence of a significant brown trout catch decline over the preceding two decades and the discontinuation of stocking for the two years under study in order to be able to assess the natural recruitment potential. In addition,

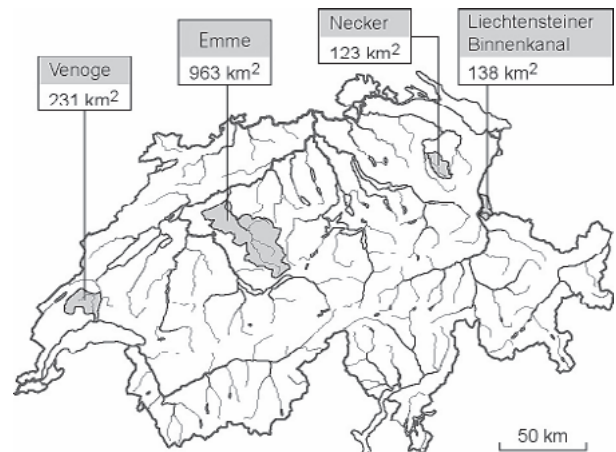


Figure 2. Location of test rivers in Switzerland with size of their catchments.

the river basins are typical in that three of them exhibit a multitude of potential causal factors. In each basin, the brown trout population was studied at three sites. These sites were either separated by barriers or the distance between the sites was great enough that migration was considered to be of minor importance.

The *Emme* river has its headwater in a steep pre-alpine region considerably influenced by spring snow-melt and seasonal flow fluctuation. Downstream, historically high occurrence of floods prompted intense river management activities in the nineteenth and twentieth centuries. The construction of dams and weirs has resulted in isolation of tributaries. Additionally, poor riparian conditions and high water extractions strongly influence the hydrology in the two downstream sites. Natural trout habitats are mostly found in the upper reach. Land use in the Emme basin (963 km²) consists of 35 % extensive agriculture (mostly downstream), 15 % intensive agriculture, 40 % forest, and 6 % developed land. Two large wastewater treatment plants (WWTPs) discharge into the downstream Emme, while a number of smaller WWTPs discharge into the river's tributaries. Catch of brown trout has declined by approximately 60 % since 1990.

The *Liechtensteiner Binnenkanal* (LBK) is a channel constructed in the 1930s for flood protection and land conversion. The flow is rather constant, and the only prominent barrier, at the mouth of the channel, was removed in 2000. Restrictions of natural habitat are mainly due to channelization leading to low variability in width and depth and a monotonous flow. This, together with input of fines from agriculture, settlements and roads has resulted in high levels of fine sediment and stream bed clogging. One WWTP treating about 4,500 person-equivalents discharges into the LBK. The LBK basin (138 km²) is dominated by forest (50 %) with only 18 % agriculture. Catch levels of trout (rainbow trout and brown trout) have

declined by more than 90 % since the mid 1980s (and 85 % since the 1970s).

The *Necker* is a pre-alpine river with natural, seasonally fluctuating flow. River morphology is only mildly disturbed, providing varied habitat for all life stages of brown trout. Primary land use in the basin (123 km²) consists of 35 % agriculture and 38 % forest. A small amount of wastewater is discharged into the *Necker* (input of four small WWTP, with treated waste of fewer than 10,000 people), but input of fines and stream bed clogging may be a problem. Angler catch has decreased by more than half since the mid 1980s.

The *Venoge* is located in the west of the Swiss plateau and flows into Lake Geneva. Habitat quality seems adequate in the upper reach but poor in the lower section. The area of the basin is 231 km² and land use includes 47 % agriculture and 34 % forest. Eighteen mostly small (2: 10,000–50,000 people, others \leq 10,000 people) WWTPs discharge to the *Venoge* or its tributaries. Catch of brown trout in the *Venoge* has decreased less than in the other studied rivers (approximately 25 % since the late 1980s). However, the largest decreases occurred before data on brown trout catch was raised separately for this river.

Data for the case studies

Twenty six parameters were selected as potential causes or intermediate indicators and used in weight-of-evidence tests. In the following sections, these parameters and their measurement methods are shortly described. More details can be found in the cited references (cf also Table 1).

Biological data. One basic question was, whether the assumption of a decline in fish stocks in the four catchment areas can be made from the observed catch declines over the last two decades. Anglers in Switzerland have to record and submit the species, number, size, location and date of all fish caught and retained to the appropriate canton. However, since the number of unsuccessful trips and the duration of trips are not generally recorded, total effort and catch per unit effort cannot be calculated. Furthermore, stocking measures and fisheries management differing from canton to canton influences fish stocks additionally in an unforeseeable way. Population data of quantitative measurements were too scarce and methodologically difficult to interpret for sufficiently supporting the basic assumption of stock declines (Fischnetz, 2004). Although changes in fishery management and angling behaviour (e.g. changed recreational activities) contributed to the catch decline, data on trends in angler permits and catch data allow to conclude that a real reduction of fish stocks occurred: Between 1980 and 2000, the number of angling permits sold for rivers and streams decreased by 23 %. A representative survey of anglers fishing in riv-

ers and streams showed that the number of angling trips per permit declined from an average of 27 (1980) to 22 (2000) (Fischnetz, 2004). Nevertheless, the ratio of successful angling trips declined from 78 % to 24 %, while the trip duration remained the same. Since successful trips and total annual catch per angler (reduction from 49 fish in 1980 to 25 fish in 2000) dropped much more than the number of permits and trips per permit, we concluded that there is a real reduction of the fish stock and that this decline forced the anglers to adapt their behaviour.

The biological surveys in this study included investigation of population abundance, recruitment, reproductive impairment and health indicators.

Population abundance was measured and re-calculated as the total number of brown trout per hectare and was performed by quantitative electrofishing, with two consecutive passes with lock nets at upper and lower ends (De Lury, 1947; Schager and Peter, 2004). Abundance data were classified according to comparable data from reference streams in Swiss midlands and local conditions such as altitude, steepness and hydrological regime into the classes 1 = very good; 2 = good; 3 = sufficient; 4 = insufficient; 5 = poor according to the modular stepwise procedure for comprehensive assessment of streams in Switzerland, step F, for fish (Schager and Peter, 2003; Schager and Peter, 2004).

Surveys over 100–200 m stretches were carried out at each site in spring (April/May), summer (Jul/Aug) and autumn (Oct/Nov/Dec) of 2002 and in summer (Jul/Aug) and autumn (Oct/Nov) of 2003. Due to the very low abundance in the river *Emme*, sites D1 and D2 were fished only at specific structures (Schager and Peter, 2004). Destructive biomarkers were measured only during the summer sampling dates, when 20 juvenile brown trout were collected at each site by electrofishing and sacrificed. A sample of 20 fish was not possible at *Emme* D1 (n = 19) and D2 (n = 6) in 2002, at *Emme* D1 (n = 14), *Necker* HW (n = 14), D2 (n = 16) and *LBK* HW (n = 19), D1 (n = 13), D2 (n = 15) in 2003 and no fish were caught for sampling at *Emme* D2 in 2003, *LBK* D2 in 2002 and *Venoge* HW in 2003.

As an indicator of *recruitment*, late summer fry or “young-of-the-year” abundance (age 0⁺) was analyzed separately from the other life stages and is compared with the relationship age 0/ >1 . The classification was done according to the modular stepwise procedure for comprehensive assessment of streams in Switzerland, step F, for fish (Schager and Peter, 2004; Schager et al., 2007) and data were ranked in a 5-step classification scheme, with “5” the poorest, and therefore, insufficient recruitment success (personal communication, E. Schager, Eawag). Density at this life stage can be related to egg and young-of-the-year survival as well as habitat limitations (Elliott, 1994) although these relations can be complicated by density dependence (Borsuk et al., 2005).

Table 1. All primary and intermediate parameters used for the weight-of-evidence approach and the possible answers on the seven questions. Some of the questions (exposure, correlation, threshold) are answered by the results of the site-specific studies, while others (plausibility, specificity, experiment, removal) are mainly answered by data available from other investigations.

Parameter	Plausibility	Exposure	Correlation	(Exceedance of) critical value	Experiment	Removal experiment	Specificity	Conclusion
final effect								
brown trout abundance								
recruitment	yes	yes/no/n. d.	yes/no/n. d.	class 5 (Schager, pers. commun.)	yes (f. review: Elliott, 1994; Crisp, 2000)	yes (dito)	no	u/poss/lik./very lik.
ELS-survival rates	yes	yes/no/n. d.	yes/no/n. d.	< 80% (Bernet and Segner, 2004)	yes (Bernet and Segner, 2004; Kobler, 2004)	n. d.	no	u/poss/lik./very lik.
intermediate factors								
VTG-induced	yes	yes/no/n. d.	yes/no/n. d.	1000 ng/L (Vethaak et al., 2002)	yes (1) (Pawlowski et al., 2004)	n. d.	no	u/poss/lik./very lik.
condition factor	yes	yes/no/n. d.	yes/no/n. d.	0.8 < Median < 1.2 (Bernet and Segner, 2004)	n. d.	n. d.	no	u/poss/lik./very lik.
HSI-elevated	yes	yes/no/n. d.	yes/no/n. d.	sign. above reference (Zimmerli et al., 2007)	yes (Bernet and Segner, 2004)	n. d.	no	u/poss/lik./very lik.
hist. liver index	yes	yes/no/n. d.	yes/no/n. d.	> 10 (Bernet and Segner, 2004)	yes (Fischnetz, 2004)	n. d.	no	u/poss/lik./very lik.
EROD	yes	yes/no/n. d.	yes/no/n. d.	50 pmol/mg/min (Zimmerli et al., 2007)	yes (f. review: Whyte et al., 2000)	n. d.	no	u/poss/lik./very lik.
PKD	yes	yes/no/n. d.	yes/no/n. d.	positive (Clifton-Hadley et al., 1987)	yes (Schubiger, 2003)	n. d.	yes	u/poss/lik./very lik.
NH ₄ -N	yes	yes/no/n. d.	yes/no/n. d.	0.2 mg N/L (GSchV, 1998)	yes (Burton and Pitt, 2002)	n. d.	no	u/poss/lik./very lik.
NO ₂ -N	yes	yes/no/n. d.	yes/no/n. d.	0.0015 mg N/L (PNEC) (LOEC in Russo, 1985)	yes (Russo, 1985)	yes (Rodriguez-Moreno and Tarazona, 1994)	no	u/poss/lik./very lik.
Atrazin	yes	yes/no/n. d.	yes/no/n. d.	20,000 ng/L (PNEC) (Götz et al., 2003)	yes (2)	n. d.	no	u/poss/lik./very lik.
Diazinon	yes	yes/no/n. d.	yes/no/n. d.	900 ng/L (PNEC) (Götz et al., 2003)	yes (3)	no (Brewer et al., 2001)	no	u/poss/lik./very lik.
NH ₄ -N	yes	yes/no/n. d.	yes/no/n. d.	3340 ng/L (PNEC) (Götz et al., 2003)	yes (3)	n. d.	no	u/poss/lik./very lik.
Tebutam	yes	yes/no/n. d.	yes/no/n. d.	187 ng/L (PNEC) (Götz et al., 2003)	yes (3)	n. d.	no	u/poss/lik./very lik.
PBDE	yes	yes/no/n. d.	yes/no/n. d.	n. d. -	n. d.	n. d.	no	u/poss/lik./very lik.
primary factors								

Table 1. Continued

Parameter	Plausibility	Exposure	Correlation	(Exceedance of) critical value	Experiment	Removal experiment	Specificity	Conclusion
final brown trout abundance								
estrogens	yes	yes/no/n. d.	yes/no/n. d.	0.1 ng/L (PNEC) (LOEC in Pawlowski et al., 2004)	yes (Pawlowski et al., 2004; Routledge et al., 1998)	n. d.	yes	u/poss./lik./very lik.
Ntot	yes	yes/no/n. d.	yes/no/n. d.	2 mg N/L (estimated)	yes (Borsuk et al., 2005)	n. d.	no	u/poss./lik./very lik.
% wastewater	yes	yes/no/n. d.	yes/no/n. d.	10 %	yes (Bernet and Segner, 2004)	n. d.	no	u/poss./lik./very lik.
large average width	yes	yes/no/n. d.	yes/no/n. d.	< 5m	yes (Schager et al., 2007)	n. d.	no	u/poss./lik./very lik.
% riffles	yes	yes/no/n. d.	yes/no/n. d.	n. d.	yes (Schager et al., 2007)	n. d.	no	u/poss./lik./very lik.
limited connectivity	yes	yes/no/n. d.	yes/no/n. d.	n. d.	n. d.	n. d.	no	u/poss./lik./very lik.
inner siltation	yes	yes/no/n. d.	yes/no/n. d.	class 4 or 5	yes (Schager et al., 2007)	n. d.	no	u/poss./lik./very lik.
insufficient shading	yes	yes/no/n. d.	yes/no/n. d.	n. d.	n. d.	n. d.	no	u/poss./lik./very lik.
insufficient food benthos	yes	yes/no/n. d.	yes/no/n. d.	< 20 g/m ²	n. d.	n. d.	no	u/poss./lik./very lik.
elevated T health	yes	yes/no/n. d.	yes/no/n. d.	9 °C (egg) 12 °C (fry) 19 °C (adult)	(Humpesch, 1985; Schmeing-Engberding, 1953; Crisp, 1996)	n. d.	no	u/poss./lik./very lik.
T PKD	yes	yes/no/n. d.	yes/no/n. d.	15 °C	yes (Schubiger, 2003)	n. d.	yes	u/poss./lik./very lik.
discharge (Q) with gravel transport	yes	yes/no/n. d.	yes/no/n. d.	Q ₀ > 0	(Santschi, 2003)	n. d.	yes	u/poss./lik./very lik.

n. d.= not determined; u.= unlikely; poss. = possible; lik. = likely; very lik. = very likely

(1) only coincidence, no causal relationships were up to now described.

(2) <http://www.epa.gov/ppsrdd1/reregistration/atrazine>; Aquire: www.epa.gov/ecotox/; agritox: www.inra.fr/agritox/; Tomelin, 1997; UBA, 1998.

(3) AQUIRE: www.epa.gov/ecotox/; AGRITOX: www.inra.fr/agritox/; Umweltbundesamt., 1998. Anhörung zu Zielvorgaben für Pflanzenschutzmittel/wirkstoffe. Intern Bericht. Tomelin, 1997. The Pesticide Manual. British Crop Protection Council, Farnham, UK.

primary factors

Reproductive impairment was assessed by two parameters: vitellogenin induction and experimental early life stage (ELS) tests of eggs and embryos. **Plasma vitellogenin (VTG)** was analyzed by means of a competitive brown trout VTG enzyme linked immunosorbent assay (bt-VTG ELISA) according to Körner and coauthors (2007). VTG is considered to be elevated when it is above 1,000 ng/mL in males or juveniles (Vethaak et al., 2002). **Early life stage (ELS)** tests were designed as egg incubation experiments with brown trout eggs incubated either in Vibert-boxes (in Necker in winter 2002/03) or on-gravel exposed plates (in Venoge and Emme in winter 2001/02; (Kobler, 2004; Schager and Peter, 2004; Roos, personal communication). Survival rates were measured as both the percentage of eggs reaching the eying stage and those which hatch. An adverse effect is considered to be an egg or embryo mortality exceeding 20% (Bernet and Segner, 2004).

As individual *health indicators*, we measured the condition factor, the hepatosomatic index, the histological liver index, 7-ethoxyresorufin-O-deethylase (EROD) activity and proliferative kidney disease (PKD) infection.

The *condition factor* is calculated from length and weight data as $CF = (\text{weight} \times 100) / \text{length}^3$. Decline in condition factor has been observed in fish subjected to stress from a variety of adverse environmental conditions, including low food abundance (for review: Goede and Barton, 1990). A CF below 0.8 and above 1.2 indicates a deviation from the normal status (Bernet and Segner, 2004).

The *hepatosomatic index*, reflecting energy storage capacity, is calculated as $HSI = 100 \times \text{liver weight} / (\text{body weight} - \text{liver weight})$. Data were measured and the index was calculated by Zimmerli and co-authors (Zimmerli et al., 2007). As a threshold, a significant elevation or decrease compared to the reference value of the given river was used.

For the *histological liver index*, female and male individuals from each site and season were investigated (Zimmerli et al., 2007) and the pathological changes were classified according to Bernet and coauthors (Bernet et al., 1999). A score of 10 was selected as the threshold above which alterations were deemed to have adverse effects.

The activity of one key detoxifying enzyme, the 7-ethoxyresorufin-O-deethylase (EROD) activity was measured in the liver to indicate exposure to dioxin-like xenobiotics, including dioxins and furanes, polychlorinated biphenyls (PCB's) as well as polycyclic aromatic hydrocarbons (PAH's) (Whyte et al., 2000). According to the available literature for brown trout under non-exposed (control) conditions activity, values below 50 pmol/mg/min were reported (Whyte et al., 2000; Behrens and Segner, 2005), though 50 pmol/mg/min was taken as the threshold indicating induction of EROD expression (Zimmerli et al., 2007).

Proliferative kidney disease (PKD) is the most common infection disease affecting feral brown trout in Switzerland and leads to substantial mortalities (Wahli et al., 2002). It is caused by a myxozoan parasite *Tetracapsuloides bryosalmonae*. A cumulative mortality associated with a clinical outbreak of PKD of more than 95% was reached in brown trout after the water temperature remained at least two weeks over 15°C (Schubiger, 2003). Young-of-year fish are especially vulnerable to PKD because they are exposed to the parasite for the first time (Wahli et al., 2002). A diagnosis of PKD infection was based on macroscopical, histological and immunohistochemical evaluations of the kidney (Zimmerli et al., 2007).

Physico-chemical data. During 2002, chemical analyses of river water samples were measured monthly in cantonal laboratories (Environmental Protection Office Canton St. Gallen; Environmental Protection Office Liechtenstein; Water and Soil Protection Laboratory of the Canton Bern; Water and Soil Protection Laboratory of the Canton Waadt). The parameters analysed included: dissolved organic carbon, nitrate, nitrite, ammonium, chloride, orthophosphate and total phosphorus. The water temperature was measured by means of data loggers at each site once or twice per hour during the entire year (data provided by E. Schager, Eawag).

The toxicity and proportion of ammonium/ammonia ($\text{NH}_4\text{-N}/\text{NH}_3\text{-N}$) is primarily dependent on the pH level. The recommended pH-dependent water quality criteria for ammonium (average concentration 30 days) at a pH of 9 is 0.25 mg $\text{NH}_4\text{-N}/\text{L}$ (Burton and Pitt, 2002). The quality aims for $\text{NH}_4\text{-N}$ in running waters according to the Swiss water pollution decree lies even below this value (0.2 mg N/L; (GSchV, 1998) and was used as a threshold.

Environmental nitrite concentrations of 0.015 mg/L $\text{NO}_2\text{-N}$ increase methemoglobin levels in rainbow trout blood (Russo, 1985). The PNEC of 0.0015 mg $\text{NO}_2\text{-N}/\text{L}$ was defined, considering a security factor of 10. Toxicity of $\text{NO}_2\text{-N}$ can be reduced by chloride, depending on its concentration (Russo, 1985). Accordingly a doubling of the Cl-concentration increased the LC_{50} (4d)-level by a factor of 2. Although it was reported that the Cl-concentration only delays $\text{NO}_2\text{-N}$ toxicity (Bartlett and Neumann, 1998), the thresholds were adjusted for each site according to the 80% of the measured Cl-concentrations (0–5 mg Cl/L or n.d. – 0.0015 mg $\text{NO}_2\text{-N -N}/\text{L}$, 5–10 mg Cl/L – 0.003 mg $\text{NO}_2\text{-N -N}/\text{L}$, 10–15 mg Cl/L – 0.0045 mg $\text{NO}_2\text{-N -N}/\text{L}$, 15–20 mg Cl/L – 0.006 mg $\text{NO}_2\text{-N -N}/\text{L}$).

The pesticides atrazine, desethylatrazine, diazinon, diuron, dimethenamide, isoproturon, metolachlor, tebutam, dicamba, (4-chloro-2-methylphenoxy)acetic acid (MCPA), mecoprop and sulcotrion were measured at D2 of each river from one to six times monthly during the

five months in and after the main application seasons of spring and summer (Götz et al., 2003). Only data for pesticides with a risk quotient > 1 are given in Table 2. We considered the PNEC and risk quotients calculated by Götz et al. (Götz et al., 2003, based on AQUIRE: www.epa.gov/ecotox/; AGRITOX: www.inra.fr/agritox/).

Flame retardants PBDE were measured in the liver and bile of brown trout, captured between April and May 2002 at the sampling sites in the test areas (n= 3-21 fish). The given values (Table 2) represent the average concentration of all samples per site (Hartmann et al., 2006). At present, no toxicity or effect levels are known for PBDE.

Estrogen levels were calculated as estradiol equivalents from the number of inhabitants and their theoretical intake, degradation in WWTP and discharge (Strehler and Scheurer, 2003). It includes the known co-occurrence and concentration additivity of E1, E2, and EE2 and their reported relations of 10:1:0.5 (Johnson et al., 2000). The lowest effect level (LOEC) for fish found in the literature is 1 ng ethinylestradiol/L and a NOEC of 0.1 ng/L is reported (Pawlowski et al., 2004). Accordingly, the PNEC is 0.01 ng/L, considering a security factor of 10. However, 17-beta estradiol is at least 10x less potent than ethinylestradiol in inducing VTG (Thorpe et al., 2003), resulting in a PNEC of 0.1 ng/L and a hazard might exist above this threshold. Nonylphenol (NP) and nonylphenolethoxylates (NP1EO, NP2EO) were also calculated but did not contribute noteworthy (M. J.-F. Suter, Eawag, written communication).

As an integrated measure of point source pollution, the percentage of river flow comprised of wastewater was calculated from the number of inhabitants served by the discharging treatment plants and the low flow value of the river, according to Körner et al. (2005):

Percent effluent = $Q_T / (Q_T + Q_{347})$, where Q_T is the average dry weather treatment plant discharge and Q_{347} is the river discharge which is equalled or exceeded for 347 days of the year. Wastewater percentages greater than 10% were considered to be critical (GSchV, 1998). As a measure of combined point and non-point source pollution, we used the results of Zobrist and Reichert (2006), who found that mean annual concentrations of total nitrogen, nitrate, phosphate, chloride, and potassium could be predicted from basin land use and population size. We used their results to calculate the predicted mean annual total nitrogen concentration at each survey site. This is the same method that was used by Borsuk et al. (2005) whose results indicate that 2.0 mg N/L can be used as an approximate threshold for ELS effects.

Habitat data. Habitat and streambed quality were scored according to data raised by several investigators (Schager and Peter, 2003; 2004; Scheurer, 2004). Among the parameters considered were: width, % riffles, longitudinal

connectivity, riverbed siltation, and shade. Width, % riffles and riverbed siltation are reported to influence recruitment (Schager and Peter, 2007). Siltation was scored using the 5-level method of Schälchli et al. (2002) where levels 4 and 5 are assumed to have a significant impact (Schager and Peter, 2007; Schlager, personal communication). Other habitat parameters were measured on a continuous scale, as described by Schager and Peter (2004).

Data on the availability of benthic prey resources in the study areas were obtained by literature review (Scheurer, 2004). Whereas some studies dealt with macrozoobenthos as indicators for water quality, only sources where quantitative macrozoobenthos data were raised were taken into account [Emme: (Vuille, 1997); LBK: n. d.; Necker: (Frutiger, 1979; Imhof, 1994); Venoge: n. d.]. Vuille (1997) suggested that a prey density below 20 g/m² is critical. Besides benthic food availability, external inputs also play an important role in some seasons, and the percentage shade was used as an indicator for this type food availability (Schager and Peter, 2004). Macroinvertebrate biomass and fish biomass are lowest in heavily shaded areas and in open channels without riparian vegetation, but highest in ecotones with intermediate complexity (Zalewski et al., 1998).

The resident brown trout is a cold-water salmonid and many studies were published on temperature preferences of the different life-stages which also depend from the investigated population and acclimation (compiled in Crisp, 1996). Roughly, the preferred temperature range is between 4 °C and 19 °C for adults. Eggs are very temperature sensitive, and the optimal temperature for their development is around 5 °C, with increased mortality below 1 °C and above 9 °C (Humpesch, 1985). As a consequence, a threshold of 9 °C was selected for egg development and 19 °C for adverse effects on adults. Besides direct effects, indirect effects on food organisms or on the health status are of concern. A clinical outbreak of the proliferative kidney disease (PKD) occurs when ambient water temperature surpasses 15 °C for more than 2 weeks and can cause mortalities of more than 95% (Schubiger, 2003). Consequently, temperature data were checked for the number of weeks in which water temperature surpasses the threshold of 15 °C.

The hydrologic regime can have an important influence on egg incubation and fry survival and, therefore, on recruitment. High flows during the winter intra-gravel period can cause egg pocket washout. For a particular streambed, the flow magnitude at which egg washout occurs can be estimated using river width, bed slope and gravel size (Santschi, 2003). The frequency of exceedances of this discharge then provides a relevant measure of impact. We used the results of Santschi (2003) for the corresponding case study sites as inputs for our analysis.

Table 2. Data of potential factors considered for the weight-of-evidence approach. See text for further explanations. HW: headwater, D1: downstream site 1 (middle stream reach), D2: downstream site 2 (further downstream of D1).

Parameters	Emme			LBK			Necker			Venoge			Reference		
	D2	D1	HW	D2	D1	HW	D2	D1	HW	D2	D1	HW	D2	D1	HW
brown trout abundance [1-5]	5	5	3	5	5	1	4.6	3	3	n.d.	n.d.	2.3	n.d.	n.d.	Schager and Peter, 2004 Mean of 2-5 samplings
recruitment [1-5]	5	4	5	5	5	2.5	4.5	3.25	4.7	n.d.	n.d.	5	n.d.	n.d.	Schager and Peter, 2004 Mean of 2-5 samplings
ELS -survival rate [%]	13	70	n.d.	n.d.	n.d.	n.d.	0	0	92	0	9	84	0	9	Schager and Peter, 2004
VTG induced	no	no	no	no	no	no	no	no	no	no	no	no	no	no	Körner et al., 2007
condition factor above/below threshold	no	no	no	no	no	above	no	no	no	no	no	no	no	no	Körner et al., 2007
HSI elevated	yes	no	no	no	no	no	no	no	no	no	yes	no	yes	no	Zimmerli et al., 2007
histological liver index	18	16	13	21	20	13	16	18	14	16	15	18	16	15	Zimmerli et al., 2007
EROD above threshold	no	no	no	yes	yes	no	no	no	no	no	no	no	yes	no	Zimmerli et al., 2007
PKD above threshold	yes	yes	no	no	no	no	no	no	no	no	yes	no	yes	no	Zimmerli et al., 2007
NH ₄ -N [mg/L]	0.31	0.09	0.01	0.02	0.45	0.08	0.03	0.03	<0.01	0.16	0.09	0.02	0.16	0.09	1
NO ₂ -N [mg/L]	0.033	0.022	0.001	0.007	0.020	<0.001	0.007	0.008	0.001	0.046	0.036	0.011	0.046	0.036	1
Cl [mg/L]	n.d.	n.d.	n.d.	3.2	10.8	2.9	3.8	3.6	1.3	20.8	17.7	8.1	20.8	17.7	1
Atrazine [med ng/L] (min-max)	13 (7-728)	n.d.	n.d.	² <QL-11	n.d.	n.d.	⁵ <QL-11	n.d.	n.d.	⁸⁰ (23-764)	n.d.	n.d.	n.d.	n.d.	Götz et al., 2003
Diazinon [med ng/L] (min-max)	⁴ <QL-26	n.d.	n.d.	<QL-27	n.d.	n.d.	<QL-5	n.d.	n.d.	⁸ (1-16)	n.d.	n.d.	n.d.	n.d.	Götz et al., 2003
Diuron [med ng/L] (min-max)	<QL-113	n.d.	n.d.	<QL-19	n.d.	n.d.	<QL-5	n.d.	n.d.	¹¹ (4-103)	n.d.	n.d.	n.d.	n.d.	Götz et al., 2003

Table 2. Continued

Parameters	Emme			LBK			Necker			Venoge			Reference
	D2	D1	HW	D2	D1	HW	D2	D1	HW	D2	D1	HW	
Tebutam [med ng/L] (min-max)	<QL (<QL - 27)	n.d.	n.d.	<QL (<QL)	n.d.	n.d.	<QL (<QL)	n.d.	n.d.	<QL (<QL - 285)	n.d.	n.d.	Götz et al., 2003
PBDE [ng/g Lipid] in liver / in bile	n.d.	156/1.2	n.d.	1286/7.5	2501/23.8	1379/1.5	n.d./1.0	137/1.0	n.d./1.2	217/52	38/3	n.d./36	Hartmann et al., 2006
estrogens [ng/L]	0.247	0.097	0	n.d.	n.d.	n.d.	0.119	0.12	0	0.253	0.260	n.d.	Strehler and Scheurer, 2003
N _{tot} [mg/L]	5.69	3.87	2.54	1.13	1.18	1.08	2.88	2.77	1.20	8.96	7.76	5.16	Zobrist and Reichert, 2006
% wastewater	3	2	n.d.	4	10	0	2	1	0	6	5	2	Körner et al., 2005
average width [m]	28	32	14	8	4.6	3.4	11	14	5.6	11	16	5	Schager and Peter, 2004
% riffles	12	100	50	0	0	0	40	23	38	0	15	40	Schager and Peter, 2004; Schager, personal commun.
connectivity [1-5]	5	5	3	2	2	2	1	3	3	2	3	3	Scheurer, 2004
inner siltation [1-5]	1	2	1	4	4	4	2	3	1	5	5	1	Schager and Peter, 2004
shading [% of river bank]	2	2	10	20	5	90	5	10	90	70	50	90	Schager, personal commun.
food benthos [g/m ²]	20-30	10-20	20-30	n.d.	n.d.	n.d.	40	40	12	n.d.	n.d.	n.d.	Scheurer, 2004
T water [duration elevated]	4w > 15C 1w > 9C	2w > 15C	2w > 15C	no	4w > 9C	no	3w > 15C	3w > 15C	no	7w > 15C	7w > 15C	2w > 15C	Scheurer, 2004
discharge with gravel transport [events/yr]	0.22	0.22	0.22	n.d.	n.d.	n.d.	0.19	0.19	0.19	1	1	1	Santschi, 2003

¹ Values are presented as 80th percentile of monthly measured samples in 2002. Data kindly provided by cantonal authorities VD, BE and SG and of the Principality of Liechtenstein.

Primary vs. intermediate factors

Of the twenty causal factors we investigated, some can be considered to be primary causes because they are closely linked to anthropogenic impacts, while others should be treated as intermediate in nature because they cannot be controlled directly but are actually the effects of the primary causes (Fig. 3). For the latter factors, which are generally related to health and reproduction, data were raised in different forms; we transformed them to the qualitative levels of “good”, “medium” or “poor”. In particular, recruitment data were raised and classified in 5 classes (Schager and Peter, 2004; Schager et al., 2007); these classes were transformed to “good” (class 1 and 2), “medium” (Class 3) and “poor” (class 4 and 5); for the ELS, the raised percentage values were classified in 5 classes (Bernet and Segner, 2004) and we adapted them again to the 3-step classification as outlined for recruitment data. For the histological liver index, a value above a score of 10 was assessed as slightly impaired (“medium”) and above 20 as impaired (“poor”), as suggested by Bernet and Segner (2004). This corresponds to the importance of this parameter proposed by Zimmerli et al. (2007). In the case of VTG induction, the situation was assessed as poor (i.e. indicating a previous exposure to (xeno)estrogens) when the mean of the group sampled at a specific site was significantly elevated (Körner et al., 2007). Additionally, conditions above the threshold for EROD (50 pmol/mg/min) were assessed as “poor” (Zimmerli et al., 2007).

To apply the weight-of-evidence procedure, we linked the primary causes to the adverse, intermediate effect. Often it was necessary to decide which of the possible relations between primary factor and intermediate factor is the most obvious and relevant, since some causes may influence more than one intermediate factor and several primary factors may influence the same intermediate factor. For example, some chemical factors are known (or

suspected) to affect health (Götz et al., 2003; Bernet and Segner, 2004; Zimmerli et al., 2007). More specifically, NO₂-N was shown to affect liver structure (Michael et al., 1987). Accordingly, NO₂-N was related to the histological liver index. However, effects of NO₂-N on several enzymatic activities of blood and various tissues also have been demonstrated (Das et al., 2004), which would have justified a link between NO₂-N and general condition as well. Nevertheless, we decided to link NO₂-N to the liver histology since this effect is more specific than general condition. Exact links used in the analysis are shown in Table 3.

Results

In the following, the seven questions to which the weight-of-evidence analysis was applied were answered based on the data available.

Plausibility question (1)

Experts in fisheries, environmental chemistry, fish biology and pathology determined the causative factors to be plausible and worth investigating during the first stage of the Fischnetz project (Burkhardt-Holm et al., 2002; Burkhardt-Holm, 2006). Therefore, in terms of WOE, they passed the **plausibility** test. In the process of providing the literature basis for all the various research projects, the theoretical plausibility of the investigated factors was further confirmed.

Site specific questions (2–4)

Site specific values of all considered primary and intermediate causes are shown in Table 2. Starting with the

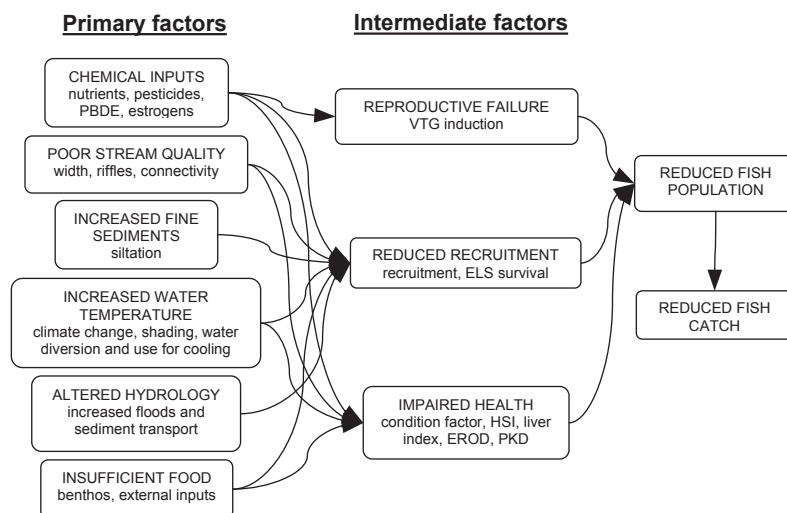


Figure 3. This boxes-and-arrows diagram shows the relations between the primary and intermediate factors and effects.

Table 3. Results of the weight-of-evidence assessments. Column 2 presents parameters investigated affecting either the brown trout abundance as such (intermediate factors) or, in the lower part, primary factors which affect intermediate factors. Example: insufficient recruitment results in low trout abundance. Nitrite results in an increased histological liver index which indicates a bad health status and might indirectly lead to low trout abundance. Note: intermediate factors were not considered for the WOE but assessed according to their general trend at this site of the river. HW: Headwater, D1: downstream site 1 (middle stream reach), D2: downstream site 2 (further downstream of D1).

	Parameter	Intermediate factors	Emme			LBK			Necker			Venoge		
			D2	D1	HW	D2	D1	HW	D2	D1	HW	D2	D1	HW
intermediate factors	final effect	brown trout abundance	↘	↘	→	↘	↘	↗	↘	→	→	—	—	↗
		recruitment	↘	↘	↘	↘	↘	→	↘	→	↘	—	—	↘
		ELS-survival rates	↘	↗	—	—	—	—	↘	↘	↗	↘	↘	↗
		VTG-induced	↗	↘	↗	↗	↘	↘	↗	↘	↘	↘	↗	↘
		condition factor	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗	↗
		HSI-elevated	↘	↗	↗	↗	↗	↗	↗	↗	↗	↘	↘	↗
		hist. liver index	→	→	→	↘	↘	→	→	→	→	→	→	→
		EROD	↗	↗	↗	↘	↘	↗	↗	↗	↗	↘	↗	↗
primary factors	PKD	brown trout abundance	●	●	×	×	×	×	×	×	×	●	●	×
	NH ₄ -N	hist. liver index	⊙	×	×	×	⊙	×	×	×	×	×	×	×
	NO ₂ -N	hist. liver index	●	●	×	×	●	×	●	●	×	●	●	●
	Atrazin	hist. liver index	×	—	—	×	—	—	×	—	—	×	—	—
	Diazinon	hist. liver index	×	—	—	×	—	—	×	—	—	×	—	—
	Diuron	hist. liver index	×	—	—	×	—	—	×	—	—	×	—	—
	Tebutam	hist. liver index	×	—	—	×	—	—	×	—	—	○	—	—
	PBDE	hist. liver index	—	○	—	×	○	○	×	×	×	×	×	×
	estrogens	VTG induction	×	×	×	—	—	—	×	×	×	○	○	—
	N _{tot}	reproduction	×	×	×	×	×	×	×	×	×	○	○	○
	% wastewater	hist. liver index	×	×	—	×	⊙	×	×	×	×	×	×	×
	large average width	reproduction	×	×	×	⊙	×	×	×	×	×	○	○	×
	% riffles	reproduction	⊙	×	○	×	×	×	×	×	×	○	○	○
	limited connectivity	brown trout abundance	○	○	○	×	×	×	×	×	×	×	×	×
	inner siltation	reproduction	×	×	×	×	×	×	×	×	×	○	○	×
	unsufficient shading	condition factor	×	×	×	×	×	×	×	×	×	×	×	×
	unsufficient food benthos	condition factor	×	×	×	—	—	—	×	×	×	—	—	—
	unsuitable T health	reproduction/bt abundance	⊙	×	×	×	⊙	×	×	×	×	⊙	×	×
	unsuitable T PKD	PKD	●	●	●	×	×	×	×	×	×	●	●	×
	discharge with gravel transport	reproduction	○	○	○	—	—	—	○	○	○	○	○	○

Legend: — n.d. ↘ poor → average ↗ good
 × unlikely ○ possible ⊙ likely ● very likely

intermediate causes, it can be seen from the table that the downstream populations are generally subject to low reproductive success, as indicated by critically low ELS survival. However, VTG was only elevated in 10 out of 197 males analysed and the average was not elevated at any of the sites (Körner et al., 2007).

Recruitment, as determined by the abundance of late summer young-of-the-year, was found to be impaired in all rivers and at all sites. However, a downstream gradient was only observed at the LBK. Brown trout abundance was very good to moderate at the headwater site and deteriorated downstream in all rivers.

Condition factor was never below the threshold value of 0.9, and therefore provides no positive causal evidence. Downstream sites at the Emme and Venoge showed elevated HSI, and the two downstream sites at LBK and D2 at the Venoge showed elevated values for EROD. The latter sites and D1 at the Venoge were the only ones with evidence of PKD infection. As these were also locations with very low abundance, a positive correlation is concluded. Water temperatures which favour PKD outbreak were also found at these sites.

Water quality parameters indicated high levels of nitrogen compounds at all of the Venoge sites, and the two most downstream sites in the Emme and the Necker. Additionally, ammonium and nitrite appeared to be elevated at the LBK D1 site. These observations were generally consistent with wastewater percentages greater than 1 % at this site.

According to the ecotoxicological measurements and assessments, there was exposure of fish to tebutam only during a short time period in Venoge, whereas for all other of the measured pesticides and for the other rivers, critical pesticide exposure to fish was not identified. When critical effect levels for aquatic biota other than fish are considered, a risk is indicated for diazinon in Emme and Venoge and, for the latter river, also for diuron, since the measured median concentrations of these compounds in the river water are in the range of the environmental quality standard (EQS). When considering maximal instead of median concentrations, additionally atrazine, diuron and metolachlor in Emme and Venoge, and diazinon and diuron in LBK and Necker raise a potential risk. Since measurements were made only at the most downstream sites, no correlations with the histological liver index were possible. The site-specific assessment of PBDE was hampered by the lack of thresholds, though detectable levels were found at all LBK sites, Emme D1, Necker D1, and Venoge D1 and D2.

Estrogenic potentials were at levels of concern at nearly all the sites where data were recorded, except for those headwater sites where there are no wastewater inputs. A hypothesized correlation with VTG induction is not supported by the data.

Habitat quality is generally quite high at the Emme HW site, all Necker sites, and the two downstream

Venoge sites, but at least partially impaired at the most upstream LBK and the most upstream Venoge locations (Schager and Peter, 2004). Siltation may be a concern in the LBK and two downstream Venoge sites. Other locations have relatively poor morphology. There is only evidence of food limitation at the Necker HW and Emme D1, but for the other two rivers there are no data and a correlation with condition factor is not apparent.

In the rivers Emme and Necker, gravel transport is initiated by winter floods approximately every 5 years. In contrast, the river Venoge experiences extended gravel transport nearly every winter season. Accordingly, in all these rivers, high winter floods with gravel transport are possible factors that impair reproduction of brown trout. Data are not available on this factor for LBK.

Questions to be answered by literature data (5–7)

PKD, estrogenic exposure, and gravel bed washout by floods are the only causative factors that cause **specific effects**. These effects can, however, be modulated in their strength by environmental confounding factors. PKD is an infectious disease of salmonids caused by the myxozoan parasite *Tetracapsuloides bryosalmonae* which can be specifically identified by a serial combination of three different methods (Wahli et al., 2007). High water temperature, as well as slow-flowing water and high amount of organic pollutants can also favour the settlement, distribution and proliferation of filter-feeding bryozoans, which serve as intermediate host for the parasite (Gay et al., 2001). Accordingly, these factors may favour the distribution of the parasite. River water temperatures increased in the last 25 years in Swiss rivers (Hari et al., 2006). This led to a shift in brown trout habitat up-river and contributed to an increase in PKD incidence at the habitat's lower boundaries (Hari et al., 2006).

Estrogens are of key importance for reproductive fitness, and VTG induction in male fish is established as a specific biomarker of exposure to environmental estrogens (e.g. Sumpter and Jobling, 1995). As a consequence, VTG induction specifically indicates a preceding exposure to estrogens – but no statements on the duration of this exposure and concentration of estrogens can be made. Significant inductions were not found at the test areas (Körner et al., 2007). However, it has to be kept in mind that VTG response can be modulated by temperature influence and stress parameters (King et al., 2003; Berg et al., 2004).

Large spates with movement of gravel may cause washout of salmonid eggs and fry, or physically damage them, leading subsequently to their death (Crisp, 2000; Massa, 2000). Massa (2000) showed that even small gravel transport can lead to damage of offspring. Therefore, populations with abnormally low numbers of young-of-the-year are likely to have been exposed to washout.

Experimental and observational results can be used as evidence for causation for many of the intermediate and primary factors (Table 1). For example, a severe disturbance of embryonic recruitment of brown trout due to fine sediment particles and siltation was described by several authors (Turnpenny and Williams, 1980; Luckenbach et al., 2001; Kobler, 2004). In a multiple stepwise regression analysis, (Eklöv et al., 1999) showed that stream size was the most important component for density of age 0 brown trout with higher densities found in smaller streams. Shading was also strongly associated with high densities of age 0 brown trout (Eklöv et al., 1999). Shading is important for the regulation of the water temperature and in consequence might decrease stress. Vegetation at river banks is also known as a source for allochthonous food input into the rivers. In small rivers, up to 80% of gastric contents of brown trout is composed of terrestrial input (Gisler, 1991). In addition, river banks with extensive vegetation build a barrier for sediment input. However, too heavy shading causes a decrease in productivity of a river (Zalewski et al., 1998). In consequence, shading is important not only for age 0, but indirectly for the fitness of all age classes and was therefore related to the adverse effect of hepatosomatic index. The productivity of an ecosystem is often expressed in amount of food benthos, and a threshold of 20 g/m² was defined (Vuille, 1997). The amount of food contributes to the health and fitness of fish, one of the resulting indicators is the condition factor. In addition, the percentage of riffles and the connectivity are further parameters used to describe the state of the physical habitat and limit the abundance of brown trout, if insufficient. In respect to connectivity, spawning areas are mostly located in headwaters and small tributaries from where the juveniles could migrate to main streams if connectivity is provided (Bagliniere and Maisse, 2002; Schager et al., 2007).

Removal of the stressor leading to the amelioration of the situation could be used as supporting evidence for a cause-effect relation according to question 7. For our study, only few data, were available. An experimental removal of nitrite lead to a recovery of several physiological parameters (Azevedo et al., 2004; Rodriguez-Moreno and Tarazona, 1994) and nitrite is detoxified by trout hepatocytes (Doblender and Lackner, 1996). Accordingly a regeneration of liver structure can be assumed as well. In contrast, after removal of diazinon, no recovery was observed (Brewer et al., 2001). For the other factors, no appropriate data could be found to evaluate the effects of removal.

Weight of evidence conclusions

The weight of evidence suggests that PKD is a very likely factor for the low abundance at the two most downstream sites of Venoge and Emme.

Further, nitrite, partly in addition with ammonia or the wastewater percentage as described in the method section: “physio-chemical data”, are very likely causes of adverse effects on the liver index at the two downstream sites of Emme and Necker, the D1 site at LBK and all sites in Venoge.

Of the micropollutants, only tebutam was assessed as a possible factor at the downstream site of the Venoge and PBDE at D1 in Emme as well as at D1 and HS of LBK.

Of the parameters characterizing habitat, the river bed in LBK D2 and in Venoge D1 and D2 the average width is assessed as a likely or possible factor influencing recruitment. Also, likely contributing to this adverse effect is the low percentage of riffles in Emme D2, which is possibly contributing in Emme HW and all sites in Venoge. The limited connectivity is a possible factor for insufficient brown trout abundance at all sites in the Emme, whereas siltation might possibly affect the recruitment in the two downstream sites of Venoge. Insufficient shading is a possible factor at all sites of Venoge for an adverse effect on the hepatosomatic index.

The elevated water temperature in winter above 9°C at LBK D1 and Emme D2, as well as in Venoge D2 where water temperature surpasses the optimal temperature threshold for growth of 19°C leads to an assessment of these factors as likely.

Discharge with gravel transport in winter possibly affects successful recruitment in all rivers except LBK.

Discussion

This study was designed to identify the most likely factors for the low abundance of brown trout in the four test areas. Data on the brown trout population, water quality and habitat in the investigated rivers were different enough to define distinct patterns of impairments. However, many stressors overlapped in their temporal and spatial occurrence. Since extrapolation from effects on lower levels of the biological hierarchy (e.g. individual parameters) to the population level is difficult, this evaluation differentiated between primary factors (anthropogenic or natural stressors) and their effects on intermediate factors. Clearly, we have to differentiate between primary factors which affect, for example the intermediate factor recruitment only (such as unsuitable temperature for eggs) and those affecting the population as a whole. We looked at the single primary factors and evaluated them for their relation to the most direct and dominant adverse effects only. However, other relations could also exist, but their investigation is hampered by lack of knowledge on the interactions between factors. For example, synthetic chemicals might also affect food organisms of fish and as a consequence, lead to insufficient nutritional status of fish. However, too little is known on

this effect under natural conditions to investigate these relations with the weight-of-evidence approach.

In all rivers, the headwater sites were characterized by the occurrence of the least number of stressors, supporting our decision to apply the gradient approach and to select these sites as reference conditions.

Additionally, in all rivers at least two of the studied parameters were identified as candidate causes contributing to the observed low brown trout abundance. The application of the WOE was successful in making the available evidence transparent and improves the likelihood that causes are identified.

We identified nine candidate causes for the river Emme with their number increasing along the downstream gradient. At all sites, the summer temperature regime can contribute to PKD infection at all sites, and the high correlation with the low abundance and the low recruitment at these sites make this factor very likely responsible for the decline in brown trout abundance. Evidence for PKD as a causative agent for decline in fish abundance is supported by the study of Schubiger (2003). However, this disease was not identified at the headwater site. This is probably due to the migration barriers and the great distance between D1 and HW. The slightly elevated histological liver index could be due to water pollution, as indicated by nitrogen compounds. Water pollution is known to affect fish liver structure (Bernet et al., 2000; Bernet and Segner, 2004) and, for example, nitrite and ammonia can induce a range of liver pathological alterations (Michael et al., 1987). Stress, due to insufficient habitat parameters could also contribute to increase in liver alterations. The very poor morphology of the two downstream sites, the uniform habitat structure and connectivity to the tributaries aggravate the situation, in particular for the offspring. Especially in case of spate in winter, the probability of devastating effects on eggs or recently emerged fry is high and certainly contributes to low recruitment success and low trout abundance.

The river LBK was characterized by the poor situation at the site D1, where high concentrations of ammonia, nitrite and high percentage of wastewater, together with unsuitable winter temperature affecting survival of offspring all contribute to poor health and brown trout abundance. Additionally, at this site, the highest concentrations of flame retardants were measured in fish. Since concentrations are low in the WWTP effluent, inputs from agriculture or atmospheric deposition have to be considered (Hartmann et al., 2006). Possibly, the high histological liver index at this (and the further downstream site) is due to this poor water quality. Increased histological liver and kidney indices, together with the occurrence of liver tumours were reported in previous studies (Bassi et al., 2001; Schneeberger, 1995).

PKD was not diagnosed at LBK and the water temperature never surpassed 15 °C during both years of in-

vestigation. However, in former years, PKD was identified (Bassi et al., 2001) and could have contributed to the reported fish decline in LBK. The total fish abundance was stated as high along the whole river (Schager and Peter, 2004), with a high predominance of rainbow trout at the two downstream sites where the brown trout abundance was poor. A competition for spawning sites and feed can lead to a decrease in brown trout abundance (Scott and Irvine, 2000). The inner siltation could have aggravated the situation. The consideration of the river-specific context led us to conclude, more comprehensively, that the combination of poor health status and competition possibly has led to the decrease in brown trout abundance and recruitment in LBK.

Fish decline in the Necker was most difficult to explain by the factors investigated. Whereas in most rivers, elevated nitrogen compounds are accompanied by other stressors, high nitrite concentrations are the only very likely water quality factor at the two downstream sites in the Necker. Our experiments revealed poor early life-stage survival rates at these sites. Together with the possible contribution of discharge with gravel transport in the winter, which may cause the total loss of an age-group on average every fifth year, nitrite concentrations might have led to poor recruitment in the Necker. PKD was never diagnosed.

In the Venoge, 12 factors were identified as possible, likely or very likely contributing to reduced abundance, recruitment or impaired fish health. It is striking that all these factors occur at the most downstream site, 9 are still present at the middle site, and even at the most upstream site, 4 factors contribute. This is even more astonishing since data for two very important parameters, recruitment and brown trout abundance could not be raised due to mixing of the population with migrating sea trout at the two downstream sites. Hence the weight of evidence analysis was compromised and likely underestimates the contributing factors.

The results at the Venoge reflect the intensive agriculture and the inputs of numerous, although small, sewage treatment plants. Tebutam possibly poses a risk for fish health. Considering the not-identified peak concentrations, the not measured sites more upstream in the agricultural zone as well as the not covered critical time windows, pesticide concentrations might still be high enough to affect brown trout. For example, the organophosphorous pesticide, diazinon, led to a decline in estradiol levels in bluegill sunfish, was suggested to disrupt the hormonal pathways and, as a consequence can affect the reproductive capacity (Maxwell and Dutta, 2005). Similarly, the algicide diuron, acts as an anti-androgenic compound in bioassays (Bauer et al., 1998). Receptor mediated effects have generally a very low threshold and evidence for such effects should be regarded seriously. PKD is very likely a prominent cause for the reduced

brown trout abundance at the two downstream sites, where for more than 7 weeks the temperature surpasses 15 °C. Further, inner siltation at these sites in combination with the high frequency of spates with gravel transport (one per winter) certainly contribute to the low reproductive success in the Venoge. However, it has to be taken into consideration that the two downstream sites belong to the barbel zone (epipotamal river), and hence, a high abundance of brown trout cannot be expected. Still, migration between the sites is hampered and a restoration could most probably help in restoring a natural brown trout population along the whole river.

We hypothesized 20 candidate causes for the test areas. For a conclusive assessment on the potential causes of the fish decline, more parameters would have to be included. Predation by fish-eating birds, predatory fish, competing species, intraspecific and intracohort competition as well as angler catch could act as potential stressors, but they were not included, due to a lack of data. Data on angler catch and stocking were available, but no thresholds and experimental data were present. Accordingly, angler catch and stocking would be possible causes in our assessment. In fact, for some of them more data are currently raised and they are studied for their relevance (Mürner, 2005).

A criterion in selecting our sites was the wadeability of the river to allow for a quantification of the fish abundance. Other researchers were confronted with the same problem and chose a mixed sampling approach (Norton et al., 2002). However, since in our study population density was the most crucial endpoint, we decided for a quantitative fishing method along the whole river (De Lury, 1947). Accordingly, small rivers were selected which were most often not long enough to allow a selection of more than three sites far apart enough to allow a distinction.

In almost no cases was a correlation statistically proven, but rather was assessed subjectively, but independently, by the authors. A prerequisite for a relation was the association of agents and effects along the river. We judged a correlation as positive when at least the headwater and one of the downstream sites of the river showed a correlation by visual inspection of the data. A further problem is that scale intervals are different in effects and parameters. Thus, correlations will not necessarily be linear.

Threshold values are well confirmed for some stressors, especially chemicals with non-specific effects. Problems arise (I) due to temporal and spatial variability (Gerecke et al., 2002); (II) substances acting at a receptor and therefore do not have a threshold value, (III) thresholds below analytically detectable values, or (IV) mixture effects (Silva et al., 2002; Brian et al., 2005). Besides, many stressors were never investigated for thresholds or these can only be determined for a particular site in question. Furthermore, some effects become only manifested

later in life (Johnstone et al., 1978) or even in next generations (Schwaiger et al., 2002).

In a separate study within the Fischnetz project, a dynamic population model was developed to consider the relative importance of natural and anthropogenic influence factors on brown trout abundance (Borsuk et al., 2005). This was done using a measure of causal strength based on a comparison of predicted brown trout abundance under both actual and hypothetical reference conditions in the same four river basins investigated in the present study. Similar to our results, it was found that the relative impact of the different stress factors differs by location. Habitat factors were found to be very important at many of the sites, potentially responsible for population reductions of over 50 % in nine of the locations. The difference in this finding, compared to the present study, may be due to the way in which habitat quality and its impact were quantified. In the modelling study, four habitat factors (depth and width variability, dominant substrate size, and stream bank structure) in addition to those considered here were assumed to set the upper limit on the capacity of age 0 brown trout. This is in contrast to the diverse intermediate factors that we considered to be the main effects of habitat impairment (see Table 2).

Borsuk et al. (2005) also found that PKD was fairly important, causing reductions of over 25 % at some locations. While we did not consider angler catch as a factor affecting brown trout abundance, they found that excessive angler catch was likely to be influential at all sites in the Emme and in the downstream sites in the Venoge, where it may be responsible for reductions as high as 50 %. The effects of siltation and water quality were more ambiguous, potentially causing either large reductions or large increases and being very uncertain in any case. In contrast to our study, the model of Borsuk et al. (2005) did not address fish health parameters or the effect of specific pollutants, such as pesticides, flame retardants or environmental estrogens. However, in a future project, the possible effects of estrogenic substances will be included.

Conclusions

We conclude that PKD, whose clinical outbreak is aggravated by confounding factors, such as increased river water temperature, is a very likely single parameter for the decline of brown trout abundance at the sites of the test areas where it occurs. Water pollution, as indicated by elevated levels of nitrogen compounds, may pose a serious risk at several sites, in particular those downstream of sewage treatment plants. Several habitat parameters, such as large width, low percentage of riffles or elevated winter temperatures, were identified as factors likely contributing to an impaired health, recruitment or low brown trout abundance at single sites. At most sites,

multiple factors are needed to act jointly to cause the decline in fish. This is apparent when, for example, at the headwater site in LBK, very good brown trout abundance was observed although two of the measured habitat parameters were insufficient (0% riffle and strong inner siltation). Thus, although brown trout is a very demanding species, some compensation in its requirements can be observed. This also has implications on the comparability: the fish population in each river basin should be considered individually and comparisons between rivers are not reasonable.

Acknowledgments

We thank all members of the Fischnetz project leadership committee and Katja Knauer, Nathalie Chèvre, Eva Schager and Oliver Körner for stimulating discussions. We thank Mark Borsuk for contributing to the early stages of this work and for assisting with the figures and text. Thanks are due to the staff of the cantonal authorities of Waadt, Thurgau and Berne and of the Principality of Liechtenstein for their help in raising data on water quality, fish population and habitat parameters. Funding was provided by the Fischnetz project which was supported by the Swiss Federal Institute for Environmental Science and Technology, the Swiss Agency for the Environment, Forests and Landscape, all 26 cantons of Switzerland, the Principality of Liechtenstein, the Swiss Society of Chemical Industries (SGCI), the Swiss Fishery Federation, the Center for Fish and Wildlife Medicine of the University of Bern, and the University of Basel. Thanks are due to helpful comments and suggestions of three anonymous reviewers.

References

- Adams, M., 2003. Establishing causality between environmental stressors and effects on aquatic ecosystems. *Human and Ecological Risk Assessment* **9**: 17–35.
- Azevedo, M., M. A. Souza and C. A. Freire, 2004. Reversibility of deleterious effects of the pisciculture byproduct nitrite on cultured Nile tilapia (*Oreochromis niloticus*). *Aquatic Living Resources* **17**(1): 19–23.
- Bagliniere, J. L. and G. Maisse, 2002. The biology of brown trout, *Salmo trutta* L., in the Scorff River, Brittany: a synthesis of studies from 1972 to 1997. *Productions Animales* **15**: 319–331.
- Bartlett, F. and D. Neumann, 1998. Sensitivity of brown trout alevins (*Salmo trutta* L.) to nitrite at different chloride concentrations. *Bulletin of Environmental Contamination and Toxicology* **60**: 340–346.
- Bassi, L., U. Baumann, M. Eugster, H. R. Hunziker, T. Keller, T. Kindle, R. Riederer, T. Rüdiger, C. Ruhlé and E. Zwicker, 2001. Gesundheitszustand der Fische im Rheintal – Untersuchungen 1997 bis 2000. Synthesebericht Arbeitsgruppe Gesundheitszustand der Fische im Rheintal, Amt für Umweltschutz (AFU), St. Gallen.
- Bauer, E. R. S., H. H. D. Meyer, P. Stahlschmidt-Allner and H. Sauerwein, 1998. Application of an androgen receptor assay for the characterisation of the androgenic or antiandrogenic activity of various phenylurea herbicides and their derivatives. *Analyst* **123**: 2485–2487.
- Behrens A. and H. Segner, 2005. Cytochrome P4501A induction in brown trout exposed to small streams of an urbanized area: results of a five-year-study. *Environmental Pollution* **136**: 231–242.
- Berg, A. H., L. Westerlund and P. E. Olsson, 2004. Regulation of Arctic char (*Salvelinus alpinus*) egg shell proteins and vitellogenin during reproduction and in response to 17 beta-estradiol and cortisol. *General and Comparative Endocrinology* **135**: 276–285.
- Bernet, D., H. Schmidt, W. Meier, P. Burkhardt-Holm and T. Wahli, 1999. Histopathology in fish: Proposal for a method to assess aquatic pollution. *Journal of Fish Diseases* **22**: 25–34.
- Bernet, D., H. Schmidt, T. Wahli and P. Burkhardt-Holm, 2000. Effects of waste water on fish health: an integrated approach to biomarker responses in brown trout (*Salmo trutta* L.). *Journal of Aquatic Ecosystem Stress and Recovery* **8**: 143–151.
- Bernet, D. and H. Segner, 2004. Effektstudie: Zusammenstellung von Fischgesundheitsdaten in der Schweiz. Fischnetz-Publikation, Teilprojekt 01/19. Zentrum für Fisch- und Wildtiermedizin, Universität Bern und Eawag, Bern/Dübendorf.
- Borsuk, M. E., P. Reichert, A. Peter, E. Schager and P. Burkhardt-Holm, 2005. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. *Ecological Modelling* **192**: 224–244.
- Brewer, S. K., E. E. Little, A. J. DeLonay, S. L. Beauvais, S. B. Jones and M. R. Ellersieck, 2001. Behavioral dysfunctions correlate to altered physiology in rainbow trout (*Oncorhynchus mykiss*) exposed to cholinesterase-inhibiting chemicals. *Archives of Environmental Contamination and Toxicology* **40**: 70–76.
- Brian, J. V., C. A. Harris, M. Scholze, T. Backhaus, P. Booy, M. Lamoree, G. Pojana, N. Jonkers, T. Runnalls, A. Bonfà, A. Marcomini and J. P. Sumpter, 2005. Accurate prediction of the response of freshwater fish to a mixture of estrogenic chemicals. *Environmental Health Perspectives* **113**: 721–728.
- Burkhardt-Holm, P., 2006. Fischnetz – a transdisciplinary project in Switzerland involving fishermen, authorities, scientists and chemical industry. In: TD-net, (ed), *Transdisciplinary Handbook*, in press.
- Burkhardt-Holm, P., W. Giger, H. Güttinger, A. Peter, K. Scheurer, M. J.-F. Suter, U. Ochsenbein, H. Segner and E. Staub, 2005. Where have all the fish gone? The reasons why fish catches in Swiss rivers are declining. *Environmental Science and Technology* **39**: 441 A–447 A.
- Burkhardt-Holm, P., A. Peter and H. Segner, 2002. Decline of fish catch in Switzerland. *Project Fishnet: A balance between analysis and synthesis. Aquatic Sciences* **64**: 36–54.
- Burton, G. A. and R. E. Pitt, 2002. *Stormwater effects handbook. A tool for watershed managers, scientists and engineers.* Lewis Publishers, CRC Press, Boca Raton, USA.
- Clifton-Hadley, R. S., D. Bucke and R. H. Richards, 1987. A study of the sequential clinical and pathological changes during proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. *Journal of Fish Diseases* **10**: 335–352.
- Crisp, D. T., 1996. Environmental requirements of common riverine European salmonid fish species in fresh water with particular reference to physical and chemical aspects. *Hydrobiologia* **323**: 201–221.
- Crisp, D. T., 2000. *Trout and salmon: ecology, conservation and rehabilitation.* Blackwell Science, Oxford.
- Das, P. C., S. Ayyappan, B. K. Das and J. K. Jena, 2004. Nitrite toxicity in Indian major carps: sublethal effect on selected enzymes in fingerlings of *Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*. *Comparative Biochemistry and Physiology, Part C* **138**: 3–10.
- De Lury, D. B., 1947. On the estimation of biological populations. *Biometrics* **3**: 145–166.

- Doblender, C. and R. Lackner, 1996. Metabolism and detoxification of nitrite by trout hepatocytes. *Biochimica et Biophysica Acta* **1289**: 270–274.
- Eklöv, A. G., L. A. Greenberg, C. Brönmark, P. Larsson and O. Berglund, 1999. Influence of water quality, habitat and species richness on brown trout populations. *Journal of Fish Biology* **54**: 1–234.
- Elliott, J. M., 1994. *Quantitative ecology and the brown trout*. Oxford University Press, Oxford.
- Fischnetz, 2004. Final Report of Project Fischnetz (in German). Eawag Dübendorf and BUWAL Bern, Switzerland.
- Forbes, V. E. and P. Calow, 2002. Applying weight-of-evidence in retrospective ecological risk assessment when quantitative data are limited. *Human and Ecological Risk Assessment* **8**: 1625–1639.
- Friedl, C., 1999. *Fischfangrückgang in schweizerischen Fließgewässern*. BUWAL, Bern.
- Frutiger, A., 1979. *Untersuchungen zur Ökologie der räuberischen Steinfliege *Dinocras Cephalotes* Curt. (Plecoptera: Perlidae) in einem Fließgewässer der schweizerischen Voralpen*. Dissertation, ETH, Zürich.
- Gay, M., B. Okamura and P. de Kinkelin, 2001. Evidence that infectious stages of *Tetracapsula bryosalmonae* for rainbow trout, *Oncorhynchus mykiss*, are present throughout the year. *Diseases of Aquatic Organisms* **46**: 31–40.
- Gerecke, A. C., M. Schäfer, H. P. Singer, S. R. Müller, R. P. Schwarzenbach, M. Sägger, U. Ochsenbein and G. Popow, 2002. Sources of pesticides in surface waters in Switzerland: Pesticide load through waste water treatment plants – current situation and reduction potential. *Chemosphere* **48**: 307–315.
- Gisler, B., 1991. Vergleich des Fressverhaltens von Bachforellen (*Salmo trutta fario* L.) mit der Makroinvertebratendrift. Diplomarbeit, Eawag, Dübendorf.
- Goede, R. W. and B. A. Barton, 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. *American Fisheries Society Symposium* **8**: 93–108.
- GSchV, 1998. Gewässerschutzverordnung (814.201), Council of Ministers, Bern, Switzerland.
- Götz, C., N. Chèvre, H. Singer and S. Müller, 2003. Emme, Necker, Liechtensteiner Binnenkanal, Venoge: Gebietscharakterisierung, Pestizidmessungen, Toxizitätsabschätzung. Fischnetz-Publikation, Teilprojekt 01/15. Eawag, Dübendorf.
- Hari, E. R., D. M. Livingstone, R. Siber, P. Burkhardt-Holm and H. Guetinger, 2006. Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. *Global Change Biology* **12**: 10–26.
- Hartmann, P. C., P. Burkhardt-Holm and G. Walter, 2006. Occurrence of polybrominated diphenyl ethers (PBDEs) in brown trout bile and liver from Swiss rivers. *Environmental Pollution*, online first: DOI:10.1016/j.envpol.2006.06.004.
- Humpesch, U. H., 1985. Inter-specific and intra-specific variation in hatching success and embryonic-development of 5 species of Salmonids and *Thymallus thymallus*. *Archiv für Hydrobiologie* **104**: 129–144.
- Huertas, M., E. Gisbert, A. Rodriguez, L. Cardona, P. Williot and F. Castello-Orvay, 2002. Acute exposure of Siberian sturgeon (*Acipenser baeri*, Brandt) yearlings to nitrite: median-lethal concentration (LC₅₀) determination, haematological changes and nitrite accumulation in selected tissues. *Aquatic Toxicology* **57**: 257–266.
- Imhof, A., 1994. *Habitatsansprüche und Verhalten von *Perla grandis rambur* (Plecoptera: Perlidae) und anderen räuberischen Steinfliegenlarven*. Dissertation, ETH, Zürich, Switzerland.
- Johnson, A. C., A. Belfroid and A. Di Corcia, 2000. Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. *Science of the Total Environment* **256**: 163–173.
- Johnstone, R., T. H. Simpson and A. F. Youngson, 1978. Sex reversal in salmonid culture. *Aquaculture* **13**: 115–134.
- King, H. R., N. W. Pankhurst, M. Watts and P. M. Pankhurst, 2003. Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. *Journal of Fish Biology* **63**: 153–167.
- Kobler, B., 2004. *Effects of treated wastewater on trout: A case study of a Swiss river*. PhD Thesis, Swiss Federal Institute of Technology, Zurich, Switzerland.
- Körner, O., E. L. M. Vermeirssen and P. Burkhardt-Holm, 2005. Intersex in feral brown trout from Swiss midland rivers. *Journal of Fish Biology* **67**: 1734–1740.
- Körner, O., E. L. M. Vermeirssen and P. Burkhardt-Holm, 2007. Reproductive health of brown trout inhabiting Swiss rivers with declining fish catch. *Aquatic Sciences* **69**(1): DOI 10.1007/s00027-006-0842-5.
- Luckenbach, T., M. Kilian, R. Triebkorn and A. Oberemm, 2001. Fish early life stage tests as a tool to assess embryotoxic potentials in small streams. *Journal of Aquatic Ecosystem Stress and Recovery* **8**: 355–370.
- Massa, F., 2000. *Sédiments, physico-chimie du compartiment interstitiel et développement embryon-larvaire de la truite commune (*Salmo trutta*): Etude en milieu naturel anthropisé et en conditions contrôlées*. Université Paris Grignon, Paris.
- Maxwell, L. B. and H. M. Dutta, 2005. Diazinon-induced endocrine disruption in bluegill sunfish, *Lepomis macrochirus*. *Ecotoxicology and Environmental Safety* **60**: 21–27.
- Michael M.I., A. M. Hilmy, N. A. El-Domiaty and K. Wershana, 1987. Serum transaminases activity and histopathological changes in *Clarias lazera* chronically exposed to nitrite. *Comparative Biochemistry and Physiology* **86C**: 255–262.
- Mürner, A., 2005. *Fischmodell praxistauglich*, Diplomarbeit, ETH Zürich.
- Norton, S. B., S. M. Cormier, G. W. Suter II, B. Subramanian, E. Lin, D. Altwater and B. Counts, 2002. Determining probable causes of ecological impairment in the little Scioto river, Ohio, USA: Part 1. Listing candidate causes and analyzing evidence. *Environmental Toxicology and Chemistry* **21**: 1112–1124.
- Pawlowski, S., R. van Aerle, C. R. Tyler and T. Braunbeck, 2004. Effects of 17 α -ethinylestradiol in a fathead minnow (*Pimephales promelas*) gonadal recrudescence assay. *Ecotoxicology and Environmental Safety* **57**: 330–345.
- Rodriguez-Moreno, P. A. and J. V. Tarazona, 1994. Nitrite-induced methemoglobin formation and recovery in rainbow trout (*Oncorhynchus mykiss*) at high chloride concentrations. *Bulletin of Environmental Contamination and Toxicology* **53**: 113–119.
- Routledge, E. J., D. Sheahan, C. Desbrow, G. C. Brighty, M. Waldock and J. P. Sumpter, 1998. Identification of estrogenic chemicals in STW effluent: 2. In vivo responses in trout and roach. *Environmental Science and Technology* **32**: 1559–1565.
- Russo, R. C., 1985. Ammonia, nitrite and nitrate. In: G. M. Rand and S. R. Petrocelli (eds.), *Fundamentals of Aquatic Toxicology*, Hemisphere Publishing Corporation, N.Y., pp. 455–471.
- Santschi, D., 2003. *Zeitliche Veränderung der winterlichen Abflusscharakteristik schweizerischer Fließgewässer*. Diplomarbeit. Universität Bern.
- Schager, E. and A. Peter, editors, 2003. *Methoden zur Untersuchung und Beurteilung der Fließgewässer: Fische Stufe F*. BUWAL, Bern.
- Schager, E. and A. Peter, 2004. *Testgebiete – Fischbestand & Lebensraum*. Fischnetz-Publikation, Teilprojekt 00/16. Eawag, Kastanienbaum.
- Schager, E., A. Peter and P. Burkhardt-Holm, 2007. Status of young-of-the-year brown trout (*Salmo trutta fario*) in Swiss streams: factors influencing YOY trout recruitment. *Aquatic Sciences* **69**(1): DOI 10.1007/s00027-007-0845-x.
- Scheurer, K., 2004. *Zwischenbericht zu den Untersuchungen 2001–2002 in den vier Testgebieten Emme, Liechtensteiner Binnenkanal, Necker, Venoge*. Fischnetz-Publikation, Teilprojekt 00/16. Eawag, Dübendorf.

- Schmeing-Engberding, F., 1953. Die Vorzugstemperaturen einiger Knochenfische und ihre physiologische Bedeutung. *Zeitschrift für Fischerei und deren Hilfswissenschaften* **2**: 125–155.
- Schmidt-Posthaus, H., D. Bernet, T. Wahli, W. Meier and P. Burkhardt-Holm, 2001. Morphological organ alterations and infectious diseases in brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) exposed to polluted river water. *Diseases of Aquatic Organisms* **44**: 161–170.
- Schneeberger, U., 1995. Abklärungen zum Gesundheitszustand von Regenbogenforelle (*Oncorhynchus mykiss*), Bachforelle (*Salmo trutta fario*) und Groppe (*Cottus gobio*) im Liechtensteiner-, Werdenberger- und Rheintaler-Binnenkanal. Dissertation, Universität Bern, Bern.
- Schubiger, C. B., 2003. Proliferative Nierenerkrankung (PKD): Diagnostische Nachweismethoden und Untersuchung krankheitsfördernder Faktoren. Inaugural-Dissertation, Universität Bern, Bern.
- Schwaiger, J., U. Mallow, H. Ferling, S. Knoerr, T. Braunbeck, W. Kalbfus and R. D. Negele, 2002. How estrogenic is nonylphenol? A transgenerational study using rainbow trout (*Oncorhynchus mykiss*) as a test organism. *Aquatic Toxicology* **59**: 177–189.
- Schälchli, Abegg and Hunzinger, 2002. Innere Kolmation – Methoden zur Erkennung und Bewertung. Eawag, Dübendorf.
- Scott, D. and J. R. Irvine, 2000. Competitive exclusion of brown trout *Salmo trutta* L., by rainbow trout *Oncorhynchus mykiss* Walbaum, in lake tributaries, New Zealand. *Fisheries Management and Ecology* **7**: 225–237.
- Silva, E., N. Rajapakse and A. Kortenkamp, 2002. Something from “nothing” – eight weak estrogenic chemicals combined at concentrations below NOECs produce significant mixture effects. *Environmental Science and Technology* **36**: 1751–1756.
- Strehler, A. and K. Scheurer, 2003. Synthese Ökotox. Abschätzung der Konzentration östrogenen Stoffe in Fließgewässern. Fischnetz-Publikation, Eawag, Dübendorf.
- Sumpter, J. P. and S. Jobling, 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental Health Perspectives* **103 Suppl 7**: 173–178.
- Suter, G. and S. Bartell, 1993. Ecosystem-Level effects. In: G. W. Suter (ed.), *Ecological Risk Assessment*, Lewis Publishers, Boca Raton, pp. 275–308.
- Suter, I. G. W., S. B. Norton and S. M. Cormier, 2002. A methodology for inferring the causes of observed impairments in aquatic ecosystems. *Environmental Toxicology and Chemistry* **21**: 1101–1111.
- Thorpe, K. L., R. I. Cummings, T. H. Hutchinson, M. Scholze, G. Brighty, J. P. Sumpter and C. R. Tyler, 2003. Relative potencies and combination effects of steroidal estrogens in fish. *Environmental Science & Technology* **37**: 1142–1149.
- Turnpenny, A. W. H. and R. Williams, 1980. Effects of sedimentation on the gravels of an industrial river system. *Journal of Fish Biology* **17**: 681–693.
- Vethaak, A. D., J. Lahr, R. V. Kuiper, G. C. M. Grinwis, T. R. Rankouhi, J. P. Giesy and A. Gerritsen, 2002. Estrogenic effects in fish in the Netherlands: some preliminary results. *Toxicology* **181-182**: 147–150.
- Vuille, T., 1997. Ertragsvermögen der Patentgewässer im Kanton Bern. Fischereiinspektorat des Kantons Bern, Bern.
- Wahli, T., D. Bernet, P. Steiner and H. Schmidt-Posthaus, 2007. Geographical distribution of *Tetracapsuloides bryosalmonae* infected fish in Swiss rivers: an update. *Aquatic Sciences* **69(1)**: DOI 10.1007/s00027-006-0843-4.
- Wahli, T., R. Knuesel, D. Bernet, H. Segner, D. Pugovkin, P. Burkhardt-Holm, M. Escher and H. Schmidt-Posthaus, 2002. Proliferative kidney disease in Switzerland: current state of knowledge. *Journal of Fish Diseases* **25**: 491–500.
- Whyte, J. J., R. E. Jung, C. J. Schmitt and D. E. Tillitt, 2000. Ethoxyresorufin-O-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Critical Reviews in Toxicology* **30**: 347–570.
- Zalewski, M., B. Bis, M. Lapinska, P. Frankiewicz and W. Puchalski, 1998. The importance of the riparian ecotone and river hydraulics for sustainable basinscale restoration scenarios. *Aquatic Conservation – Marine and Freshwater Ecosystems* **8**: 287–307.
- Zimmerli, S., D. Bernet, P. Burkhardt-Holm, H. Schmidt-Posthaus, P. Vonlanthen, T. Wahli and H. Segner, 2007. Assessment of fish health status in four Swiss rivers showing a decline of brown trout catches. *Aquatic Sciences* **69(1)**: DOI 10.1007/s00027-006-0844-3.
- Zobrist, J. and P. Reichert, 2006. Bayesian estimation of export coefficients from diffuse and point sources in Swiss watersheds. *Journal of Hydrology* **329**: 207–223.

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