EFFECTS OF ACID STRESS ON THE DISTRIBUTION AND REPRODUCTIVE SUCCESS OF FRESHWATER FISH IN DUTCH SOFT WATERS

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ABSTRACT

The distribution and reproductive success of fish were examined in Dutch soft waters of varying pH. In total, 20 fish species were encountered. The lowest pH-values at which fish species were observed varied remarkably. *Umbra pygmaea* and *Tinca tinca* appeared to be extremely acid tolerant and occurred in waters with pH-values below 4.0. *Abramis brama, Cyprinus carpio, Esox lucius, Misgurnus fossilis, Perca fluviatilis* and *Scardinius erythrophthalmus* tolerated moderately acidified water with pH-values between 4.0 and 5.0. The remaining species were only recorded in waters with pH-values above 5.0. Only 17 species showed successful reproduction in Dutch softwater ecosystems. For *Abramis brama, Blicca bjoerkna, Cyprinus carpio, Esox lucius* and *Scardinius erythrophthalmus* especially the reproductive phase seemed to be very sensitive to low pH. Waters with non-lethal pH-values showed ageing populations of these fishes. The reproductive failure of these species at sublethal pH may be due to impaired gonadal maturation, spawning, fertilization, embryonic development and hatching. In extremely acid waters (pH<4.0) only *Umbra pygmaea* and *Tinca tinca* exhibited successful reproduction. The reproductive cycle of *Umbra pygmaea* was studied more in detail. Normal gonadal maturation of *Umbra pygmaea* and fertilization, development and hatching of its eggs were observed among a wide pH-range ($3.5 \leq pH < 8.0$).

INTRODUCTION

Some coastal dunes and the pleistocene sandy soils in the southern and eastern part of The Netherlands are highly weathered and exhibit low acid neutralizing capacities. In these areas thousands of softwater ecosystems are situated. During the last decades many of them have been adversely affected by acidification (van Dam *et al.*, 1981; Roelofs, 1983; Leuven *et al.*, 1986; Schuurkes, 1986). Particularly the lack of buffering capacity makes them very vulnerable to acid shock events and/or continuous loadings with acidifying substances. Recently it has been pointed out that at least 59% of the Dutch soft waters have been acidified to some extent (Leuven *et al.*, 1986). Acidification of these systems has been largely attributed to acidifying precipitation resulting from anthropogenous emissions of SO_2, NO_x and NH_3 (Schuurkes, 1986; Schuurkes *et al.*, 1986).

In Scandinavia and eastern North-America acidification of rivers and lakes cause impoverishment of fish communities and a low productivity or even the loss of entire fish assemblages (Drabløs and Tollan, 1980, Overrein *et al.*, 1980; Johnson, 1982). Although evidence on the ecological impact of acidifying precipitation in Dutch surface waters is accumulating, the fishery aspects remained poorly understood (Leuven and Oyen, 1987). Almost all of the previous studies concerning the effects of acidification on fish have been conducted in systems which show great geomorphological differences with the acid-susceptible systems in The Netherlands. In the present paper the relation between the pH of soft inland waters and the distribution of freshwater fishes is described. Furthermore, the available data concerning the effects of acid stress on the reproductive success of fish are summarized.

MATERIAL AND METHODS

During 1983-1984 the distribution pattern and reproductive success of freshwater fish were examined in 91 lentic soft waters (alkalinity < 2 $meq.l^{-1}$) in The Netherlands. Most sampling sites (moorland pools and small lakes) are situated in the southern and eastern part of the country. A few (dune) pools are located on the island of Terschelling. The afore mentioned areas are highly sensitive to acidifying deposition (Leuven *et al.*, 1986).

Different types of fishing gear (i.e. two fykes, two trammel nets and a seine net) were used in order to obtain a reasonably complete list of fish species. Additionally, each body of water was sampled with dip nets during the (post) spawning period of several fish species. The presence of eggs, larvae and fry (indicating reproductive success) were investigated. The applied sampling procedure was appropriate for small as well as large fishes (Leuven and Oyen, 1987).

From each locality water samples were taken. Chemical analyses of nutrients, major ions and toxic metals (i.e. aluminium and cadmium) were carried out according to Dederen *et al.* (1986). The pH was measured with a Metrohm model E488 pH-meter and a model EA 152 combined electrode. Alkalinity was estimated by titration of 100 ml water with 0.01 M HCl down to pH 4.2. Detailed geomorphological and physicochemical data of the sampling sites are given by Oyen (1984) and Leuven and Oyen (1987).

The reproductive cycle of the acid tolerant mudminnow *Umbra pygmaea* was studied more in detail. From March 1983 until May 1985 regularly samples of approximately 5-40 individuals of *Umbra pygmaea* were collected in The Groot Huisven. This acidified moorland pool (pH=4.5) is situated East of Oisterwijk (51°35'N, 5°16'E) and has a surface area of 5 ha and a maximum depth of 1.5 m. The physico-chemical properties of the Groot-Huisven are given by Dederen *et al.* (1986). All fishes captured were preserved in 70% ethanol. The Gonado Somatic Index (GSI) of a fish was calculated as:

$$GSI = (\text{gonad weight}).(\text{body weight} - \text{gonad weight})^{-1} \times 100\%.$$

From July 1984 to May 1985 the testes or ovary of specimens of *Umbra pygmaea* from six moorland pools were examined histologically following autopsy of approximately 10 males and 10 females from each set of samples. The pH-values of these pools varied from 3.65 to 4.98. Central parts of the testis and ovary were fixed in calcium-formaldehyde and embedded in paraffin wax. Sections of 10 µm thick were stained with haemalum-eosin. The lamellae of the ovary contain oogonia and oocytes in follicles at various stages of development. Previtellogenic, vitellogenic, post-vitellogenic and atretic follicles were distinguished according to Richter *et al.* (1982). The relative numbers of follicles in these stages were calculated. The developmental stage of the testis could be classified with an arbitrary choosen Spermatozoa Index (SI) which ranged from 0 to 5. A SI value of 0 was used for fishes with undeveloped, spent or regressed testes. SI 2.5 was applied for developing testes with cysts mainly containing spermatogonia, spermatocytes and spermatids, and few spermatozoa. For ripe testes with cysts completely filled with spermatozoa the maximum SI-value of 5 was used.

RESULTS

The available information concerning the presence of fish in the sampling sites is summarized in Table 1. With respect to the pH of the water the sampling localities are divided into three major pH-classes. In total, 20 fish species have been recorded in Dutch soft waters. Most of them show low frequencies of occurrence in the sampled bodies of water. Nevertheless, in slightly acid and alkaline soft waters the presence percentages of *Esox* *lucius, Cyprinus carpio, Perca fluviatilis, Rutilus rutilus, Scardinius erythrophthalmus* and *Tinca tinca* are rather high (>25%). Only 7 species have been encountered incidentally in moderately acidified waters. *Tinca tinca* has been recorded once in a extremely acid water. *Umbra pygmaea* is even very common in waters with pH-values below 4.0.

Table 1. Frequencies of occurrence (%) of several fish species caught in Dutch soft waters among three different pH-classes.

Species	Non-acidified pH≥5.0 (n = 35)	Moderately acidified 4.0≤pH<5.0 (n = 34)	Extremely acidified pH<4.0 (n = 22)
Ctenopharyngodon idella (Val.)	5.7	NR	NR
Carassius carassius (L.)	2.8	NR	NR
Cobitis taenia L.	8.6	NR	NR
Lepomis gibbosus (L.)	2.8	NR	NR
Blicca bjoerkna (L.)	14.3	NR	NR
Gasterosteus aculeatus L.	8.6	NR	NR
Ictalurus nebulosus (Le Sueur)	5.7	NR	NR
Anguilla anguilla (L.)	14.3	NR	NR
Rutilus rutilus (L.)	40.0	NR	NR
Pungitius pungitius (L.)	5.7	NR	NR
Alburnus alburnus (L.)	8.6	NR	NR
Carassius auratus (L.)	17.1	NR	NR
Misgurnus fossilis (L.)	2.8	2.9	NR
Cyprinus carpio L.	25.7	2.9	NR
Perca fluviatilis L.	42.9	2.9	NR
Abramis brama (L.)	22.8	2.9	NR
Scardinius erythrophthalmus (L.)	34.2	2.9	NR
Esox lucius L.	42.9	11.6	NR
Tinca tinca (L.)	37.1	2.9	4.5
Umbra pygmaea (De Kay)	17.1	8.7	50.0

(n: number of sampling sites; NR: not recorded during this study).

The lowest pH-values at which fish species have been recorded show remarkable interspecific differences and vary between 3.5 and 9.5 (Fig. 1). For *Blicca bjoerkna, Cyprinus carpio, Esox lucius, Rutilus rutilus* and *Scardinius erythrophthalmus* the lowest pH of occurrence differs from the minimum pH at which these species can successfully reproduce. In waters with sublethal pH-values, under-yearlings of these fishes have not been noticed, whereas only specimens from older year classes have been recorded.

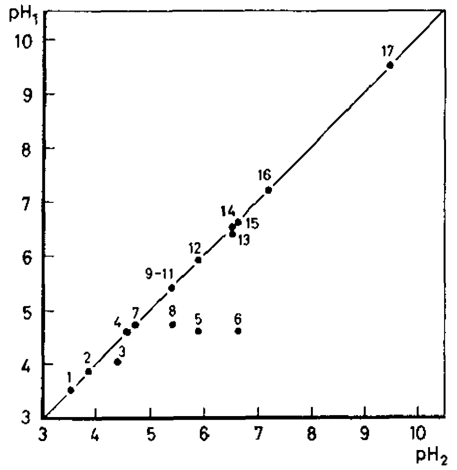

Fig. 1. Lowest pH-values for occurrence (pH_1) and successful reproduction (pH_2) of 17 fish species in Dutch soft waters. 1. *Umbra pygmaea* 2. *Tinca tinca* 3. *Esox lucius* 4. *Perca fluviatilis* 5. *Scardinius erythrophthalmus* 6. *Abramis brama* 7. *Misgurnus fossilis* 8. *Cyprinus carpio* 9. *Carassius auratus* 10. *Alburnus alburnus* 11. *Ictalurus nebulosus* 12. *Rutilus rutilus* 13. *Blicca bjoerkna* 14. *Lepomis gibbosus* 15. *Cobitis taenia* 16. *Gasterosteus aculeatus* 17. *Carassius carassius*.

Fig. 2 illustrates the length-frequency distributions of *Perca fluviatilis* and *Scardinius erythrophthalmus* in three moorland pools. In the two slightly acid pools (Kanaalven and Groot Schaapsloopven) the length distributions of *Scardinius erythrophthalmus* were rather normal and indicated good recruitment. The *Scardinius erythrophthalmus* populations are generally dominated by young fishes. In the acidified water (Groot Aderven) only large specimens were present. The same phenomenon has also been observed for *Perca fluviatilis*. However, for this species spawning and partly hatching of eggs have been recorded in the Groot Aderven. At low pH the length distribution of *Perca fluviatilis* shows lack of young fishes, indicating mortality of fry.

The Gonado Somatic Index of *Umbra pygmaea* collected from an acidified moorland pool is presented in Fig. 3. The data have been obtained during two breeding seasons. Gonadal development starts in Autumn and the highest average GSI is reached during Spring. The maximum GSI-values are 10.5% and 25.4% for males and females, respectively. Spawning of *Umbra pygmaea* always occurs from April to May. Gonadal maturation was observed in all fishes older than 1 year and under-yearlings with a length exceeding 4.7 cm.

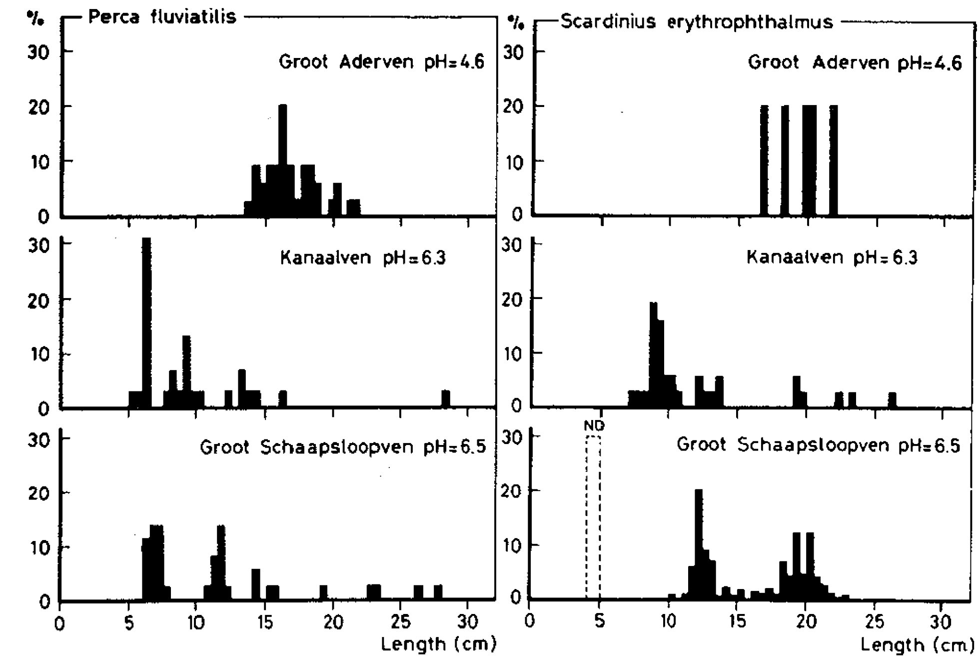

Fig. 2. The length distribution of *Scardinius erythrophthalmus* and *Perca fluviatilis* in three moorland pools differing in pH (ND: not determined but most abundant length class).

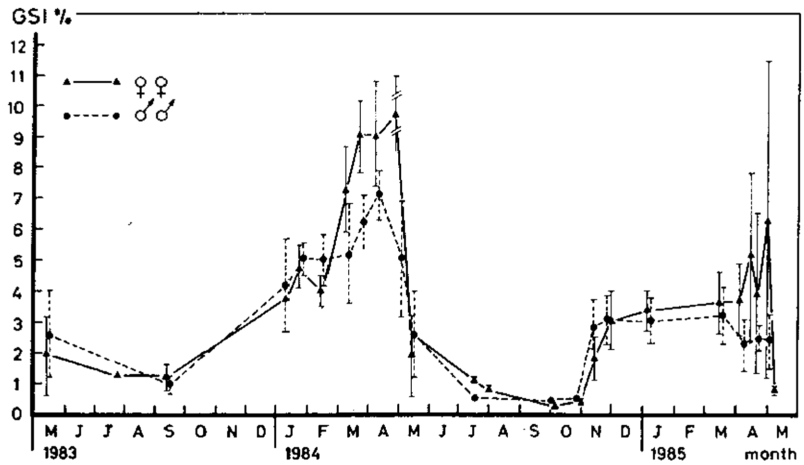

Fig. 3. The average Gonado Somatic Index (GSI), with standard deviations, of *Umbra pygmaea* in the Groot Huisven during the period 1983-1985. The highest GSI for females (May 1984) has a standard deviation of 10.5%.

The results of histological examinations of the ovary and testis of *Umbra pygmaea* are presented in Fig. 4 and 5. The vitellogenesis of the oocytes started in August (Fig. 4a). From the beginning of January follicles with post-vitellogenic oocytes were present. At the end of April the females contained ovary with predominantly post-vitellogenic oocytes and the males testes with ripe spermatozoa (Fig. 4a,b). During normal ovarian development, phagocytic resorption of oocytes or atresia is frequently observed. Atretic follicles are most abundant at the beginning of vitellogenesis, whereas, lowest values have been recorded during the pre-spawning period. From August until April atresia of oocytes is rather constant. Atresia is generally restricted to the later stages of oocyte development. The data suggest a continuous slow "turnover" from vitellogenic and post-vitellogenic oocytes to atretic follicles.

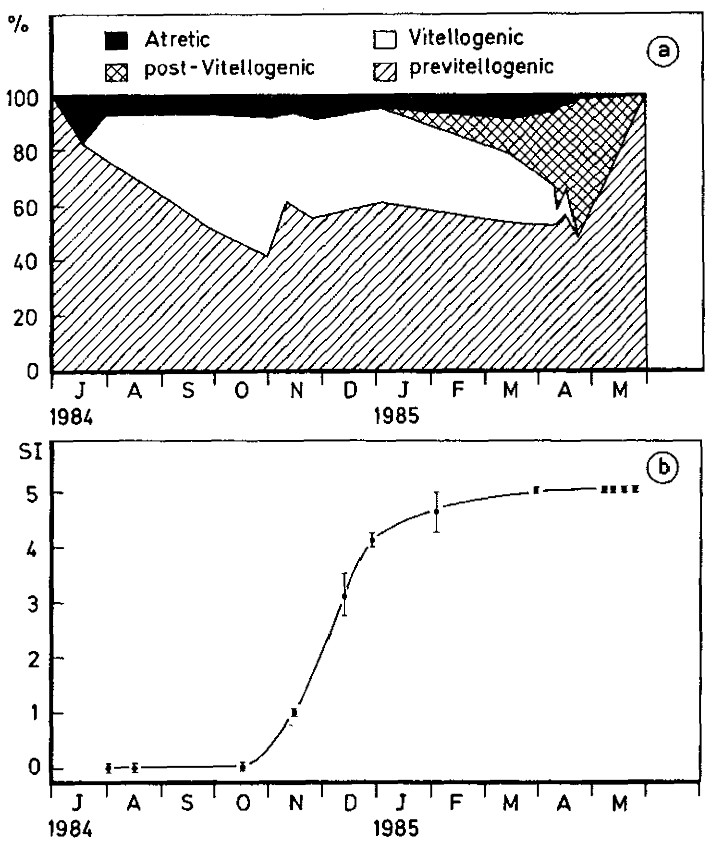

Fig. 4. Gonadal development of *Umbra pygmaea* in the Groot Huisven during 1984-1985. a) Development of ovary indicated by relative number of oocytes at various developmental stages. b) Development of testes indicated by Spermatozoa Index (SI).

The reproduction of *Umbra pygmaea* seems not to be affected by low pH (Fig. 5). Although the percentages of atretic follicles in the ovary of females from six populations are significantly different (Kruskal-Wallis test $p<0.05$) there is no significant correlation with the acidity of the water (Trend test of Terpstra $p>0.05$). The eggs and fry of *Umbra pygmaea* have been observed in waters with a pH$\geq$3.5.

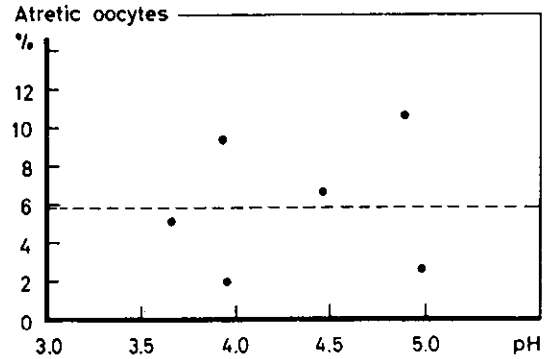

Fig. 5. The percentage of follicles with atretic oocytes in the ovary of *Umbra pygmaea* from six moorland pools varying in pH.

DISCUSSION

Dutch soft waters differ remarkably in the composition of their fish assemblages. This phenomenon can be largely attributed to the deleterious effects of acidifying precipitation. Most soft waters have been acidified to some extent (Leuven *et al.*, 1986). Comparisons of recent fishery data with historical ones reveal that in many acidified sampling localities fish species or even entire fish assemblages have disappeared (Leuven *et al.*, 1986; Leuven and Oyen, 1987). In Belgian bog pools the fish populations have also diminished drastically from about 1940 onwards (Vangenechten *et al*, 1984). These pools are more or less similar to the Dutch moorland pools. The percentage of moderately and extremely acid waters which harbour well developed fish populations is small, and the number of species in such waters is strongly reduced in comparison with less acid soft water habitats. In The Netherlands only the East American mudminnow *Umbra pygmaea* is common and abundant in strongly acidified waters. This species has been introduced in western Europe at the beginning of this century and in The Netherlands its distribution has been rapidly extended during recent decades (Dederen *et al.*, 1986).

In Scandinavia and eastern North America it has already been established during several field surveys that the distribution and abundance of fish species may be limited by the acidity of surface waters (EIFAC, 1969; Wright and Snekvik, 1978; Fromm, 1980; Rahel and Magnuson, 1983; Kelso and

Gunn, 1984; Wiener *et al.*, 1984; Haines *et al.*, 1986; Rahel, 1986). Osmoregulatory stress, impaired gas exchange, reproductive failure, and changes in the predator-prey relations are considered to be the main reasons for the mortality of fish in acidified environments (EIFAC, 1969; Fromm, 1980; Overrein *et al.*, 1980; Howells *et al.*, 1983; McDonald, 1985).

Apart from the differences in pH, the sampling localities remarkably vary in many other physico-chemical properties and morphometric factors (Oyen, 1984). In Dutch soft waters the species richness and the composition of fish assemblages are also related to the alkalinity, conductivity, trophic level, and the concentrations of major ions and some toxic metals, e.g. aluminium and cadmium (Leuven and Oyen, 1987). The results of several other field surveys and experimental studies show that the above mentioned parameters are indeed important factors structuring fish assemblages in low alkaline waters (Wright and Snekvik, 1978; Howells *et al.*, 1983; Kelso and Gunn, 1984; Morris and Krueger, 1984; Haines *et al.*, 1986). Morphometric factors and the lack of water inlets or outlets appear to be of minor importance for the species richness of soft waters in The Netherlands (Leuven and Oyen, 1987).

The lowest pH of occurrence of fish species showed large interspecific differences (Fig. 1). Wiener (1983) and Rahel and Magnuson (1984) obtained similar results for species occurring in lakes in northern Wisconsin (USA). For *Carassius auratus, Cyprinus carpio, Misgurnus fossilis, Scardinius erythrophthalmus, Tinca tinca* and *Umbra pygmaea* the present pH-limits are the lowest values ever recorded under field conditions. The lowest pH limit for the originally North-American species *Lepomis gibbosus* (pH=4.5) has been observed its their natural distribution area (Beamish, 1974). In some Scandinavian lakes *Abramis brama, Esox lucius, Perca fluviatilis* and *Rutilus rutilus* have been noticed at pH-values between 3.5-3.8 (EIFAC, 1969). However, these sites are very heterogeneous with respect to the hydrogen ion concentrations. Probably, these systems provide refuge areas with higher pH-values.

Our data show that the adults of several species tolerate pH-levels at which reproduction is not successful anymore. The reproduction process may be negatively affected by reduced spawning (Mount, 1973; Beamish, 1974; Ruby *et al.*, 1977) decreased functioning of gametes (EIFAC, 1969), failure of fertilized eggs to develop (Mount, 1973; Drabløs and Tollan, 1980 Overrein *et al.*, 1980) or to hatch (Peterson *et al.*, 1980), and hatching of deformed fry (Runn *et al.*, 1977). In waters with non-lethal pH-values the populations of several species show recruitment failure. Ageing of fish populations in recently acidified bodies of water has been reported by several other investigators (Rosseland *et al.*, 1980; Kelso and Gunn, 1984; Mills, 1984). Therefore minimum pH-values for fish species recorded from recently acidified waters are generally lower than those found for naturally acid systems. The occurrence of reproductive impairment at sublethal pH-levels illustrates the importance of analysis the whole life cycle for establishing water quality criteria for freshwater fish.

Umbra pygmaea appears to be extremely acid tolerant. This species shows several morphological and physiological adaptations to acid stress (Dederen *et al.*, 1986). Histological examinations reveal that in *Umbra pygmaea* normal development of ovary and testes occur among a wide pH-range. In *Umbra pygmaea* oocytic atresia is not related to the pH of the habitats. In acid-sensitive fish species acid stress increases the rate of oocyte atresia. At pH 6.0 and lower female *Jordanella floridae* displayed large numbers of early oocytes, which was accompanied by an inability to produce post-vitellogenic oocytes (Ruby *et al.*, 1977). Also for *Ambloplites rupestris* and *Lepomis gibbosus* from a lake with pH 4.5, resorption of eggs has been described (Beamish, 1974). In *Umbra pygmaea* we have observed normal gonadal maturation, spawning fertilization, egg development and hatching at pH 3.5.

The ability of *Umbra pygmaea* to survive and reproduce successfully in extremely acid water is quite exceptional, and has probably been developed in the course of a long period of evolution. Cultural acidification of softwater ecosystems is currently increasing too fast to allow such adaptations to be expected on the part of other species (Wendelaar Bonga and Dederen, 1986). Continuing acidification will thus almost certainly lead to a further decline in fish stocks.

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