Journal of Fish Biology (1993) **42**, 411–419

Glochidia of the freshwater mussel, *Anodonta anatina*, affecting the anadromous European smelt (*Osmerus eperlanus*) from the Eider estuary, Germany

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(*Received 15 January 1991, Accepted 21 July 1992*)

During a 2-year survey on external disease conditions of fish between January 1988 and December 1989, 0·2% of 40 011 smelt, *Osmerus eperlanus*, from 30 stations in the German Wadden Sea and estuaries of tributary rivers, were infected with glochidia of the freshwater mussel *Anodonta anatina*. Seasonality was marked, with glochidia only being observed in March and April 1989, when prevalences were 37% and 15% respectively, in fish 10–20 cm long at the most heavily infected site. A marked increase in prevalence in fish 15 cm in length and longer, suggested that only temporarily resident spawning fish (as opposed to resident juveniles) were infected. Infection was almost exclusively restricted to the Eider estuary, where prevalence decreased with increasing salinity. It is concluded that the glochidia carried by smelt returning from their freshwater spawning sites in the Eider River to the sea would have died, thus representing a loss to the 1989 cohort of *A. anatina* from the Eider. The potential importance of the smelt–*A. anatina* relationship as indicator for the detection of (a) spawning mussel populations and of (b) possible ecological effects of climate changes is noted.

Key words: *Anodonta anatina*; glochidia; European smelt; Eider estuary; ecological effects of climate changes.

I. INTRODUCTION

Anodonta anatina (L.) belongs to the freshwater mussel family Unionidae. Larval stages (glochidia) of the freshwater mussels are known as temporary ectoparasites of fish. After being released from the mother mussel, glochidia attach preferably to fins and gills of suitable host fish. After successful attachment, encystment and metamorphosis of glochidia takes place until young mussels detach and settle on the sediment for further growth and development. Depending on water temperature, glochidia parasitize their host specimens for 2–8 weeks.

It has been shown experimentally that unionid glochidia become attached to many species of fish and even to tadpoles and axolotls (Cheng, 1973). Development and metamorphosis of the glochidia were not completed unless they became attached to a specific species of fish, thus demonstrating host specificity. If the glochidia became attached to an incompatible host, they were sloughed off before metamorphosis was completed.

Members of the family Unionidae are distributed worldwide whereas the occurrence of the genus *Anodonta* is restricted to the northern hemisphere. In Germany, two species of the genus *Anodonta* and one species of the morphologically similar genus *Pseudanodonta* occur. *Anodonta cygnea* (L.) is the largest European

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0022–1112/93/030411+09 $08.00/0

freshwater mussel. It inhabits waters with low current. *Anodonta anatina* (L.) settles in waters with strong current as well as within surf zones of big lakes. In northern Germany, *A. anatina* is a widely distributed species (Wiese, 1991). This species is much more prevalent than *A. cygnea* and is common in almost all larger inshore waters including the freshwater parts of tributary rivers of the North Sea. Little is known about the distribution of *A. anatina* within the lower reaches of these rivers. Due to its longevity, its maturation period of several years, the obligatory parasitic stage, as well as its sensitivity to anthropogenic water pollution, members of the Unionidae are endangered worldwide. Some species are already extinct (Fechter & Falkner, 1990). In suitable locations, *A. anatina* reaches an age of more than 15 years. Populations of this still-common species in northern Germany are decreasing in number and therefore have to be considered endangered (Weise, 1990).

Little is known about the natural host range of *A. anatina* glochidia. So far, only pure freshwater fish species are known as hosts. In this report a mass infection with glochidia of *A. anatina* is described on anadromous European smelt (*Osmerus eperlanus* L.) on their spring spawning run in the Eider estuary.

The anadromous smelt occurs along the coasts of western and northern Europe. The freshwater regions of streams are used for annual spring spawning runs, and as nursery areas for juveniles (Möller, 1988). During spring, smelt is the most abundant species in many rivers. In the lower Elbe River, for example, it contributes up to 60% of the commercial anchor net catches (Möller, 1989). For spawning, smelt use a wide variety of substrates, though gravel and sand may be preferred (Nellbring, 1989).

II. MATERIAL AND METHODS

SAMPLING

As part of a general survey on fish diseases in the German Wadden Sea from January 1988 to December 1989, smelt were caught using chartered commercial shrimp trawlers (Anders & Möller, 1991). Three stations (1, 3, 5) were surveyed monthly in the estuary of the Elbe and four stations (11–14) in the Eider estuary. An additional 23 stations, distributed over the whole Wadden Sea, were surveyed at 3-month intervals (Fig. 1). Fishing hauls were accompanied by measurement of temperature and salinity of the surface water. All samples were examined by only two experienced persons to minimize identification mistakes. Altogether 40 011 smelt within a range of 10–27 cm total body length were examined on board ship.

GROSS EXAMINATION

Immediately after capture, all fish were examined macroscopically for the presence of externally visible disease signs and parasites. Presence or absence of glochidial infection on the fins was noted. Gills were not examined. Severity of infection was not quantified due to time restraints, imposed by the other aspects of the survey. Total body length of fish was determined to the centimeter value below the measured one. As it was known from previous surveys that smelt shorter than 10 cm in length did not show externally visible signs of disease or parasites, and owing to low numbers of fish longer than 20 cm, only specimens measuring 10–20 cm were considered for epizootiological calculations.

MICROSCOPICAL EXAMINATION

For identification of the condition, a few smelt specimens with clear signs of infection were taken after each cruise and frozen. For exact identification of the glochidia species, an additional sample of smelt showing the same gross signs of glochidial infection as those

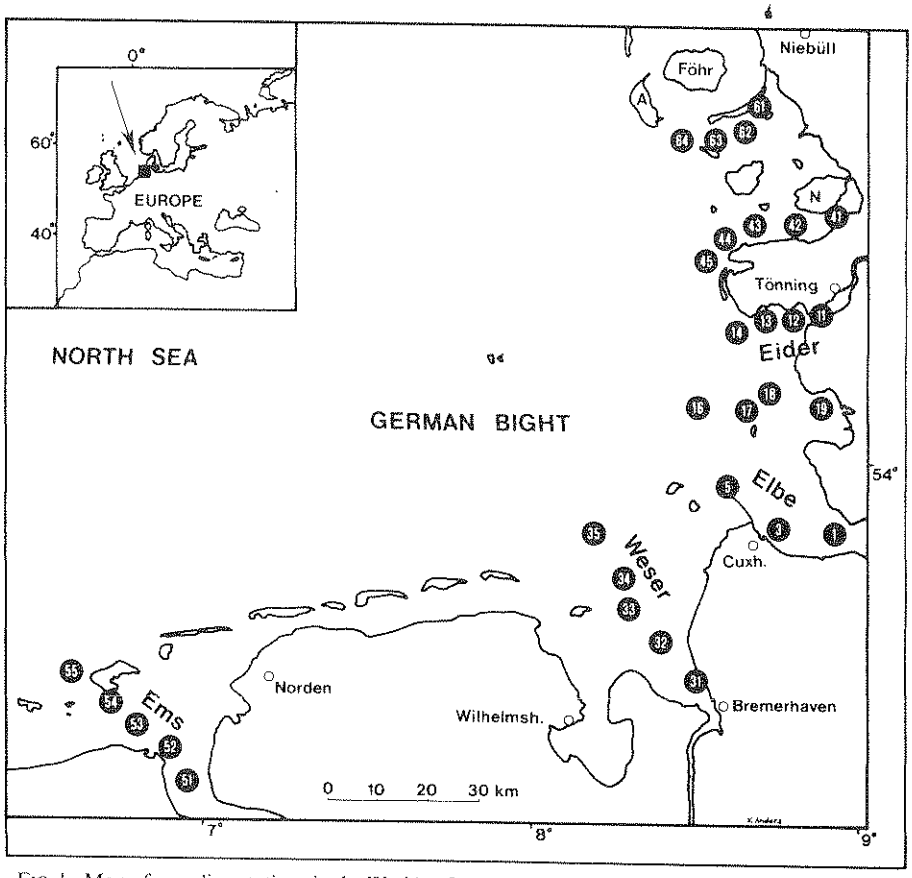


FIG. 1. Map of sampling stations in the Wadden Sea (the tidal flats off the German North Sea coast).

observed in 1989 was taken in the Eider estuary on station 11 in April 1990 and fixed immediately after capturing in 4% formaldehyde. Later in the laboratory, 10 smelt from 14–18 cm total body length infected with glochidia on the fins were examined for presence or absence of the parasite on the gills. Length and width of a total of 50 glochidia from the fins of the 10 host specimens were determined using a stereomicroscope at 50 × magnification.

HISTOPATHOLOGY

For light microscopy, fish tissue samples from infected fins were fixed immediately after capturing in chilled Bouin's solution. In the laboratory, samples were dehydrated in a graded ethanol series, sectioned at 5 μm, and routinely stained with Mayer's hematoxylin and erythrosin.

III. RESULTS

The highest numbers of smelt were caught during its spawning runs in spring (Anders & Möller, 1991). Smelt affected with the parasitic condition described here were found for the first time in March 1989 (Anders & Möller, 1992).

GROSS SIGNS OF INFECTION

Affected fins were coloured conspicuously orange–brown at their margins due to massive occurrence of glochidia. Single parasites were hardly visible to the naked

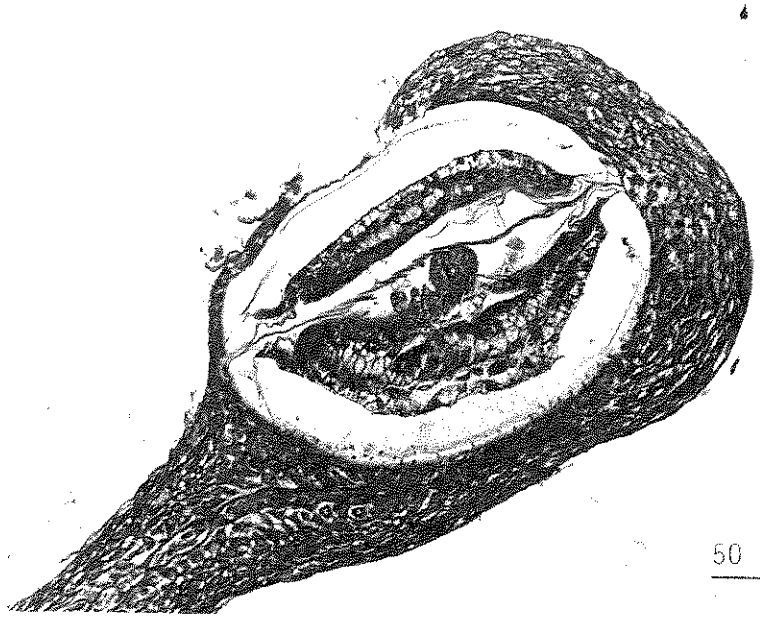


FIG. 2. Glochidium of *A. anatina* embedded in epidermal tissue of a smelt fin; hematoxylin–erythrosin stain.

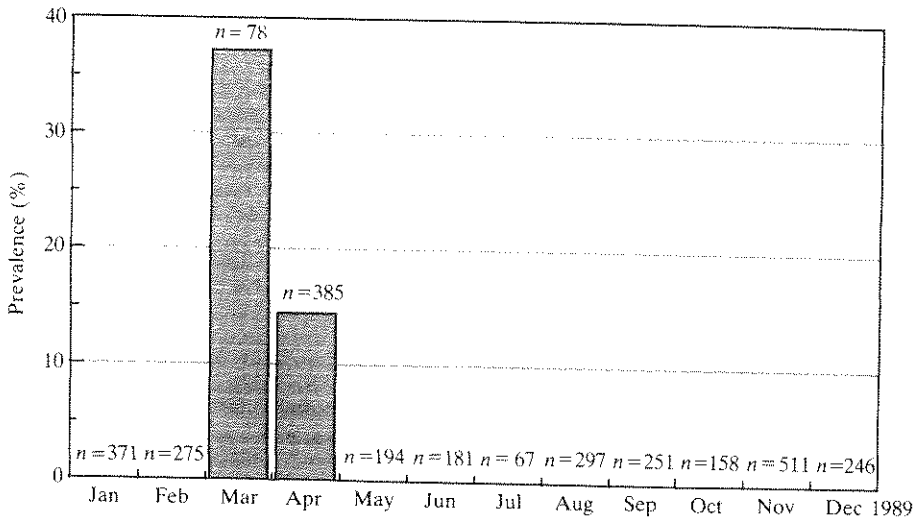


FIG. 3. Seasonal variation in prevalence of glochidia of *A. anatina* on the fins of smelt from the Eider estuary (station 11) during 1989. Number of fish (10–20 cm) examined is given above each column.

eye. Fish affected at the fins frequently harboured glochidia on the gills as well. Whereas most 'fin glochidia' were found to be covered by a thin layer of fin epidermis (Fig. 2), 'gill glochidia' frequently were deeply embedded in the soft tissue of the gill lamellae.

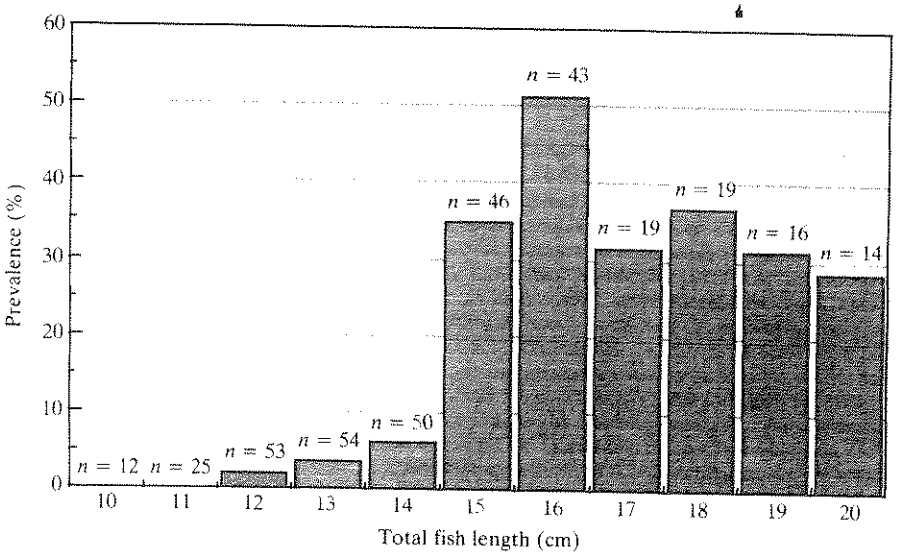


FIG. 4. Relationship between total fish length and prevalence of *A. anatina* glochidia on the fins of smelt from the Eider estuary (stations 11–12) in March 1989. Number of fish examined is given above each column.

TABLE I. Regional variation in prevalence of glochidia of *A. anatina* on the fins of smelt (10–20 cm) in the German Wadden Sea in March 1989

Region	Station number	Number examined	Prevalence (%)
Süderaue	61–64	115	0
Heverstrom	41–45	585	0·5
Eider estuary	11–14	481	15·0
Süderpiep	16–19	477	0
Elbe estuary	1, 3, 5	279	0
Weser estuary	31–35	346	0
Ems estuary	51–55	148	0

CHARACTERIZATION OF GLOCHIDIA

Glochidia were of triangular shape and of typical orange–brown colour. A larval thread could be observed frequently, especially on glochidia attached to gill tissue. Of a total of 50 glochidia from 10 different host specimens which were caught in 1990, mean length and width of shells were determined as 353 ± 12 and 356 ± 11 µm respectively. Although no glochidia of smelt caught in 1989 were measured, it is concluded from the identical morphological appearance of glochidia from both years, that all glochidia described here are to be assigned to the unionid mussel species *Anodonta anatina* (L.).

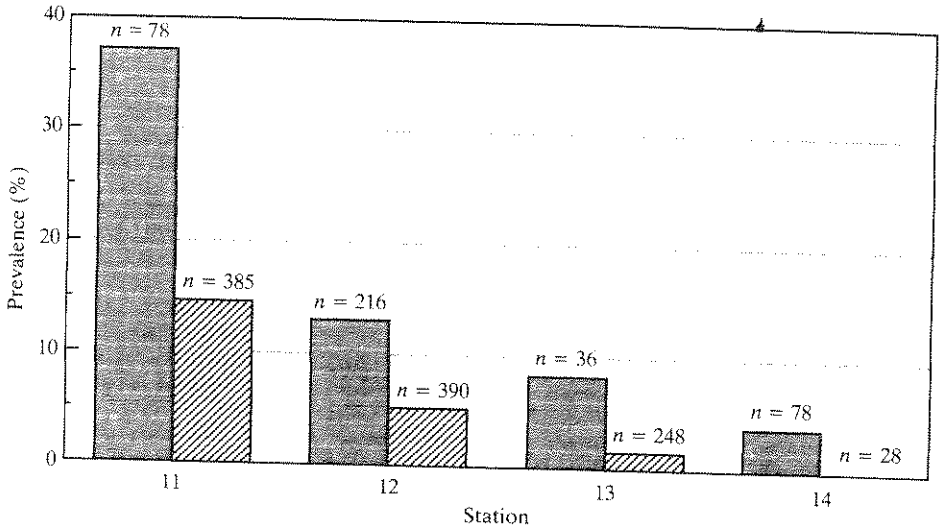


FIG. 5. Glochidia of *A. anatina* on the fins of smelt (10–20 cm) from the Eider estuary in spring 1989; prevalence at four sampling stations. Number of smelt examined are shown above the columns. ■ = March 1989, ▨ = April 1989.

TABLE II. Regional variation in salinity (‰) of surface water layers in the Eider estuary on four sampling stations in March and April 1989

Station	11	12	13	14
March	12·8	18·2	21·0	24·2
April	7·7	16·2	20·1	24·0

EPIZOOTIOLOGY

Annual variation

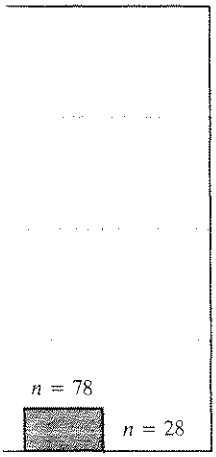
In 1988, smelt with typical signs of infection were not seen in any of the samples.

Seasonality

In 1989, a clear seasonality of infection was observed in smelt from the Eider estuary (Fig. 3). Infection was restricted to the months of March and April when prevalences reached maximum values of 37·2% and 14·5% respectively. No infection was observed during the other months.

Relationship with length of host

The smallest smelt infected measured 12 cm and the longest infected 24 cm total body length. There was a clear relationship between glochidia prevalence and total body length of infected fish (Fig. 4). A sharp increase of prevalence occurred in fish of 15 cm length and longer.



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Geographical distribution

On comparison with catches from the whole German Wadden Sea area, only smelt from the Eider estuary (stations 10–14) and from station 41 in the Heverstrom (Table I) as well as four 22–24 cm long specimens from station 61 in the Süderaue (data not presented) showed typical signs of infection. Highest mean prevalences of 15·4% and 7·6% in March and April 1989 respectively, were found in fish from the Eider estuary stations 11–14.

Relationship with salinity gradient

During March and April 1989, prevalences were highest on the innermost station 11 of the Eider estuary and decreased towards the open sea while salinities increased (Fig. 5, Table II).

IV. DISCUSSION

The condition of European smelt described in this study had never been seen in smelt or in any other fish species although extensive field studies in estuaries tributary to the Wadden Sea, especially in the Elbe estuary, have been carried out in the past (Möller, 1981, 1984*a*,*b*, 1988; Anders & Möller, 1987; Anders, 1988, 1989). Even during the first year of this survey the condition was not observed.

In addition to the morphological characteristics of glochidia described above, there are other facts that support their classification as *Anodonta anatina*. (1) *A. anatina* is the most abundant mussel species in the Eider River. (2) Glochidia of the genera *Unio* and *Margaritifera* do not occur on fins but affect the gills of their host fish only (Bednarczuk, 1986; Bauer, 1987; Maass, 1987). (3) In contrast to *Unio* and *Margaritifera* species, *Anodonta* spp. are known as typical long-term and winter breeders (Hüby, 1988). Glochidia are released preferably in December/January and March/April. (4) The present glochidia could not belong to the genus *Pseudanodonta*, which has no larval thread, is grey–white in colour and smaller (340 μm width, 290 μm length) (Hüby, 1988).

The pronounced seasonality of infection we observed coincides well with the spawning period of European smelt in the Eider River which lies within the reproduction period of *A. anatina* in central Europe. The infection period found in smelt is considerably shorter than that reported for attachment of *A. anatina* glochidia to brown trout *Salmo trutta* L. (Campbell, 1974). It is assumed that it simply reflects the duration of stay of mature smelt for spawning in the pure freshwater regions of the Eider River (upstream of station 11), as adult smelt specimens are absent from these areas during the rest of the year.

Results on the relationship between glochidia prevalence and total body length of host fish, which revealed a sharp increase in prevalence in 15 cm long smelt and longer specimens (Fig. 4), are well in accordance with the fact that the majority of smelt specimens become mature by the end of the second year of their life, which corresponds to a total body length of 13–16 cm (Lillelund, 1961; Möller, 1984*a*). It is concluded that the uptake of glochidia is directly linked to the spawning behaviour of potential fish hosts and therefore restricted to mature specimens only. It is known that smelt prefer a sandy bottom for spawning which is the preferred sediment for spawning mussels as well. The temporal cohabitation represents a

perfect chance for spawning mussels, as soon as a fish casts a shadow over the mussel, the latter expels thousands of glochidia which become attached to fins and gills of the spawning fish. This mechanism for ensuring contact between glochidia and their fish hosts has been confirmed in the laboratory for other species (Cheng, 1973).

Although smelt were abundant in the whole area of investigation, and the innermost stations of Elbe and Eider estuaries (stations 1, 3 and 10, 11 respectively) showed similar hydrographical conditions (temperature and salinity) in March and April 1989, glochidia were found only on specimens from the Eider and nearby locations. It is argued here that the occurrence of large *A. anatina* broodstocks in lower reaches of north German rivers tributary to the North Sea are restricted to the Eider, which in contrast to the Elbe and Weser, is not affected by industrial pollution. Thus, smelt might be useful indicators for detection of 'healthy' populations of *A. anatina*.

The distinct decrease of prevalence from the inner towards the outer estuary is explained most easily by the fact that *A. anatina* glochidia do not tolerate salinities above 3‰ (Jaeckel, 1962). As the smelt migrates from its spawning place in the river to its feeding grounds along the open coast, the glochidia obviously die off. The fact that glochidia were found occasionally on marine stations (e.g. 41, 61) leads to the hypothesis that parasites deeply embedded in host tissue might have been able to survive but are doomed as soon as their parasitic life stage is completed.

In conclusion, the mass occurrence of *A. anatina* glochidia on smelt in the Eider estuary and nearby locations of the German Wadden Sea was remarkable, and has never been observed at other times in many years of monitoring. We suggest that the abnormally mild winter of 1988/89 may have contributed to a shortened period of coincidental spawning of smelt and mussels, due to faster spawning and therefore earlier return of smelt to the sea in 1989, before glochidia completed their parasitic development. Since *A. anatina* cannot tolerate salinities in excess of 3‰, the glochidia carried by the returning smelt would have died, thus representing a loss to the 1989 cohort of mussels.

Further studies are needed to show whether the situation described here was an exceptional case. More information is needed on the potential usefulness of the smelt—*A. anatina* relationship as an indicator for (a) detection of endangered mussel broodstocks in rivers tributary to the North Sea and (b) identification of possible effects of climate changes on the behavioural ecology of aquatic organisms.

We cordially thank Prof. Dr H. Möller (Kiel) and Dr S. Poynton (Baltimore) for helpful and stimulating suggestions and their critical reading of the manuscript. The names of Dipl. Biol. Ingrid Hilger and Mandy Kierspel are gratefully acknowledged to stand in for all busy hands which helped during the fishing cruises. Finally, we appreciated Dipl. Oecotroph. Sabine Holst's help with computer graphics. This study was part of an extensive field survey on fish diseases in the German Wadden Sea in 1988/89 which was financed by the Governments of Schleswig-Holstein and Niedersachsen (Germany) and the German Federal Environmental Agency.

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