EPIDÉMIOLOGIE ET CONTRÔLE DES INFECTIONS A *PSEUDODACTYLOGYRUS* DANS LES ELEVAGES INTENSIFS D'ANGUILLES : DONNÉES ACTUELLES.

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RÉSUMÉ

Dans une première partie, l'auteur considère successivement divers aspects qui conditionnent l'épidémiologie des infections observées, chez l'Anguille européenne, par *Pseudodactylogyrus anguillae* et *P. bini,* en élevage intensif, en eau chaude.

La température optimale pour la croissance des Anguilles est aussi celle qui correspond à une reproduction optimale de ces deux Monogènes. L'influence des conditions de recyclage de l'eau et de son traitement par le formol sur les réinfestations parasitaires sont discutées. Il existe une distribution différentielle des deux espèces congénériques sur les branchies, qui varie en fonction de la taille de l'hôte. Les relations entre la taille de l'hôte et la charge parasitaire sont discutées en fonction des conditions d'élevage et les conditions naturelles. Une relative résistance à la réinfestation parasitaire a été observée.

Dans une seconde partie, une étude thérapeutique basée sur les effets antihelmintiques des 22 produits est présentée. Si le mebendazole s'est avéré le plus efficace, des phénomènes de résistance après traitements répétés sont évoqués.

EPIDEMIOLOGY AND CONTROL OF *PSEUDODACTYLOGYRUS* INFECTIONS IN INTENSIVE EEL CULTURE SYSTEMS : RECENT TRENDS.

ABSTRACT

The research efforts conducted to elucidate the epidemiology of *Pseudodactylogyrus* infections in the European eel *Anguilla anguilla* are described. Subjects as temperature dependent reproduction of gill monogeneans, biotic factors influencing the infection level, the microhabitats of the congeneric parasites, the relation between host size and parasite load and the development of host responses to infection are considered. In addition the investigations performed in the laboratory and in the eel farm in order to develop an effective anthelmintic treatment for control of *Pseudodactylogyrus* infections are presented.

INTRODUCTION

During the 1980's production of the European eel (*Anguilla anguilla*) in farm systems based on recycling of heated freshwater proved to be a profitable aquacultural activity in Denmark.

Initially the eel farmers faced a number of technical and biological problems, among which infections with the gill parasitic monogeneans *Pseudodactylogyrus anguillae* and P. bini were dominating.

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These platyhelminths were introduced from the Pacific area, and first reported from Europe by Golovin (1977), Molnár (1983) and Lambert et al. (1984). The genus *Pseudodactylogyrus* is now widespread in populations of *Anguilla anguilla* in European natural waters (Køie 1991).

The present paper describes the research on the epidemiology of this parasite infection in farmed eel and the successful search for effective control methods.

EPIDEMIOLOGY

Temperature dependent reproduction.

The water in the intensive eel culture systems is artificially heated to approximately 25 °C, which temperature is near optimum for growth of the European eel. Unfortunately this temperature level is also optimal for reproduction of *Pseudodactylogyrus anguillae* and *P. bini*. Egg production is maximal and egg and postlarval development for both congeneric parasites are fastest at 25 to 30 °C. The survival time of the adult monogeneans is at least 50 to 60 days at the prevailing farm temperature (Buchmann 1988 d, 1990). The short parasite generation time of 10-12 days in the farms creates a basis for explosive increases in parasite occurrence. Thus, parasite burdens of several hundreds monogeneans per host (even fingerling eels) were occasionally observed in the eel farms.

In wild populations of eels, seasonal occurrence of *Pseudodactylogyrus* spp. (Imada & Muroga 1977, Køie 1988, Nie & Kennedy 1991) has also been explained by temperature variations.

Other factors influencing reproduction.

Although temperature is the major abiotic determinant of parasite reproduction other factors might influence the occurrence of *Pseudodactylogyrus* spp. under intensive eel farm conditions. During a survey in an intensive eel culture system, in which the water (25 °C) was recycled using biofilters, and the eels were fed continuously with dry feed, no reinfections of eels were recorded for several weeks, despite the presence of adult egg laying monogeneans on the eel gills (Buchmann 1988 a).

However, few days after formaldehyde treatment of the fish tank water (which will reduce the microbial activity in biofilter and fish tank) or removal of biofilm on fish tank walls, surges of settling parasite larvae appeared. It was worth noting that these activities did not significantly influence the turbidity, pH, oxygen-saturation or ammonia level in the surface water of the fish tanks.

Several explanations to these observations have been offered. Theoretically, it could be suggested that 1) Anaerobic zones in the biofilter or in the biofilm on the fish tank wall are able to inhibit parasite egg development or hatching, 2) Compounds affecting survival of eggs or larvae could be released from microorganisms 3) The microfauna consisting of turbellarians, copepods and rotifers could predate on soft shelled newly laid parasite eggs or oncomiracidia. Formaldehyde reduction or mechanical removal of these three factors might produce a surge of hatched larvae and thereby the observed reinfection of eels. The possibility that a host immune response was responsible for the absence of reinfection was considered less likely, because treatment of eels with the immunosuppresive steroid dexamethasone did not elicit reinfection.

The question is still unsolved, but although egg predation of turbellarians were reported this factor is obviously not the sole cause, as reinfection free periods in infected eel farms with no turbellarians present in the biofilm have been noted.

Microhabitats of parasites.

The two congeneric species of *Pseudodactylogyrus* are distributed differently on the gill apparatus of *Anguilla anguilla*. In addition the location of parasites varies also according to the size or stage of the host. Generally, *P. bini* occupies the anterior gill arches and are more distally placed on the gill filament, whereas *P. anguillae* mostly is found on the posterior gill arches and located on the proximal part of the gill filament. Both species are most numerous in the dorsal and bent part of the hemibranch, but *P. bini* has a broader amplitude and is often present on the ventral and median part. The two congeneric species did not show a clear predilection for neither the right or left side of the gill apparatus nor the anterior or posterior hemibranch (Table 1). Numerous authors (e.g. Wootten 1974, Lambert & Maillard 1975, Hanek & Fernando 1978, Rohde 1982, Koskivaara et al. 1992) have described similar preferences of gill parasites for host microhabitats.

Tableau I : Distribution spatiale habituelle de *P. bini* PB et *P. anguillae* PA sur les branchies d'*Anguilla anguilla*. ( ) : observations variables (d'après Buchmann, 1989 a).

Table I : Most commonly observed spatial distribution of *P. bini* PB and *P. anguillae* PA on the gill apparatus of *Anguilla anguilla*. (): Variable observations. (After Buchmann, 1989 a).

	Glass-eels 6.5-7.2 cm	Pigmented eels 8-14 cm	Pigmented eels 21-42 cm
Side:			
Right	-	(PB)	-
Left	(PB)	-	(PB)
Arch 1	PB	PB	-
Arch 2	PB	PB	PB
Arch 3	PA	PA	PA/PB
Arch 4	PA	PA	PA/PB
Hemibranch:			
Dorsal	PA	PA/PB	PA/PB
Median	-	PB	-
Ventral	PB	-	-
Anterior	(PB)	-	-
Posterior	-	(PA)	-
Filament:			
Proximal	PA/PB	PA	PA
Median	-	PB	PA
Distal	-	PB	PB

Relation between host size and parasite number.

A common observation in eel farms, where eels in a particular fish tank are exposed to identical environmental parameters, is a significant positive correlation between the number of settling larvae and the host size. Increasing host size is correlated to an increase in the total gill surface area (Hughes 1966), providing more space for the parasite larvae to attach. However, due to interactions between the host and the parasites, the positive correlations will become less distinct during the post infection period (Buchmann 1989 b).

Therefore, such associations will not always be recorded in natural populations, where also a number of other parameters are likely to influence the abundance of parasites in eels. Thus Køie (1988) found a slight decrease in parasite number with increasing host size, whereas Nie & Kennedy (1991) observed a weak increase of intensity in larger eels.

Parasite/host interactions.

a. Different survival of species.

Although a considerable number of larvae settle on the eel gills and develop to the adult stage, a marked reduction in parasite burden is often noted in the weeks following infection. During this proces development of hyperplasia and cell proliferation of infected eel gills is observed (Buchmann 1988 c).

It is noteworthy that in eels infected with the two congeners particularly *P. anguillae* is experiencing a rapid decrease, whereas the decline in the number of *P. bini* is less pronounced. This latter species, which has larger body size and a more sedentary life style than the former, is often found partly embedded in a marked gill tissue reaction (Buchmann 1988 b). Some specimens of *P. bini,* a species possessing small hamuli, even seems to improve its gill attachment through this tissue reaction. Improved gill attachment of monogeneans through host tissue reaction has previously been described from other parasite-host systems (e.g. Llewellyn & Simmons 1984, Roubal 1988). *P. anguillae* is smaller, has larger hamuli and moves more easily on the gills. It is possible that this species requires an intact gill structure for optimal attachment. Thus, although the different population dynamics of the two species could be explained by interspecific interactions they could at least partly be mediated by the host reaction.

b. Acquired resistance to reinfection.

The possibility of fish being protected against reinfections with monogeneans after an initial infection became evident following the studies of Jahn & Kuhn (1932) and Nigrelli & Breder (1934) on monogeneans from fishes in the New York Aquarium.

A protective response of fish towards monogenean infections was described by Vladimirov (1971), who elucidated the resistance of a Ropsha carp hybrid to *Dactylogyrus vastator*. Host reactions against monogeneans were also studied by Paperna (1964), Molnár (1971), Lester & Adams (1974) and Scott (1985).

Recent studies on the *Anguilla anguilla / Pseudodactylogyrus* system have demonstrated that a previous infection offers a slight relative resistance against reinfection. Small pigmented eels were experimentally infected by *Pseudodactylogyrus* spp., whereafter the parasites were eradicated with mebendazole. Reinfection both 14 and 33 days post treatment resulted in a significantly lower number of *Pseudodactylogyrus* spp. on previously infected eels compared to previously non-infected (but likewise mebendazole treated) control group eels (Slotved & Buchmann 1993). The resistance is relative and does not prevent reinfection totally.

The mechanisms of this aquired resistance to reinfection is under investigation. It has been demonstrated that large infected European eels are able to mount a very weak humoral immune response towards antigens in *Pseudodactylogyrus bini* (Buchmann 1993a). However, this response is extremely weak compared to the humoral responses of eels towards antigens in the endoparasitic swimbladder nematode *Anguillicola crassus* and the endoparasitic microsporean *Pleistophora anguillarum*, which were studied using the same western blot technique (Buchmann et al. 1991, 1992 b). Therefore, cellular reactions and non-specific mechanisms are suggested to be involved in the resistance mechanisms.

MEDICAL CONTROL.

During the first years of commercial eel farming in Denmark, formaldehyde treatment (50 to 100 ppm) of the fish tank water once to several times a week was the preferred control method of *Pseudodactylogyrus* infections. This practice kept the infection at a lower but often unpredictable level. Due to the allergenic and suspected carcinogenic nature of this chemical, search for alternative methods were implemented.

Based on the first laboratory trials of Székely & Molnár (1987) mebendazole was tested under commercial eel farm conditions in Denmark (Buchmann & Bjerregaard 1990) and proved to exhibit excellent anthelmintic activity towards *Pseudodactylogyrus* spp. This drug, used as bath treatment (0.5 to 1.0 ppm), is now in common use in our intensive eel culture systems.

In a series of laboratory trials a total of 22 drugs were tested for their anthelmintic effect on the eel monogeneans. Small pigmented eels, naturally or experimentally infected by *Pseudodactylogyrus* spp., were exposed in a standardized bath treatment to the drugs (Table 2). This approach provides a good basis for comparison of drugs, as the previous studies on drug effect on *Pseudodactylogyrus* are based on differing in vitro and in vivo designs.

Tableau II : Effets de 22 médicaments chez *Pseudodactylogyrus spp.* d'Anguilles pigmentées (8-14 cm). Traitement en bain aéré à 24-27°C pendant 24-25 heures. Les Anguilles sont transférées dans une eau non traitée après le bain et examinées 3-4 jours après, d'après Buchmann 1993b.

Table II : Effect of 22 drugs on *Pseudodactylogyrus* spp. on pigmented eels (body length 8-14 cm). Water bath treatment (aerated) at 24-27 °C, 24-25 hours exposure time. Eels were transferred to non-medicated water after treatment and examined for parasites after additionally 3-4 days. From Buchmann 1993b.

Drug	Concentration (mg/l) resulting in Complete	Partial Parasite eradication	Signs of toxicity
Albendazole	10	1	-
Fenbendazole		1	-
Flubendazole		1	-
Luxabendazole	10	1	-
Mebendazole	1		-
Oxibendazole		1	?
Parbendazole		50	?
Thiabendazole			+
Triclabendazole			-
Metrifonate		1	+
Pyrantel citrate		100	?
Morantel tartrate		100	-
Ivermectin			+
Bithionol			+
Niclosamide	1		+
Rafoxanide		1	+
Bunamidine hydro-chloride			+
Praziquantel	10		-
Levamisole-hydrochloride			+
Phenolsulfon-pthalein			+
Toltrazuril			+
Sodium chloride		30 000	+

It was shown that mebendazole and niclosamide in a concentration of 1 mg/l and albendazole, luxabendazole and praziquantel in a concentration of 10 mg/l were able to eradicate all specimens of *Pseudodactylogyrus* spp. in the experiments. Niclosamide, however, showed high acute toxicity to eels and should not be used in intensive eel farms.

Mebendazole is an excellent anthelmintic under practical culture conditions and is at present widely applied. Unfortunately, a risk for development of drug resistant *Pseudodactylogyrus* parasites is imposed by erratic use of anthelmintics. In laboratory systems it has been demonstrated that seven exposures of *Pseudodactylogyrus* populations to subtherapeutic dosages of mebendazole during a seven month period resulted in selection of partly drug resistant parasite populations (Buchmann et al. 1992 a). Development of drug resistance in eel culture enterprises would be of great concern as only few alternatives to mebendazole exist. A number of benzimidazoles are partly effective, but due to the common mode of action (tubulin binding) in this drug group (Lacey 1988), side resistance to these substances is likely to occur. Praziquantel is the only non-benzimidazole having satisfactory effect on the gill monogeneans without eliciting acute toxic reactions in eels. Alternation in use between this isoquinoline and benzimidazoles will theoretically (Anderson & Waller 1985) delay emergence of drug resistance in these gill monogeneans.

CONCLUSIONS

Research in epidemiology and control of *Pseudodactylogyrus* infections in intensive eel culture systems have demonstrated the high capability of parasite reproduction under the prevailing temperature conditions (25 °C) in eel farms. This has resulted in mass occurrences of the parasites, which have caused morbidity and mortality of *Anguilla anguilla* in the systems.

The two congeneric gill monogeneans show some ecological differences. *P. bini* occupies mostly the anterior gill arches and are placed distally on the gill filaments, whereas *P. anguillae* mainly infects the posterior gill arches at the proximal part of the filament. It has been observed that a pronounced cellular reaction often is associated with *P. bini,* which species exhibit a superior survival compared to *P. anguillae* in mixed infections.

The number of parasites infecting the host is positively correlated to host size. However, the host reactions influence the parasite burden and a relative acquired resistance to challenge infection has been demonstrated. A very weak humoral immune response of infected eels to antigens in *P. bini* has been found, but cellular reactions are likely to be involved in the response.

After laboratory screening of 22 anthelmintics it was concluded that mebendazole, when used as bath treatment, is the most effective drug against infections with *Pseudodactylogyrus* spp. This drug is now widely used in intensive eel farm systems. Treatments should be conducted with care as it has been demonstrated that mebendazole resistant strains of gill monogeneans are readily selected in the laboratory after repeated subtherapeutical drug exposure.

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