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Study of *Diplectanum aequans* (Monogenea), parasite of sea bass (*Dicentrarchus labrax*) in intensive farming

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Abstract

The distribution of the parasite along three axes of sea bass gill structures shows differences among adult and juvenile forms. Along the anter-posterior axis, the adult presence is higher in the two first gill arches while juveniles were distributed on 2nd and 3rd gill arches. The medium-lateral axis shows tendency of adults to be distributed at the extremity of lamellae, while juveniles were located on the internal face of each hemibranchia. Along the dorso-ventral axis, both adults and juveniles were located mainly on the central areas of each hemi-branchia. Differences in distribution have been related to water flow through the gill system and to a loss of the juvenile forms during migration. Indications are also given to minimize the acute impact on the receiving environment, due to chemical antiparasitic treatments.

KEYWORDS: Intensive farming, *Diplectanum aequans*, Sea bass, Antiparasitic treatments.

Introduction

An important part of protecting the earth's water resources from possible sources of pollution is reducing to a minimum the environmental impact of the prophylactic and therapeutic pharmaceutics used in aquatic animal breeding (Hoffman and Meyer, 1974).

Sea bass (*Dicentrarchus labrax*) raised in farms are commonly parasitized by a variety of organisms, among which the trematode worm, *Diplectanum aequans* (Trematoda, Monogenea), which settle on its gills (Paperna and Baudin Laurencin, 1979; Ghittino, 1985). The use of formaline as an external disinfectant and anti-parasitic is permitted by Italian law and often practised (Sarti, 1988). This method is however not very effective against *D. aequans*.

This report presents the results of a study on the distribution of these parasites on the branchial arches of farmed *D. labrax*, as well as the first data from therapeutic trials of the anti-parasitic drugs Ranide® , Neguvon® and mebendazole.

Methods and materials

For the study of parasite distribution along gill structures, one-year-old sea basses, naturally parasitized, were chosen from a population artificially bred in a palustrine and euryhaline environment.

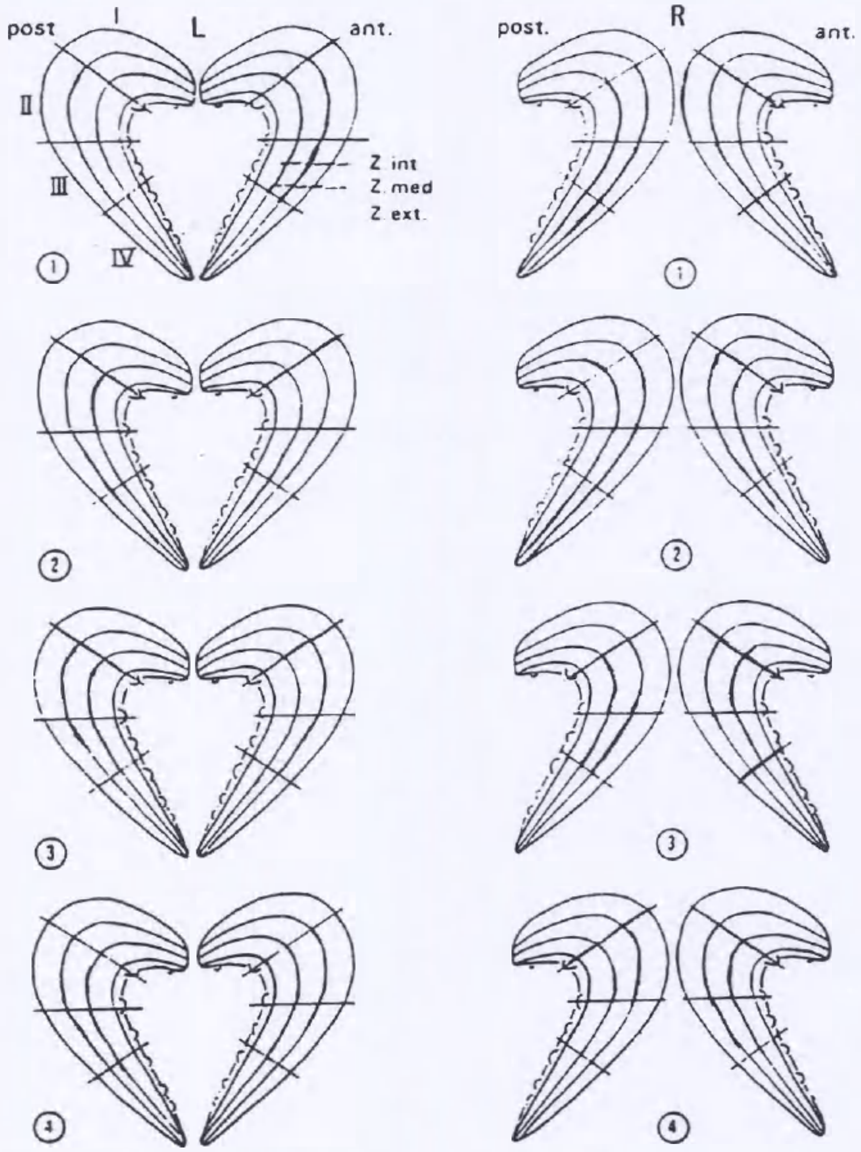
For this purpose, we referred to the schemes proposed by Lambert and Maillard (1975) who studied the distribution of *Diplectanum aequans* and *Diplectanum laubieri* parasites on gills during congeneric infestation in *Dicentrarchus labrax*. Consequently, the branchial apparatus has been subdivided in various portions along three axes:

1) anter-posterior axis: corresponding to the branchial arches numbered ante-posteriorwise from 1 to 4. Each branchial arch has also been subdivided in anterior and posterior hemi-branchiae.
2) medium-lateral axis: each hemi-branchia has been subdivided in three areas (internal, medium and external) parallel to the cartilagineous arch.
3) dorso-ventral axis: each hemi-branchia has been further subdivided into four sectors (I, II, III, IV) perpendicular to the cartilagineous arch. The branchial apparatus is then subdivided into 192 units 24 for each gill. Observations have been carried out soon after the subjects examined were killed in order to observe fresh gills with optical microscopes.

The significance in differences between parasite frequencies in right and left gill were evaluated using t test; the significance between adult and juvenile quantities in the same location on each branchial axis were evaluated by x^2 test (Table I and II).

Therapeutic trials on *Dicentrarchus labrax* have been carried out on numerically variable samples of one-year-old sea basses chosen from a population farmed in the same palustrine environment.

Sea basses chosen for trials were put in 50 l tanks equipped with oxygenating devices and recirculation pumps, lacking filters and containing the same feeding water of the farm in which the chosen samples were bred. Other tanks with continuous changes of water and with characteristics similar to the former were employed to put in treated fish after a 24-72-h period in order to observe the appearance of possible short-term toxic phenomena.



Schematic representation of the gills of *Dicentrarchus labrax* illustrating the localization of *Diplectanum aequans*. (L) Left gills; (R) right gills; (Post) posterior hemi-branchia; (Ant) anterior hemi-branchia; (1,2,3,4) gills; (Z.int, Z.med, Z.ext) internal, middle, external portion.
1→4: anter-posterior axis.
Z.int→Z.ext: medium lateral axis.
I→IV: dorso-ventral axis.
(Lambert and Maillard, 1975)

Sea bass sub-samples chosen and separated from the same studied population, were used as checks. The study on fresh gill left arches only, revealed that the *D. aequans* infestating strength was equal to 78.5 parasites/sample.

Therapeutic trials have been carried out through closed-cycle medical baths using various concentrations and different times, of three drugs: Ranide® oral mixture (Rafoxinide 6%), Neguvon® (trichloroxiethylmethylsulfonate) and active mebendazole powder, non-soluble in water.

Results and discussion

Average weight of samples analyzed in the study of parasite distribution is of 30.11g (73g max; 12g min); the average infestation is of 92.7 parasites (477 max; 1 min). The total number of parasites found was 1 659; 349 juvenile forms in which vitellogenes had not appeared; 1 310 adult forms in which vitellogenes were most evident. Almost the total number of parasites was found in 13 of the 17 of the *D. labrax* examined samples. These 13 only have been further examined for statistic analyses with the aim of evaluating the differences in parasite distribution along the various gill portions.

The parasite distribution, both adult and juvenile, on right and on left gills (672 adults and 165 juveniles on a total number of 837), on left gills, (635 adults and 187 juveniles on a total number of 822) does not reveal any remarkable difference as shown by the relative tests t (Table I). In order to analyze the general distribution of *D. aequans* parasites, parasites present along the correspondent right and left gill portions in *D. labrax* sea bass have thus been observed complexively in the same subjects.

Table I. Tests t relating to the compared distributions of *D. aequans* individuals on *D. labrax* right and left gills

		t	DF	P
Total number of parasites	-	0.0809	24	NS
Adults	-	0.1872	24	NS
Juveniles	-	0.1736	24	NS

Table II. Tests x^2 relating to the compared frequencies of young and adult parasites along the examined three *D. labrax* gill axes

	x^2	DF	P
A-P axis	72.94	7	<0.001
M-L axis	716.12	5	<0.001
D-V axis	49.75	7	<0.001

Table III shows parasite frequency along each gill portion in all the sea bass samples analyzed; Fig. 1 schematizes total incidence of infestation along each of the three axes examined.

These results lead to the following observations: the distribution of *D. aequans* parasites, both adult and juvenile, along the three examined axes, is not even, as the parasites show preferential locations on gills. Distribution of adults is remarkably different from that of juveniles along all the three axes taken into examination. Differences along the anter-posterior axis, specially along the medium-lateral axis, are evident as is shown in Fig. 1 where relative distribution along these axes are presented. Instead, along the dorso-ventral axis parasites, without considering their stage of development, tend to localize mostly in areas II and III. The significant difference between juvenile and adult frequencies observed in the test is mainly due to their different distribution in other areas. Adult distribution is more even than juvenile distribution which is particularly high also in posterior hemi-branchiae of the IV area.

If we take particular consideration of the distribution along the anter-posterior axis, it is possible to point out the difference in parasite strength distribution on the eight portions considered (four arches - anterior and posterior hemi-branchia). Examining the anter-posterior axis, it may be observed that parasites have a higher concentration on the 1st (29.70%) and 2nd(29.62%) branchial arches than on the 3rd (20.46%) and 4th (20.22%) branchial arches. Moreover, an acute difference may be clearly observed in parasite distributions along the anterior and posterior hemibranchiae of each branchial arch. Along the 1st and 2nd branchial arches there is a higher concentration of adults on anterior hemi-branchiae, while along the 3rd and 4th they prefer posterior hemi-branchiae. Instead, there is a higher concentration of juveniles along the 2nd and 3rd branchial arches and they always prefer posterior hemi-branchiae.

Along the medium-lateral axis, where differences between adult and juvenile distribution are most evident, both adult and juvenile forms tend to concentrate particularly on gill extremities along this axis. Adult forms clearly show preference for external portions (71.22%) rather than medium (2.67%) and internal (6.11%) portions while juvenile forms concentrate particularly on internal portions (59.31%) rather than medium (37.82%) and external (2.87%) portions.

Finally, along the dorso-ventral axis, both adult and juvenile forms preferentially concentrate on medium portions II and III rather than on portions I and IV.

In our opinion, the different preferential sites for parasite localization, related to its development stage, along the gill anter-posterior axis in sea bass samples may be due to the inlet and outlet water flows into and from sea bass gills as it was assumed by Giavenni (1983). The fact that juvenile forms are proportionally more concentrated than adult forms along posterior hemi-branchiae of the 1st and 2nd branchial arches might depend on juvenile migration from the anterior to the posterior face of the same arches. Indeed, most probably, it may be due to the heavier loss in juvenile forms on anterior hemi-branchiae where the water flow, during their centrifugally oriented migration, is stronger than the one onto posterior hemi-branchiae. This was showed by Lambert and

Maillard (1975) and is enforced by data contained in this study. In fact, the different distribution shown between adult and juvenile forms along the medium-lateral axis supports the thesis of a centrifugally oriented larval migration from the branchial arch roots towards the primary lamella apexes. This migration is related to the morphological development of the parasite (Lambert and Maillard, 1974, 1975).

Table III. Schematic representation of distribution of ***Diplectanum aequans*** on the gills of sea bass ***Dicentrarchus labrax***. Gills Dx: right branchial archs; Gills Sx: left branchial archs; Ant: anterior hemi-branchia; Post: posterior hemi-branchia; 1,2,3,4: gills (anter-posterior axis); I, M, E: internal, middle, external portion (medium-lateral axis); I, II, III, IV: dorsal→ventral portion (dorso-ventral axis) (by Lambert Lambert and Maillard, 1975)

Adult forms

		gills Dx						gills Sx					
		ant			post			ant			post		
		I	M	E	I	M	E	I	M	E	I	M	E
111	I	1	12	34	1	1	4	1	6	35		1	5
	II	1	12	44	3	1	4	6	4	53	3	3	10
	III	1	4	26	1	4	6	3	4	24	3	2	4
	IV		5	17		2	6	1	3	16	1	4	7
121	I		2	18		6	4		5	24		4	13
	II	2	5	31		5	24	2	3	37	4	4	15
	III	1	4	22	1	6	11		8	27	1	10	13
	IV	4	3	14	2	6	21		3	4		4	15
131	I	1	3	9	1	2	10	2	3	8		8	7
	II		1	7	1	4	20	2	1	8		10	14
	III	4	11	4		5	29	2	4	7	2	6	15
	IV	2	3	5	1	4	21	1	1	2	2	2	13
141	I		1	6	1	6	32	1	2	10		2	23
	II	3	4	4	4	13	11		7	6	4	7	13
	III	1	17	4	1	2	15		5	7		3	11
	IV		6	4	1	3	6		2	1		3	13

Juvenile forms

		gills Dx						gills Sx					
		ant			post			ant			post		
		I	M	E	I	M	E	I	M	E	I	M	E
111	I		2					2					
	II		4		4	5		5	2		3	4	2
	III	3	1		5			4		1	4	2	
	IV				1						6	1	2
121	I		1	1	2			1	1		3		
	II	5	2		8	3		6	3		9	1	1
	III		4		12	8		2	1		5	2	
	IV	3	3		4	2		4	4		10	3	
131	I	1	2		1	2		5	4			3	
	II	6	2		6	4	1	4	3		2		
	III	1			5	3		6	3		6	6	
	IV	3	3		4	1		1	1		7	4	
141	I				3	2			1		1	5	1
	II	1	1		6	5		3	1		5	3	
	III	3			3	5		4	4		2		
	IV	3	1			3	1	3	2		1	2	

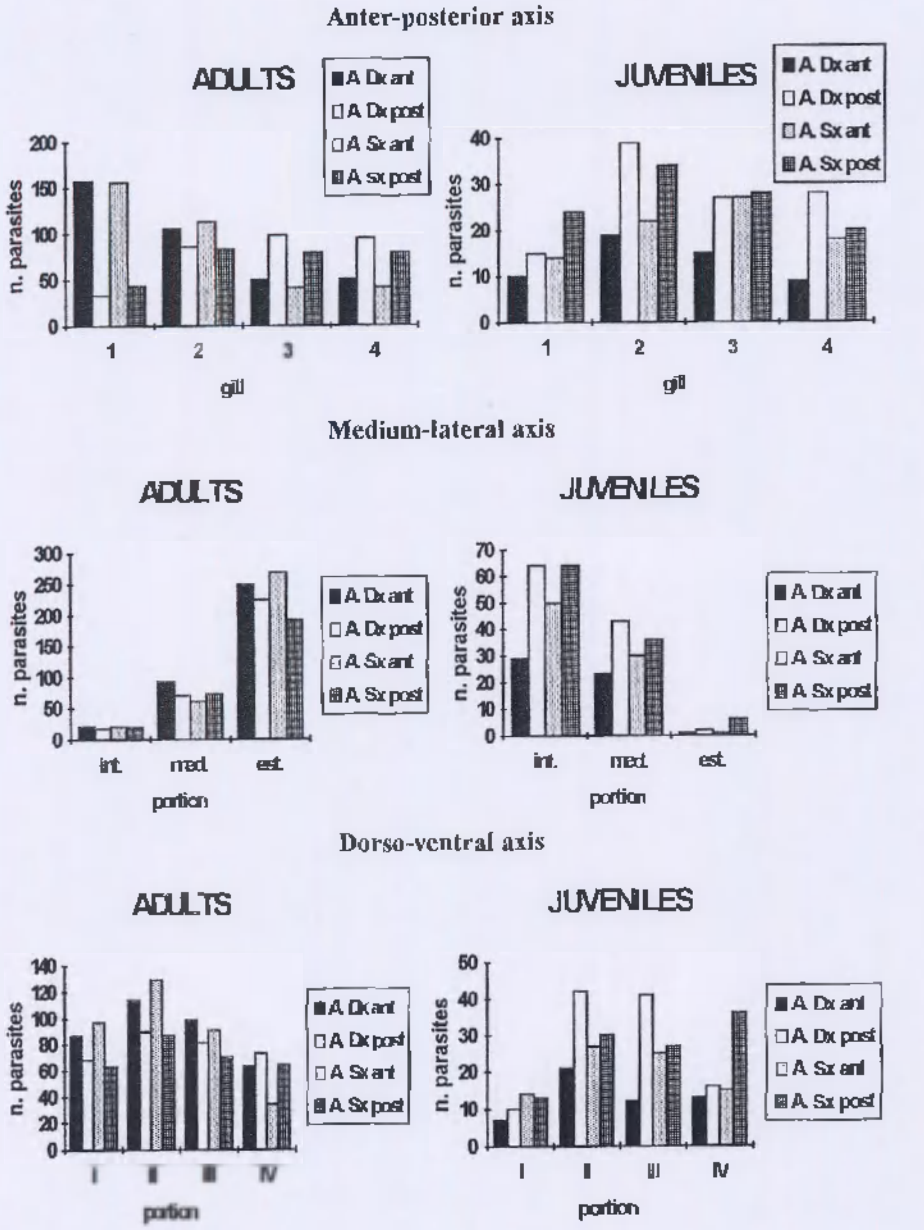


Fig. 1. Distribution of *D. aequans* on sea bass gills along the three axes considered. For explanation of symbols related to gill portions cf. text.

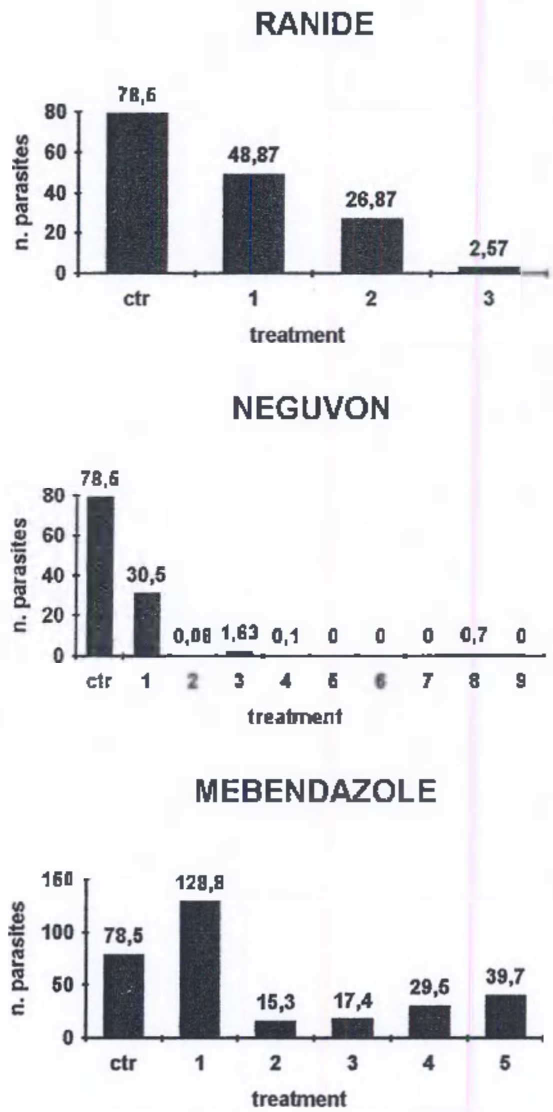


Fig. 2. Average presence of parasites (*D. aequans*) found on the four left branchial archs after treatments with Ranide, Neguvon, mebendazole. Note: ctr = control, for supplementary explanation cf. text.

Table IV. Antiparasitic treatments

Product	Trial	No. subjects	°C	S ‰	Dosage and duration	Count effected	Results (parasites present on BASx) ($\overline{X}$ = the mean)
Ranide®	1	8	16	7	60ppm/24h	After 24h	391 (max 146, min 7), $\overline{X}$=48.87
	2	8	16	7	60ppm/48h	After 24h	215 (max 36, min 10), $\overline{X}$=26.87
	3	7	15.5	7	6ppm/48h then 60ppm/24h	After 24h	18 (max 5, min 1), $\overline{X}$=2.57
Neguvon®	1	6	14	8	0.5%/15min.	After 24h	183 (max 112, min 5), $\overline{X}$=30.50
	2	12	15	8	7ppm/65h	After 24h	6 dead fish, 6 live fish 1 parasite, $\overline{X}$=0.08
	3	8	15	8	5ppm/24h	After 24h	3 dead fish, 5 live fish 13 (max 6; min 0), $\overline{X}$=1.63
	4	10	16.5	8	1ppm/48h	After 48h	1 parasite, $\overline{X}$=0, 10
	5	10	15.5	7	0.5ppm/48h	After 48h	/
	6	10	14.5	10	0.25ppm/48h	After 48h	/
	7	10	15	10	0.15ppm/48h	After 48h	/
	8	4 000/40mc*	16.5	5	0.30ppm/24h	After 48h on 10 subjects	7 (max 3, min 0), $\overline{X}$=0.70
	9	8 000/105mc**	16.5	5	0.20ppm/48h	After 48h on 10 subject	/
Mebendazole®	1***	5	16	5	25ppm/48h	End of treatment	644 (max 232, min 11),
	2****	10	15	7	5ppm/48h	After 48h	X=128.80
	3****	10	14.5	7	1ppm/48h	After 24h	153 (max 68, min 1), $\overline{X}$=15.30
	4****	10	14	11	5ppm/72h	After 48h	174 (max 162, min 0), $\overline{X}$=17.40
	5****	10	16.5	8	1ppm/72h	After 48h	295 (max 246, min 0), $\overline{X}$=29.50
							397 (max 119, min 0), $\overline{X}$=39.70

* Population under consideration.
** Infested population similar characteristics in adjacent tank.
*** Without solvent.
**** Dissolved in ethanol.

As reported in therapy Fig. 2, Ranide® showed to be only partially active against the parasite on different trials; the best response, even if incomplete, wag obtained after a first medical bath in 6ppm of pharmaceutical product (referred to its active principle) in 48h, followed by a second medical bath in 60ppm/24h (cf. Table IV, Trial No. 3).

Treatments with Neguvon® medical baths also responded differently to the various times and dosages. The best responses were obtained at very low doses (between 0.5 and 0.15ppm) during a time span of 48h (cf. Table IV, Trials No. 5, 6, 7). In fact, the result was complete disinfestation in treated fish. The trial, repeated in a $105m^3$ tank on 8 000 subjects, had the same successful result.

On the contrary, mebendazole did not respond positively (cf. Table IV, Trials No. 2, 3, 4, 5) even with altered dosages and times; moreover, the drug (not being soluble in water) must be employed only after solubilization in ethanol.

Conclusions

Observations on the distribution of *D. aequans* on sea bass have lead to the conclusion that the localization of these organisms at the branchial level is not random, but follows a pattern of preferential sites that seems to depend on both the development stage of the parasite and the differential water flow through various parts of the branchial respiratory apparatus.

The pharmaceutics which resulted to be most active against *D. aequans* were Neguvon®. Optimal response to the drug - complete disinfestation of treated fish - was obtained at very low doses. The use of such a low dosage would minimize the quantity of residues released and therefore the drug's effect on the environment.

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