Arch. Hydrobiol./Suppl. 76 (Monographische Beiträge)	4	413–423	Stuttgart, November 1987

The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.) III. Host relationships

By G. BAUER

With 4 figures and 3 tables in the text

Abstract

The host range of the Margaritiferidae seems to be restricted to salmonids only. In this paper the host relationships of the european freshwater pearl mussel (*Margaritifera margaritifera* L.) are analysed. Data on the suitability of the salmonid species occuring in pearl mussel rivers are presented and, in order to get some insight into the pearl mussel's population ecology, density dependent processes governing survival of glochidia are investigated. This study also intends to provide data for effective conservation measures for the pearl mussel.

Introduction

The mussel family Margaritiferidae has a remarkable distribution that spans the holarctic regions of North America and Eurasia (SMITH 1980). All species rely on fish hosts where their glochidia are temporary gill parasites. Data on the host range, however, are confusing.

According to the first papers on this subject most of the fish species inhabiting pearl mussel rivers can serve as hosts (HARMS 1908, MURPHY 1942, WELLMANN 1943). Recent investigations show that glochidia are extremely specialized parasites which can develop successfully only on a few salmonids (AWAKURA 1968, KARNA & MILLEMANN 1978, UTERMARK 1973, YOUNG & WILLIAMS 1984 a, b). The host range, however, varies and depends on the resident fish species. According to the published data of the pacific region, *Oncorhynchus* and *Salmo* species serve as hosts (AWAKURA 1968, KARNA & MILLEMANN 1978, TAYLOR & UYENO 1966), whereas in the atlantic drainages glochidia develop on *Salmo* and *Salvelinus* species (BAUER 1979, SMITH 1976, UTERMARK 1973, YOUNG & WILLIAMS 1984 a, b). Literature on the hosts of the european freshwater pearl mussel (*Margaritifera margaritifera* L.) exists for populations in Scotland (YOUNG & WILLIAMS 1984 a, b) and North Germany (UTERMARK 1973). To supplement these data and to allow further

0341–2881/87/0076–0413 $ 2.75

conclusions on the host relationships of Margaritiferidae, the salmonid fishes (native and introduced) occuring in pearl mussel rivers of southern Germany are analysed for their susceptibility in this paper. In order to get some insight into the population ecology of the pearl mussel, density dependent processes governing survival of glochidia are also studied for two selected salmonid species. This study also intends to provide data for effective conservation measures for the pearl mussel, which has become one of the most endangered animal species in Central Europe (Baer 1970, 1981; Bauer 1979, 1980; Dettmer 1982; Jungbluth 1971; Wells et al. 1983). If the most susceptible host species is known, its populations could be supported thus allowing the natural reproduction of the mussels to increase.

Material and methods

Glochidia were obtained from gravid mussels from a small stream of the upper Elbe River system in North Bavaria. All experiments were conducted with fish obtained from a fish hatchery, none of which had previous contact with glochidia. The age classes were termed 0+ (hatched in the year of the experiment) and 1+ (hatched one year before the experiment).

1. The host range

Fingerlings (0+) of seven salmonid species were slightly infected by exposing them for five minutes to 30×10^4 active, mature glochidia per litre. After infection, the fish were kept at 15 °C and the success and development of the infections were observed until the young mussels were released.

2. Progress of glochidial mortality in time

Fingerlings of *Salvelinus fontinalis* (4–6 cm in length), *Salmo trutta* (5.5–8.7 cm) and *Salmo salar* (3.1–5 cm) were simultaneously exposed for five minutes to the same glochidial concentration as above. Immediately after infection some fish of each species were killed and the numbers of glochidia in their gills counted. The remaining fish were kept at 9.5 °C and the fate of infection was analysed from samples taken at 1–5 weeks intervals.

3. Density dependence of glochidial mortality and age effect

Seventysix *Salmo trutta* and 117 *Salvelinus fontinalis* of two age classes were used for this experiment. *S. trutta* were 9–13 cm (0+) and 24.5–29 cm (1+) and *S. fontinalis* 4–6 cm (0+) and 16–29 cm (1+) long. We devided the fish of each species and age class into four groups which were then exposed to a glochidial suspension for different time periods starting with group 4 and proceeding with group 3, 2 and 1 (Table 1) thus producing varying infection intensities. The glochidial dose for *S. trutta* and *S. fontinalis* (group 1) was 40×10^4 and 20×10^3 per litre resp. Whenever one group was infected the concentration of free glochidia in the suspension was determined. The fish of each group were marked by cutting different fins. Immediately after infection a sample of the fish was killed (Tab. 1). For each fish the size, weight, exposure time and the dose to which it was exposed was noted and the number of glochidia in its gills determined. With these data a multiple regression was conducted for each age class leading to an equation with the initial infection intensities as dependent variables and size (or weight), exposure time and glochidial dose as independent variables (Tab. 3).

Table 1. Experimental design to investigate the relationships between glochidial mortality and infection intensity or host age respectively.

Group	*Salmo trutta* exposure time (min.)	n killed at day 0 p.i. 0+	n killed at day 0 p.i. 1+	n killed at day 43 p.i. 0+	n killed at day 43 p.i. 1+	*Salvelinus fontinalis* exposure time (min.)	n killed at day 0 p.i. 0+	n killed at day 0 p.i. 1+	n killed at day 36 p.i. 0+	n killed at day 36 p.i. 1+
1	3	4	3	5	7	5	5	15	3	10
2	6	4	3	6	5	10	5	15	3	10
3	9	4	3	6	7	15	5	14	2	8
4	15	4	3	6	6	20	4	10	2	6

The remaining fish were kept for 43 *(S. trutta)* and 36 *(S. fontinalis)* days at 9.5°C when the numbers of mussel larvae in the gills were counted. Ten larvae on each *S. trutta* were measured along their longest axis. The exposure time and the size of each fish was also noted and using the above equations, the initial infection intensities could be calculated. The density dependence of glochidial mortalities was analysed by plotting the initial against the surviving densities logarithmically (MORRIS 1963, SOLOMON 1964, 1968).

Results

(1) The host range

The salmonid species inhabiting pearl mussel rivers in southern Germany are listed in Table 2. *S. trutta* and *Thymallus thymallus* occur in all rivers. *S. salar* occurs in the Rhyne and Elbe River system, while *Hucho hucho* is only found in the Danube River system. *S. fontinalis* and *S. gairdneri* are introductions from North America.

The fate of glochidial infection suggests that these fish species differ considerably with respect to their susceptibility (Tab. 2). Glochidia develop very well

Table 2. Susceptibility of salmonids occuring in pearl mussel rivers. + = highly susceptible; glochidia developed successfully on all tested specimen. (+) = less susceptible; glochidial mortality is high; frequently all glochidia are shed. – = completely resistant.

Salmoninae	
Salmo trutta	+
Salmo salar	+
Salvelinus fontinalis	(+)
Hybrid (*S. trutta* × *S. fontinalis*)	(+)
Hucho hucho	(+)
Salmo gairdneri	–
Thymallinae	
Thymallus thymallus	–

on *S. trutta* and *S. salar*. *S. fontinalis*, the hybrid of *S. fontinalis* and *S. trutta* as well as *H. hucho* are more resistant. *S. gairdneri* and *T. thymallus* are completely resistant, repelling all parasites within a few days post infection.

(2) Progress of glochidial mortality in time

S. trutta used in this experiment were longer than *S. fontinalis* and *S. salar*. Larger fish have larger gills which allow more water to pass through them. Consequently *S. trutta* could support a larger initial glochidial load than the other two species (Fig. 1). However this doesn't influence the results.

On *S. fontinalis* the number of glochidia had decreased considerably one week p.i. (Fig. 1). This trend then continued until four weeks p.i. when all fish had lost their glochidia. The degree of infection of *S. trutta* and *S. salar* on the other hand, remained fairly constant, indicating that these hosts retained their parasites during the duration of the experiment.

(3) Density dependence of glochidial mortality and age effect

S. trutta and *S. fontinalis* were chosen for this experiment because the former is an example of an ideal host and the latter of a less suitable host. The equations for the calculation of the initial infection intensities are given in Table 3. The variation of this parameter can be explained to a high degree by fish size and exposure time whereas glochidial dose contributed only to the variation in the 1+ *S. fontinalis* because of the significant reduction of suspended glochidia after each infection. In all other cases the small numbers of glochidia withdrawn by infected fish are not important.

(a) *Salmo trutta*

Glochidial mortalities 43 days after infection vary between 24 and 78% on 0+ fish. The slope of the regression is less than one (Fig. 2) and mortality therefore must be considered as density dependent: the higher the initial infection, the higher the percentage of glochidia which is lost.

Glochidial mortalities on 1+ *S. trutta* is higher and varies between 80 and 96%. The regression line has a slope of $b=2$, indicating that mortality is inversely density dependent: the higher the initial infection, the lower are the mortality rates.

During their parasitic stage the larvae of the freshwater pearl mussel grow from 0.07 to 0.4 mm before they are released as young mussels. According to Fig. 3 growth was delayed on 1+ hosts compared to 0+ hosts where they reached nearly half their final size after 43 days.

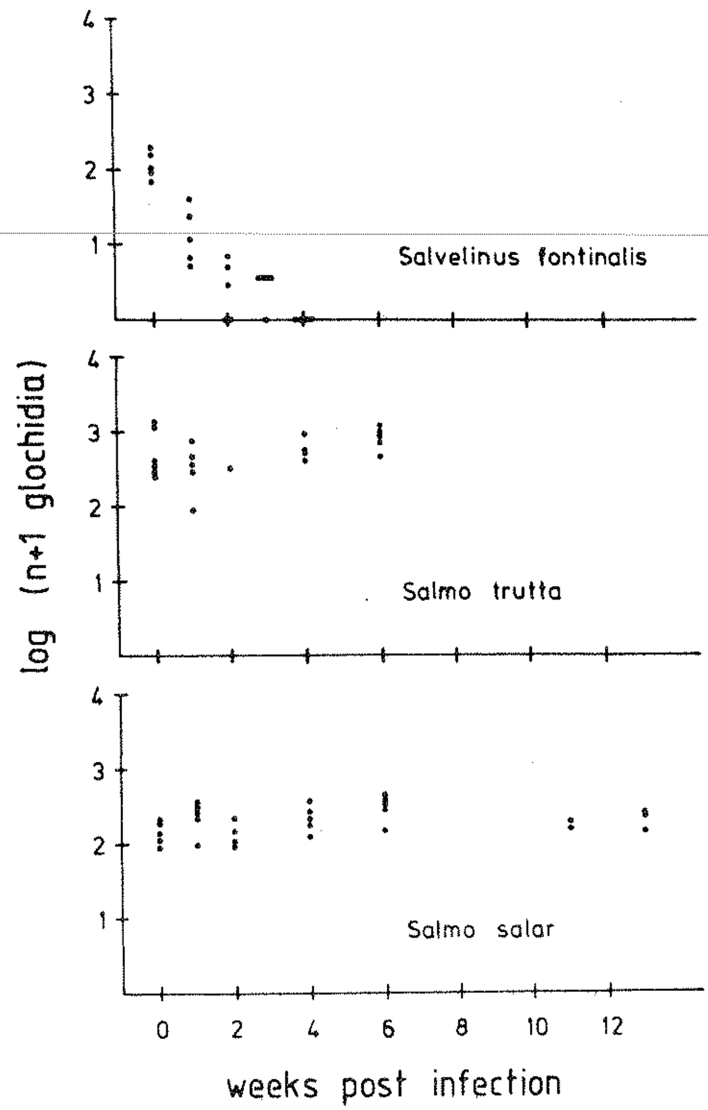

Fig. 1. Progress of glochidial mortality in time. All fish were simultaneously exposed to the same dose at day zero.

(b) *Salvelinus fontinalis*

Glochidial mortality is much higher in this species. Of the 43 fish infected, 25 had lost all glochidia (Fig. 4). In contrast to *S. trutta* the survival rates of glochidia do not show any relationship to the initial densities.

Table 3. Predictive equations for the initial infection intensities. y = initial infection intensity (glochidia per fish); l = length of fish (cm); w = weight of fish (g); t = exposure time (min.); d = glochidial dose (suspended glochidia per litre).

	Age	Equation	n	r^2
Salmo trutta	0+	$y = -10280 + 832 l + 1046 t$	16	0.94
	1+	$y = -82900 + 4394 l + 3544 t$	12	0.74
Salvelinus fontinalis	0+	$y = -228 + 123 w + 23 t$	19	0.78
	1+	$y = -38280 + 1142 l + 651 t + 1.2 d$	54	0.63

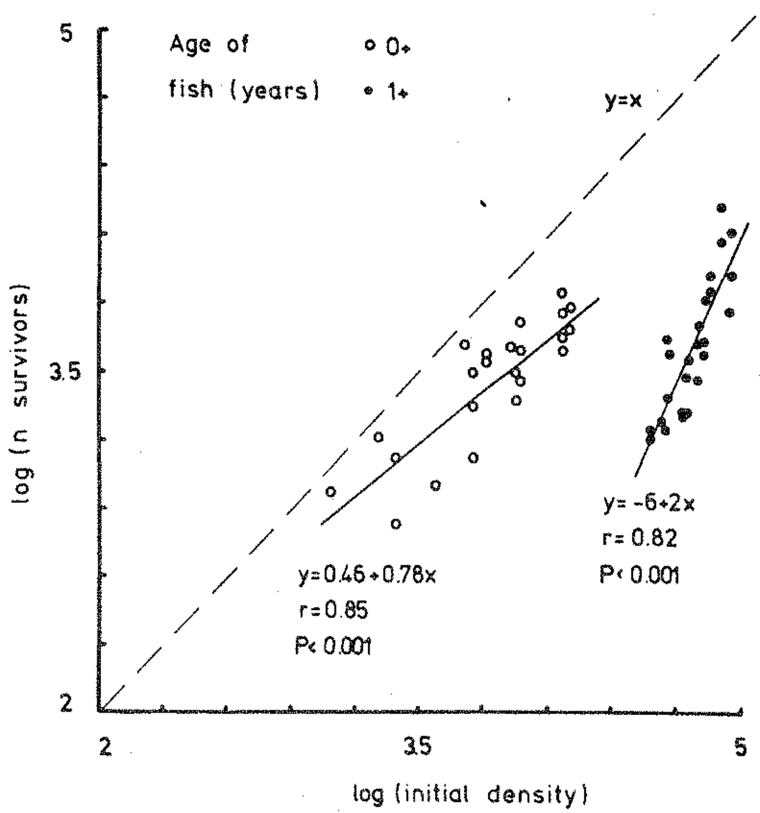

Fig. 2. Survival of glochidia on *Salmo trutta*. (Each dot represents one fish.)

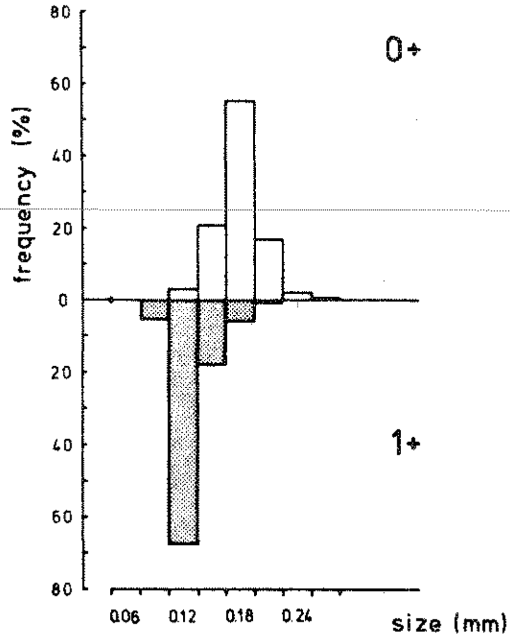

Fig. 3. Size distribution of glochidia on *Salmo trutta* 43 days p.i.

Discussion

The results of this study concerning the host specifity of *M. margaritifera* in Europe compare favourably with the findings of UTERMARK (1973) and YOUNG & WILLIAMS (1948a, b): only species of the subfamily Salmoninae can serve as hosts (Tab. 1). The native *T. thymallus,* of the subfamily Thymallinae which is commonly found in pearl mussel rivers of this study area, is completely resistant (Tab. 1).

But not all Salmoninae are accepted as hosts of pearl mussel glochidia. The suitability of *H. hucho* is also in doubt. All experiments were conducted with glochidia obtained from the Elbe River System where this salmonid doesn't occur naturally and the possibility cannot be excluded that *H. hucho* is probably less resistant to glochidia from the Danube River system. The introduced *S. gairdneri,* a host of *Margaritifera falcata* in Oregon (KARNA & MILLEMANN 1978) was shown to be completely resistant. The susceptibility of *S. fontinalis,* which is also an introduced species, is very low (Tab. 1, Fig. 1, 4). This is especially surprising as *S. fontinalis* serves as a host for *M. margaritifera* in Massachusetts (SMITH 1976).

The host range of *M. margaritifera* in the Elbe River system is apparently restricted to the two native Salmoninae namely *S. trutta* and *S. salar.* Due to water pollution the latter species became extinct in this river system so that at present

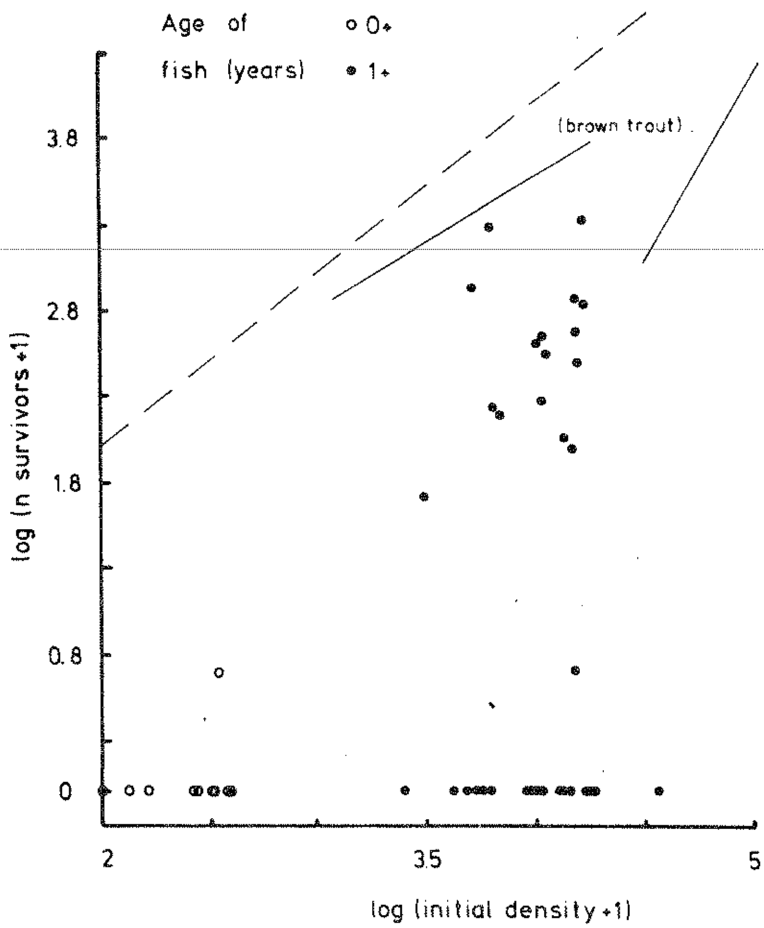

Fig. 4. Survival of glochidia on *Salvelinus fontinalis*. For comparison the regression lines of Fig. 2 also are given.

S. trutta is the only important host. In spite of its suitability as a host a remarkable high loss of glochidia may occur, even if none of the fish had been infected before. A similar phenomenon was observed by FUSTISH & MILLEMANN (1978) and YOUNG & WILLIAMS (1984a, b) who analysed the host relationship of *M. falcata* in Oregon and *M. margaritifera* in Scotland respectively. This loss depends on at least two factors namely: the age of the host and the infection intensity (Fig. 2): On 0+ fish, mortality rates are moderate and density dependent. According to the regression line in Fig. 2 mortalities should be low at initial infection intensities between 100 and 1000 glochidia. This is confirmed by the experiment on the progress of glochidial mortality in time (Fig. 1), where the fish were only slightly infected and accordingly no significant decline of glochidial numbers was observed.

The pattern of mortality on 1+ fish is quite different where mortality rates are high and exhibit a strong inverse density dependence.

Mechanisms underlying glochidial mortality in the parasitic stage were investigated by AREY (1932), BAUER & VOGEL (1987) and MEYERS et al. (1980). Immediately after infection a nonspecific tissue response may occur leading to hyperblasia and to a quick shedding of the parasites. According to WAKELIN (1976) this type of response is typical of hosts with a high degree of natural resistance. The rapid decline of glochidia in *S. fontinalis* suggests such a tissue defense mechanism.

A second type of response is the production of antibodies. Such a humoral response in fish is a delayed process (CORBEL 1975) and is therefore confined to hosts which retain the parasites for a longer period of time, e.g. to a susceptible host like *S. trutta*. The observed mortality on this host is in sharp contrast with that of *S. fontinalis*, indicating that both species have different mechanisms responsible for glochidial mortality. It has already been demonstrated that there is a humoral response to glochidiosis in *S. trutta* which leads to glochidial mortality and to delayed development of the survivors: the more intense the response (the higher mortality) the more delayed is development (BAUER & VOGEL 1987).

The results obtained from *S. trutta* indicate that glochidial mortality is mainly due to a humoral response[1]. The immune system of 0+ *S. trutta* responds only weakly to glochidiosis and the intensity of the response is enhanced if the glochidial load is high. Because of the weak response, the conditions for the parasites are very suitable and development of glochidia is enhanced. On the other hand 1+ *S. trutta* are more resistant and a strong response leads to high mortality rates and delayed development of glochidia. The response is especially intense where the glochidial load is low.

A survey of pearl mussel populations in Central Europe shows a lack of young individuals (BAUER 1983) despite the fact that the adults are fertile (BAUER 1987). In order to increase the immature population certain conservation measures are suggested. Firstly, *S. trutta* populations should be supported by providing sufficient spawning and hiding places. Secondly, no foreign salmonids should be introduced because they are unsuitable hosts and may lower the density of *S. trutta* by competing for food and territories. They might also hybridize with *S. trutta* producing offspring which are detrimental for the pearl mussel populations (Tab. 2).

An attempt was recently made to establish new pearl mussel populations by introducing infected fish into clean rivers. These attempts are most promising where 0+ *S. trutta* are used as glochidial mortality is low and glochidial development is quick on these hosts. As the mortality is only weakly density dependent high infection intensities will yield high numbers of young mussels.

[1] A tissue response seems to occur only if *S. trutta* is kept at high temperature (17°C, BAUER & VOGEL 1987).

Summary

The main hosts of *Margaritifera margaritifera* in southern Germany are the native Salmoninae *Salmo trutta* and *Salmo salar*. *Hucho hucho*, the introduced *Salvelinus fontinalis* as well as the hybrid of *S. fontinalis* and *S. trutta* are less susceptible. *Salmo gairdneri* and *Thymallus thymallus* are completely resistant (Tab. 2).

The pattern of glochidial mortality suggests that less susceptible hosts respond to infection with a rapid tissue response, whereas the susceptible host *S. trutta* shows a delayed response presumably due to a humoral factor (Fig. 1). Mortality on *S. trutta* increases with host age, it is density dependent on 0+ (less than one year old) hosts and inverse density dependent on 1+ (older than one year) hosts (Fig. 2). Conservation measures for the pearl mussel should therefore be directed at supporting populations of *S. trutta*. No foreign salmonids should be introduced into the rivers and artificial rearing of highly infected 0+ brown trout for release should be encouraged.

Zusammenfassung

Die Hauptwirte der Flußperlmuschel (*Margaritifera margaritifera* L.) sind die beiden einheimischen Salmoninae Bachforelle (*Salmo trutta*) und Lachs (*Salmo salar*). *Hucho hucho*, der eingeführte Bachsaibling sowie die Kreuzung aus Bachforelle und Bachsaibling sind kaum als Wirte geeignet. Regenbogenforelle (*Salmo gairdneri*) und Äsche (*Thymallus thymallus*) sind völlig resistent (Tab. 2).

Der Verlauf der glochidialen Mortalität legt nahe, daß weniger geeignete Wirtsarten mit einer schnellen Gewebsreaktion auf eine Infektion reagieren, während geeignete Wirte eine zeitlich verzögerte Reaktion zeigen, die vermutlich auf einer serologischen Komponente beruht (Fig. 1).

Die Mortalität der Glochidien auf Bachforellen steigt mit zunehmendem Wirtsalter. Sie ist auf Brütlingen positiv dichteabhängig, auf 1+ Fischen invers dichteabhängig (Fig. 2). Als Schutzmaßnahmen für die Perlmuschel wird vorgeschlagen, die Bachforellenpopulationen zu stützen. Es sollten keine fremden Salmoniden eingesetzt werden. Neuansiedlungsversuche sollten mit stark infizierten Forellenbrütlingen durchgeführt werden.

Acknowledgements

I am grateful to the Fischereifachberatung Oberfranken and to the staff of the fish hatchery in Aufseß. A. GEYER and J. STEIDLE provided much practical help. Mrs. E. RUMMEL typed the manuscript.

This research was supported by a grant from the Deutsche Forschungsgemeinschaft.

References

AREY, L. B. (1932): A microscopical study of glochidial immunity. – J. Morphology **53:** 367–378.

AWAKURA, T. (1968): The ecology of parasitic glochidia of the freshwater pearl mussel. – Sci. rep. Hokkaido fish hatchery **23:** 1–21.

BAER, O. (1970): Zum Rückgang der sächsischen Flußperlmuscheln. – Arch. Natursch. Landschaftsforsch. **10:** 207–209.

– (1971): Zur früheren Verbreitung der Flußperlmuschel in der Westlausitz. – Veröff. Mus. Westlausitz **9:** 53–70.

BAUER, G. (1979): Untersuchungen zur Fortpflanzungsbiologie der Flußperlmuschel im Fichtelgebirge. – Arch. Hydrobiol. **85:** 152–165.

Bauer, G. (1980): Die Situation der Flußperlmuschel in der Oberpfalz und in Niederbayern. – Ber. ANL **4:** 101–103.

– (1983): Age structure, age specific mortality rates and population trend of the freshwater pearl mussel in North Bavaria. – Arch. Hydrobiol. **98:** 523–532.

– (1987): Reproductive strategy of the freshwater pearl mussel. – J. Anim. Ec. **56:** 691–704.

Bauer, G. & Vogel, C. (1987): The parasitic stage of the freshwater pearl mussel (*Margaritifera margaritifera* L.). I. Host response to Glochidiosis. – Arch. Hydrobiol., Suppl. **76** (4): 393–402.

Corbel, M. J. (1974): The immune response in fish: a review. – J. Fish. Biol. **7:** 539–563.

Dettmer, R. (1982): Untersuchungen zur Ökologie der Flußperlmuschel in der Lutter im Vergleich mit bayerischen und schottischen Vorkommen. – Thesis, Hannover.

Fustish, C. A. & Millemann, R. E. (1978): Glochidiosis of salmonid fishes, II. – J. Paras. **64:** 155–157.

Harms, W. (1907): Zur Biologie und Entwicklungsgeschichte der Flußperlmuschel. – Zool. Anz. **31:** 814–824.

Jungbluth, J. H. (1971): Die rezenten Standorte von *Margaritifera margaritifera* in Vogelsberg und Rhön. – Mitt. d. malak. Ges. **7:** 299–302.

Karna, D. & Millemann, R. (1978): Glochidiosis of salmonid fishes. III. – J. Paras. **64:** 528–537.

Meyers, T.; Millemann, R. & Fustish, C. (1980): Glochidiosis of salmonid fishes. IV. – J. Paras. **66:** 274–281.

Morris, R. F. (1963): Predictive population equations based on key factors. – Mem. Ent. Soc. Can. **32:** 16–21.

Murphey, G. (1942): Relationship of the freshwater pearl mussel to trout in the Truckee River. – Calif. Fish Game **28:** 89–102.

Smith, D. G. (1976): Notes on the ecology of *Margaritifera margaritifera* in Central Massachusetts. – Am. Middl. Nat. **1:** 252–256.

– (1980): Anatomical studies on *Margaritifera margaritifera* and *Cumberlandia monodonta*. – Zool. J. Linn. Soc. **69:** 257–270.

Solomon, M. E. (1964): Analysis of processes involved in the natural control of insects. – [In:] Cragg, J. (ed.): Advances in ecological research: 1–57. Academic press, London.

– (1969): Logarithmic regression as a measure of population density response. – Ecology **49:** 357–358.

Taylor, D.W. & Uyeno, T. (1966): Evolution of host specifity of freshwater salmonid fishes and mussels in the north pacific region. – Venus **24:** 199–209.

Utermark, W. (1973): Untersuchungen über die Wirtsfischfrage für die Glochidien der Flußperlmuschel. – Thesis, Hannover.

Wakelin, D. (1976): Host responses. – [In:] Kennedy, L. R. (ed.): Ecological aspects of parasitiology: 115–141. North Holland Publishing company, Amsterdam.

Wellmann, G. (1943): Fischinfektion mit Glochidien der *Margaritana margaritifera*. – Z. Fischerei **41:** 358–390.

Wells, S. M.; Pyle, M. & Collins, N. M. (1983): The IUCN invertebrate red data book. – Gland: 147–156.

Young, M. & Williams, J. (1984a): The reproductive biology of the freshwater mussel in Scotland. I. Field studies. – Arch. Hydrobiol. **99:** 405–422.

– (1984b): The reproductive biology of the freshwater pearl mussel in Scotland. II. Laboratory studies. – Arch. Hydrobiol. **100:** 29–42.

Address of the author:

G. Bauer, Department of Animal Ecology, University of Bayreuth, D-8580 Bayreuth.