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INVESTIGATIONS INTO THE EFFECTS OF CONCENTRATION AND DURATION OF EXPOSURE TO FORMALIN AND MALACHITE GREEN ON THE SURVIVAL OF THE LARVAE AND JUVENILES OF THE COMMON CARP *CYPRINUS CARPIO* L. AND THE SHARPTOOTH CATFISH *CLARIAS GARIEPINUS* (BURCHELL)

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ABSTRACT

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Prophylactic dip treatments using formalin and malachite green were applied to 4-day old larvae and 12- and 20-day old juveniles of the European common carp, *Cyprinus carpio* and the African sharptooth catfish, *Clarias gariepinus*. Treatments consisted of 100 mg/ℓ malachite green for exposure periods of 10, 30 or 90 s and 200 mg/ℓ formalin, administered for 30, 60 or 90 min. Larvae and juveniles of *C. gariepinus* could be treated with 100 mg/ℓ malachite green for 10 s, or with 200 mg/ℓ formalin for 30 min, with minimum mortalities. Both chemicals affected the survival of the *C. gariepinus* juveniles, especially the 90 min exposure to formalin. Juveniles of both species were extremely sensitive to 100 mg/ℓ malachite green concentrations.

INTRODUCTION

The cultivation of the sharptooth catfish, *Clarias gariepinus* has received considerable attention because of its aquaculture potential, both in South Africa and in countries such as the Netherlands (Safriel & Bruton, 1984; Huisman & Richter, 1987). Despite progress in the culture of *C. gariepinus*, work on the treatment against diseases experienced in the various developmental stages, especially during the larval and juvenile stages, has been largely neglected. In the present study, investigations were made into the concentrations and/or application periods, at which formalin and malachite green could safely be used to treat larvae and juveniles of *C. gariepinus* and *C. carpio*, in the event of outbreaks of ectoparasitic diseases during the large-scale production of their larvae and juveniles in the hatchery.

MATERIALS AND METHODS

An aquarium set-up similar to that employed by Theron, Prinsloo & Schoonbee (1991) for the treatment of embryos of *C. carpio* and *C. gariepinus* was used in the present investigation. The spawning of both species was done according to Schoonbee & Prinsloo (1984, 1986). For the rearing of the larvae the same procedures were followed as those described by Prinsloo & Schoonbee (1986) and Polling, Schoonbee, Prinsloo & Wiid (1988).

Four-day old larvae and 12- and 20-day old juveniles (Balon, 1984) were used to evaluate the possible short-term effects of different concentrations and duration of exposure to formalin and malachite green on their survival. Due to their sensitivity to handling, the 4-day old *C. carpio* larvae were excluded from the dip treatment trials.

A small hand net was used to collect random samples of the larvae and juveniles of both species from the different holding tanks. With the net still in the tank, a small plastic dish was used to transfer the required number of specimens to separate beakers containing well-aerated, acclimated water. A total of 100 specimens per dish were counted out in this way. Those specimens showing signs of handling stress were replaced. Triplicate sets of samples for each treatment were prepared in this way.

TABLE 1 Concentrations used and duration of dip treatment followed for *Cyprinus carpio* and *Clarias gariepinus* larvae and juveniles

Chemical	Concentration mg/ℓ	Duration of treatment s (malachite green) min (formalin)		
Malachite green	100	10	30	90
Formalin	200	30	60	90

The following procedure was followed in the dip treatment of the larvae and juveniles of both species:

1. Gentle transfer of larvae/juveniles from beakers into small hand nets.

2. Simultaneous dip treatment of specimens in each hand net into dip mixture for the specified treatment period (Table 1).

3. Careful rinsing in fresh, aerated, acclimated water kept at 25 ±1,0 °C of the larvae or juveniles in the hand nets following treatment.

4. Transfer after treatment of each set of 100 larvae or juveniles from the hand nets into their own post-treatment aquariums, which were provided with aeration and kept at 25 ±1,0 °C.

As a control, replicates of 100 untreated larvae and juveniles of each species were dipped for 90 min in clean acclimated water, after which they were transferred to separate aquariums provided with well aerated, clean maturated water, kept at the same temperature as that used for the experimental aquariums. Further observations were then also made on growth performance and survival of the larvae and juveniles for each specific treatment, conducted for periods lasting for a minimum of 11 days *(C. gariepinus)*, and a maximum of 21 days *(C. carpio)*. Subsamples of not less than 20 % were taken halfway through the various observation periods for mass determinations. The size of each subsample was determined by the number of survivors following each treatment.

To determine the mean mass increments amongst juveniles after the various treatments, subsamples were taken at 8 d after treatment in each case. In both cases all remaining juveniles were sacrificed and mass-measured on the final day of observation.

The larvae and juveniles in all the experimental aquariums were fed twice daily with live zooplankton consisting of rotifers, mainly *Brachionus* spp. and Cladocera, mainly *Moina* spp. Application of food was according to Polling *et al.* (1988). Aquari-

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TABLE 2 The effects of 100 mg/ℓ malachite green and 200 mg/ℓ formalin concentrations on the survival of 4-day old *Clarias gariepinus* larvae subjected in each case to three exposure periods, as measured 72 h after treatment. Each treatment done in triplicate, at a water temperature of 25 ± 1,0 °C

Treatment type and concentration	Duration of treatment	Cumulative mean % mortalities (± S.D.) after 72 h	% mortalities	
			Min	Max
Malachite green 100 mg/ℓ	10 s	0,7 ± 0,47	0	1
	30 s	99,7 ± 0,47	99	100
	90 s	100,0 ± 0,00	100	100
Formalin 200 mg/ℓ	30 min	0,7 ± 0,47	0	1
	60 min	1,7 ± 1,70	0	4
	90 min	7,3 ± 4,19	3	13
Control	90 min	0,0 ± 0,00	0	0

S.D. = Standard deviation

TABLE 3 The effects of 100 mg/ℓ malachite green and 200 mg/ℓ formalin on the survival of 12-day old *Cyprinus carpio* and *Clarias gariepinus* junveniles, subjected in each case to three exposure periods of treatment, as measured 72 h after treatment. Each treatment done in triplicate, at a water temperature of 25 ±1,0 °C

Treatment type and concentration	Duration of treatment	Cumulative mean % mortalities after 72 h (± S.D.)		% mortalities			
				Min	Max	Min	Max
		C. carpio	*C. gariepinus*	*C. carpio*		*C. gariepinus*	
Malachite green 100 mg/ℓ	10 s	21,0 ± 8,00	0,0 ± 0,00	13	29	0	0
	30 s	80,7 ± 1,70	74,7 ± 25,77	79	83	39	99
	90 s	99,3 ± 0,47	100,0 ± 0,00	99	100	100	100
Formalin 200 mg/ℓ	30 min	23,0 ± 11,34	0,0 ± 0,00	14	39	0	0
	60 min	33,3 ± 11,15	1,0 ± 0,82	24	49	0	2
	90 min	67,0 ± 7,26	19,3 ± 7,54	57	74	14	30
Control	90 min	0,0 ± 0,00	0,0 ± 0,00	0	0	0	0

S.D. = Standard deviation

ums were cleaned either during the early morning before the first feeding time or before the second when faeces and dead zooplankton were removed by suction tube. Depending on the condition in each aquarium, 10–50 % of the water was replaced every day with clean, acclimated water kept at 25 ±1,0 °C.

Analysis of selected chemical parameters of the acclimated water used during the treatment trials were done according to APHA (1980). The water quality was also monitored in the post-treatment aquariums of the *C. gariepinus* juveniles, at 3 different periods, namely:

1. After cleaning and refilling of the aquariums with acclimated water but before the afternoon feeding, i.e. when relatively clean conditions prevailed.

2. In the morning before the first feeding. Food had thus been in the aquariums since the previous afternoon.

3. In the afternoon, after the morning feeding programme and just before the aquariums were again cleaned, i.e. at the time when conditions were the worst that they were likely to become.

RESULTS

Exposure of 4-day old larvae of C. gariepinus *to malachite green and formalin*

As mentioned earlier, mortalities caused by handling stress did not allow for dip treatments of 4-day old *C. carpio* larvae. It was, however, possible to use *C. gariepinus* larvae of the same age to evaluate their sensitivity to 100 mg/ℓ malachite green and 200 mg/ℓ formalin respectively, after three different exposure periods (Table 2). A 10-s exposure to 100 mg/ℓ malachite green caused virtually no mortalities amongst the 4-day old catfish larvae (Table 2). However, mortalities increased dramatically after an exposure period of 30 s, when more than 99 % of the larvae died within 24 h. A 90-s exposure period resulted in a 100 % mortality rate. No mortalities occurred amongst the control groups of fish (Table 2).

The 4-day old larvae showed a survival of more than 99 % when exposed for 30 min to 200 mg/ℓ formalin. Even for a 60-min exposure period, the survival was still exceptionally good, with mortalities remaining below 2 %. It was only during the 90-min exposure period that the 200 mg/ℓ formalin concentration resulted in larval mortalities of more than 7 % within 72 h after treatment (Table 2).

Exposure of 12-day old juveniles of C. carpio *and* C. gariepinus *to malachite green and formalin*

A treatment period of 10 s with 100 mg/ℓ malachite green resulted in a mean mortality rate of 21 % (Table 3) for the 12-day old *C. carpio* juveniles. After an exposure period of 30 s, this concentration led to a mortality incidence of 80 %, whilst a 90 s exposure period killed virtually all the 12-day old juveniles (99,3 % mortality, Table 3). In the case of the 12-day old *C. gariepinus* juveniles, there were no mortalities after a 10-s exposure to 100 mg/ℓ malachite green (Table 3). There was, however, a dramatic rise in juvenile mortalities of this fish, amounting to almost 75 % within 24 h, when exposed for a 30-s period to this concentration. The mortality rate increased to 100 % when the 12-day old catfish juveniles were subjected to 100 mg/ℓ malachite green for 90 s. The 12-day old juveniles of *C. carpio* appear to be more sensitive to a 10-s exposure to 100 mg/ℓ malachite green than *C. gariepinus*.

TABLE 4 The effects of 100 mg/ℓ malachite green and 200 mg/ℓ formalin on the survival of 20-day old *Cyprinus carpio* and *Clarias gariepinus* juveniles subjected in each case to three exposure periods of treatment, as measured 72 h after treatment. Each treatment done in triplicate at a water temperature of 25 ± 1,0 °C

Treatment type and concentration	Duration of treatment	Cumulative mean % of mortalities (S.D.) after 72 h		% mortalities			
				Min	Max	Min	Max
		C. carpio	*C. gariepinus*	*C. carpio*		*C. gariepinus*	
Malachite 100 mg/ℓ	10 s	29,7 ± 5,31	17,0 ± 10,23	23	36	5	30
	30 s	60,0 ± 7,26	47,3 ± 9,39	48	70	36	59
	90 s	99,3 ± 0,94	96,7 ± 3,30	98	100	92	99
Formalin 200 mg/ℓ	30 min	1,3 ± 1,25	2,0 ± 2,65	1	3	0	5
	60 min	6,0 ± 3,27	16,3 ± 6,24	2	10	8	23
	90 min	6,3 ± 5,44	61,0 ± 14,76	2	14	44	80
Control	90 min	0,0 ± 0,00	1,3 ± 0,94	0	0	0	2

S.D. = Standard deviation

TABLE 5 Calculated and empirical mean mass (in milligrams) of the surviving malachite green and formalin treated 12-day old *Cyprinus carpio* juveniles, to determine any possible short-term after-effects following treatment

Treatment type and concentration	Duration of treatment	% survival 3d after treatment	Mean mass ± S.D. 8 d post-treatment	Min	Max	C.V.	% survival 21 d post-treatment	Mean mass ± S.D. 21 d post-treatment	Min	Max	C.V.
Malachite green 100 mg/ℓ	10 s	79,5	55,4 ± 24,1	28,9	108,4	43,5	73,0	167,3 ± 101,0	22,7	610,5	60,4
	30 s	19,3	—	—	—	—	15,7	622,0 ± 406,1	153,7	1 623,2	65,3
	90 s	0,0	—	—	—	—	—	—	—	—	—
Formalin 200 mg/ℓ	30 min	77,0	49,7 ± 30,0	15,9	154,0	60,4	71,3	169,8 ± 117,0	34,7	1 002,3	68,9
	60 min	66,7	44,3 ± 16,3	23,0	85,5	36,7	62,3	192,2 ± 119,0	50,7	774,1	61,9
	90 mm	33,7	108,2 ± 40,6	34,5	180,5	37,5	28,0	385,7 ± 182,5	66,2	897,4	47,3
Control	90 min	100,0	46,9 ± 68,4	15,0	240,6	145,7	94,0	115,5 ± 69,6	43,4	379,3	60,2

S.D. = Standard deviation

C.V. = Coefficient of variability (of the mass)

Exposure of 12-day old *C. carpio* juveniles for 30 min to 200 mg/ℓ formalin resulted in a mean mortality rate of 23 %. This increased to 33 % for the 60-min exposure period and to 67 % following a 90-min exposure. For the 12-day old *C. gariepinus* juveniles subjected for 30 min to 200 mg/ℓ formalin, the survival rate was still 100 %. A mean mortality of 1 % was recorded for the 60-min exposure period. After the 90-min exposure to 200 mg/ℓ formalin, mortalities increased to more than 19 % (Table 3). Results therefore showed that the 12-day old *C. carpio* juveniles were far more sensitive to 200 mg/ℓ formalin treatment than was the case for the same age *C. gariepinus* juveniles. It was only after the 90-min dip treatment that significant mortalities began to occur amongst the *C. gariepinus* juveniles.

Exposure of 20-day old juveniles to malachite green and formalin

It is evident from Table 4 that 20-day old *C. carpio* juveniles, when exposed to 100 mg/ℓ malachite green for 10-s periods had a mortality rate of more than 29 % within 72 h after treatment. When exposed to this same concentration for 30 s, mortalities of *C. carpio* juveniles increased to 60 %, and to nearly 100 % for those treated for 90 s. Similarly, the 20-day old *C. gariepinus* juveniles exposed to this concentration of malachite green already showed a mean mortality rate of 17 % for the 10-s exposure period, increasing to more than 47 % for the 30-s exposure period and to almost 97 % when exposed for 90 s to a 100 mg/ℓ malachite green concentration.

The sensitivity of the 20-day old *C. carpio* juveniles to the same concentration of formalin as used in the dip treatment of the 12-day old juveniles showed a marked decline for all 3 periods of treatment, with almost 94 % of the juveniles surviving the 90-min exposure preiod. Here again the survival of the remaining specimens remained virtually the same when observed over a period of 72 h following treatment. The 20-day old *C. gariepinus* juveniles, on the other hand, appeared to be the most sensitive of the different larval and juvenile groups of this species when subjected for the different exposure periods to 200 mg/ℓ formalin. Where a 30-min exposure to formalin resulted in a 2 % loss of juveniles, mortalities increased to more than 16 % after the 60-min exposure period, rising to a 61 % mortality rate among 20-day old juveniles after a 90-min exposure period.

Observations on the survival and growth of the 12- and 20-day old juveniles of C. carpio *and* C. gariepinus *following treatment with formalin and malachite green*

From the results on the different malachite green treatments of the 12-day old *C. carpio* juveniles (Table 5), it can be deduced that those juveniles which survived the period of 72 h after treatment, virtually all remained alive for the rest of the observation period. A similar situation was also recorded for the various treatments with 200 mg/ℓ formalin. The percentage survival of the control group of carp juveniles, namely 94 % (Table 5), suggests that mortalities that may have occurred in the post-treatment period among the malachite green and formalin-treated juveniles might be attributable mainly to environmental factors such as handling rather than to the after-effects of the treatments with the chemicals.

TABEL 6 Calculated and empirical mean mass (in milligrams) of the surviving malachite green and formalin treated 20-day old *Cyprinus carpio* juveniles, to determine any possible short-term after-effects following treatment

Treatment type and concentration	Duration of treatment	% survival 3d after treatment	Mean mass ±S.D. 8 d post-treatment	Min	Max	C.V.	% survival 21 d post-treatment	Mean mass ±S.D. 21 d post-treatment	Min	Max	C.V.
Malachite green 100 mg/ℓ	10 s	70,0	127,5 ± 57,6	47,7	288,6	45,2	62,0	194,2 ± 86,3	48,5	521,1	44,6
	30 s	40,0	207,6 ± 56,3	137,4	329,5	27,1	38,7	342,0 ± 158,3	129,2	806,8	46,3
	90 s	1,3	—	—	—	—	1,3	2 510,7 ± 279,5	2 239,5	2 841,3	11,1
Formalin 200 mg/ℓ	30 min	98,7	78,3 ± 40,5	32,6	193,5	51,7	93,7	135,8 ± 76,3	40,2	735,3	56,2
	60 min	94,0	69,2 ± 30,8	25,0	156,8	44,5	90,0	151,1 ± 83,3	33,2	534,9	55,1
	90 mm	93,7	96,8 ± 61,8	37,6	296,3	63,8	89,7	154,0 ± 82,5	23,2	579,0	53,6
Control	90 min	100,0	79,1 ± 30,9	43,2	145,7	39,0	99,0	123,5 ± 72,6	21,2	541,1	58,5

S.D. = Standard deviation

C.V. = Coefficient of variability (of the mass)

Control group dipped in clean, acclimated water

TABLE 7 Calculated and empirical mean mass (in milligrams) of the surviving malachite green and formalin treated 4-day old *Clarias gariepinus* larvae, to determine any possible short-term after-effects following treatment

Treatment type and concentration	Duration of treatment	% survival 3 d after treatment	Mean mass ± S.D. 8 d post-treatment	Min	Max	C.V.	% survival 12 d post-treatment	Mean mass ± S.D. 12 d post-treatment	Min	Max	C.V.
Malachite green 100 mg/ℓ	10 s	99,3	70,9 ± 22,2	42,7	110,0	31,3	80,3	92,3 ± 84,6	25,1	966,7	91,7
	30 s	0,3	—	—	—	—	0,3	233,9 ± 0,0	—	—	—
	90 s	0,0	—	—	—	—	—	—	—	—	—
Formalin 200 mg/ℓ	30 min	99,3	68,4 ± 20,3	26,6	120,4	29,7	83,3	88,2 ± 28,4	16,4	202,1	32,2
	60 min	98,3	54,3 ± 19,5	22,7	102,8	35,8	90,7	75,7 ± 28,1	21,1	165,1	37,2
	90 min	92,7	162,1 ± 90,9	82,1	317,0	56,0	23,3	184,7 ± 90,4	52,0	544,0	49,0
Control	90 min	100,0	47,2 ± 21,5	12,0	88,0	45,6	91,7	72,8 ± 26,9	16,8	251,5	36,9

S.D. = Standard deviation

C.V. = Coefficient of variability (of the mass)

Where the lowest numbers of *C. carpio* juveniles survived specific treatments, e.g. 100 mg/ℓ malachite green for 30 s (Table 5), the best individual mean growths were obtained at the end of the 21 d observation period. The most servere treatment, therefore, did not show any deleterious after-effects on the growth performances of such juveniles. In most cases, a comparison of the variation in growth of the juveniles, as determined at 8 and 21 d after treatment, suggests an increase in variability of size with time, a phenomenon which is normally observed amongst healthy, untreated juveniles of this species.

In the case of the 20-day old *C. carpio* juveniles (Table 6), it can be deduced from the results that the treatment with both malachite green and formalin again did not appear to have any harmful after-effects during the observation period of 21 d. In all groups of juveniles there were significant increases in their mean mass between day 8 and 21 following the dip treatments.

The data for the control group of juveniles still showed a 99 % survival rate at the end of the observation period. There was no sign of increased variability in mass at the time of subsampling or towards the end of the 21-day observation period (Table 6).

Growth performances of the 4-day old C. gariepinus *larvae exposed to 100 mg/ℓ malachite green or 200 mg/ℓ formalin*

The 30- and 90-s treatments with 100 mg/ℓ malachite green proved to be fatal for the 4-day old *C. gariepinus* larvae with only a low survival of 0,3 % (Table 7). The few remaining juveniles, however, survived until the termination of the experiment, 12 d after treatment. In the case of formalin, an interesting and important phenomenon was observed. At 30- and 60-min exposures to 200 mg/ℓ formalin, the initial survival of juveniles following treatment was exceptionally good. This also applied to the juveniles subjected to the 90-min formalin treatment, where a 92 % survival was recorded 3 d after treatment. However, the survival of the latter juveniles declined to only 23,3 %, twelve days later. This suggests a possible delayed negative post-treatment effect on the larvae and juveniles of *C. gariepinus,* substantiated by a comparison with the survival rates of the control group (Table 6).

In the case of the 12-day old *C. gariepinus* juveniles, the 100 mg/ℓ malachite green treatment for 90 s appeared to be extremely toxic (Table 8). When analysing their survival, it appears that those individuals that survived the 10- or 30-s exposure to 100 mg/ℓ malachite green were also reasonably free of any after-effects, since virtually no further mortalities were recorded. There was, however, a decline in the mean mass of both groups of juveniles between day 8 and 11.

In the groups of formalin-treated 12-day old *C. gariepinus* juveniles (Table 8), there was a serious decline in numbers among those juveniles exposed to 200 mg/ℓ formalin for 90 min during the 11-day period following treatment. Survival was, however, slightly better than for the 4-day old larvae subjected to the same treatment (44,3 % compared to 23,3 %; Tables 7 & 8). Values for the coefficient of variability suggest a reasonably uniform size range amongst all surviving treated juveniles. There was practically a standstill in mean mass for all three groups of juveniles between day 8 and 11 following treatment with formalin (Table 8).

The 20-day old juveniles of *C. gariepinus* exposed for 90 s to 100 mg/ℓ malachite green (Table 9), again showed the most serious deleterious effects on their survival. There was, however, an improvement in

TABLE 8 Calculated and empirical mean mass (in milligrams) of the surviving malachite green and formalin treated 12-day old *Clarias gariepinus* juveniles, to determine any possible short-term after-effects following treatment

Treatment type and concentration	Duration of treatment	% survival 3 d after treatment	Mean mass ±S.D. 8 d post-treatment	Min	Max	C.V.	% survival 11 d post-treatment	Mean mass ± S.D. 11 d post-treatment	Min	Max	C.V.
Malachite green 100 mg/ℓ	10 s	100,0	110,7 ± 37,9	42,9	195,3	34,3	93,3	91,5 ± 34,5	34,4	290,7	37,7
	30 s	25,3	170,4 ± 87,1	88,3	300,2	51,1	25,3	159,6 ± 87,5	50,8	410,9	56,1
	90 s	0,0	—	—	—	—	—	—	—	—	—
Formalin 200 mg/ℓ	30 min	100,00	85,6 ± 34,6	37,9	195,6	40,4	96,0	93,5 ± 33,0	38,2	277,9	35,3
	60 min	99,0	87,4 ± 31,5	37,2	168,3	35,9	84,7	85,5 ± 36,5	22,5	327,9	42,7
	90 min	80,7	120,4 ± 46,6	61,6	251,3	38,7	44,3	118,5 ± 56,1	42,7	371,5	47,3
Control	90 min	100,0	107,7 ± 37,2	36,7	187,2	34,6	80,0	101,6 ± 40,0	28,6	270,1	39,4

S.D. = Standard deviation
C.V. = Coefficient of variability (of the mass)

TABLE 9 Calculated and empirical mean mass (in milligrams) of the surviving malachite green and formalin treated 20-day old *Clarias gariepinus* juveniles, to determine any possible short-term after-effects following treatment

Treatment type and concentration	Duration of treatment	% survival 3d after post-treatment	Mean mass ±S.D. 8 d post-treatment	Min	Max	C.V.	% survival 13 d post-treatment	Mean mass ± S.D. 13 d post-treatment	Min	Max	C.V.
Malachite green 100 mg/ℓ	10 s	83,0	141,1 ± 77,1	56,6	453,6	54,6	80,7	186,5 ± 103,1	53,2	791,1	55,3
	30 s	52,3	197,8 ± 93,9	101,7	444,4	47,5	50,0	246,0 ± 120,9	68,2	731,1	49,1
	90 s	3,3	—	—	—	—	2,3	438,3 ± 196,0	280,1	866,4	44,7
Formalin 200 mg/ℓ	30 min	98,0	157,3 ± 100,5	58,0	484,7	63,9	97,0	171,5 ± 77,3	52,8	502,5	45,1
	60 min	83,7	163,9 ± 84,7	50,7	386,6	51,7	73,0	186,3 ± 105,1	47,5	877,8	56,4
	90 min	39,0	257,6 ± 141,4	55,7	517,4	54,9	39,0	224,2 ± 123,9	50,5	834,0	55,2
Control	90 min	98,7	168,0 ± 77,7	72,0	396,9	46,2	86,7	209,0 ± 122,0	63,9	1 213,8	58,4

S.D. = Standard deviation
C.V. = Coefficient of variability (of the mass)

TABLE 10 Physical and chemical conditions of the water in 6 grow-out aquariums stocked with malachite green and formalin treated 30-day old *Clarias gariepinus* juveniles, 9–10 d after introduction. Variations in water quality conditions were determined at 15 h 00 (day 9) after removal of waste material, at 08 h 00 (day 10) before the first feeding commenced and again at 14 h 00 (day 10) before the aquariums were cleaned

Analysis		Acclimated water (n = 6) $\bar{x}$	Range	Day 9 at 15 h 00 (n = 6) $\bar{x}$	Range	Day 10 at 08 h 00 (n = 6) $\bar{x}$	Range	Day 10 at 14 h 00 (n = 6) $\bar{x}$	Range
Conductivity µs/cm		80	78–84	88	86–90	107	104–108	113	110–117
pH			7,38–7,43		7,38–7,47		7,29–7,48		7,49–7,79
Dissolved oxygen	Expressed in mg/ℓ	6,4	5,9 –6,7	6,0	5,9 –6,2	6,3	6,0 –6,6	5,9	5,3 –6,5
Nitrate (NO_3)		0,44	0,37 –0,49	5,13	4,84 –5,28	5,87	5,28 –6,60	6,01	5,72 –6,60
Nitrite (NO_2)		0,023	0,020–0,032	0,059	0,026–0,092	0,067	0,017–0,119	0,077	0,050–0,106
Ammonia (NH_3)		0,037	0,030–0,041	1,830	1,720–1,891	3,843	3,611–4,002	4,107	3,831–4,465
Phosphate (PO_4)		0,150	0,146–0,161	0,933	0,696–1,140	1,347	1,299–1,396	2,130	1,800–2,390
Alkalinity as $CaCO_3$		18	17–20	27	26–27	34	31–36	37	35–39
Ca hardness as $CaCO_3$		16	15–18	19	16–23	21	21–21	23	22–25
Total hardness as $CaCO_3$		20	18–21	25	24–26	31	28–34	32	31–33

survival rate for those treated for 30 s at this concentration compared to that of the 4- and 12-day old larvae and juveniles receiving the same treatments (compare Tables 7, 8 and 9).

Formalin at 200 mg/ℓ for a 90-min exposure period seriously affected the survival of juveniles of *C. gariepinus* immediately after treatment, but at this age (20 d post-hatching), they appeared to be more resistant than the equivalent 4- and 12-day old groups. Those juveniles that survived the various treatments did not show any further after-effects on growth during the observation period (Table 9). The coefficients of variability for mass remained fairly constant amongst all the treated groups when compared to those of the control group (Table 9).

Observations on water quality in the aquariums of the C. carpio *and* C. gariepinus *juveniles following treatment with malachite green and formalin*

The water quality in the aquariums containing the treated 20-day old *C. gariepinus* juveniles was monitored to ensure that no dangerous build-up of metabolities occurred, which could have influenced the growth or even the health of these fish. An increase in the conductivity of the holding water occurred over a period of 24 h (Table 10), suggesting a decay of dead organic material, as well as the possible release of metabolic wastes by the juveniles present in the aquariums. Aeration of the water was sufficient to maintain a dissolved oxygen level of approximately 6,0 mg/ℓ over a 24-h cycle, with pH values showing little variation, remaining between 7 and 8. Values for ammonia showed drastic increases during those times in the feeding cycle when the waste material was not removed from the aquariums, with corresponding increases but slightly lower values for nitrate and nitrite (Table 10). Values for phosphate followed a similar pattern as those for ammonia, with marked increases during those stages in the feeding cycle when there was an accumulation of organic wastes in the aquariums. The alkalinity and total hardness in the water increased in a manner

similar to that for nitrate and phosphate, but did not appear to build up to exceptionally high levels. The dilution with replacement water, after cleaning of the aquariums each day, contributed towards the periodic lowering of values for alkalinity and hardness as well as the declines in concentration of ammonia, nitrate and phosphate.

The practice followed in the removal of waste material from the aquariums, which coincided with the replacement of some of the water once in every 24-h cycle, thus resulted in the maintenance of relatively good water quality standards, as reflected by the present set of physical and chemical results (Table 10). This is of particular importance if it is taken into account that the measurements were made 9–10 d after the original introduction of the juveniles into the aquariums and that the water in the aquariums was not connected to a recirculating biological filtration system.

DISCUSSION

Post (1983) states that chemicals used for controlling or killing parasites may also exert antibiological effects on the hosts being treated. These chemicals are used at concentrations which do not permanently harm the fish, but which may exert an antifungal, antibacterial or antiparasitic effect. It is, therefore, understandable that the correct prophylactic and therapeutic concentrations, and period of application for a particular chemical, can only be determined through experimentation. These concentrations can, however, be very near the lethal limit for some fish species. Thus the chosen chemical should preferably have a large safety index and should not produce more than temporary toxicity in the host. Such transient toxicity should also be repairable and reversible, and residues of the chemical must be rendered harmless within a reasonable time after application.

The results of the treatment of the 12- and 20-day old *C. carpio* juveniles revealed that malachite green at 100 mg/ℓ is not suitable for prophylactic dip treatments of young fish against fungal and ectoparasitic diseases because of the effects on the host. Post (1983) recommended a 1 h formalin dip treatment at a concentration of 250 mg/ℓ against external pathogens of fish. During the present investigation, a formalin treatment at 200 mg/ℓ for 1 hour duration proved to be lethal for at least 33 % of the 12-day old juvenils of *C. carpio*. In the case of the 20-day old juveniles however, only a small percentage (10 %), succumbed within 21 d after treatment. Thus juveniles of *C. carpio* are clearly more tolerant of a 200 mg/ℓ formalin treatment at this age for exposure periods of up to 90 min.

Our results showed that the 4-day old *C. gariepinus* larvae can safely be treated with 100 mg/ℓ malachite green for the 10-s exposure period recommended by Post (1983) for other fish species. A slight decline in survival does, however, occur under these circumstances. The same results were obtained for the 12- and 20-day old juveniles of *C. gariepinus,* with a slight drop in survival for the 20-day old juveniles exposed for a 10-s period to a concentration of 100 mg/ℓ malachite green. This exposure period is also listed as safe by Post (1983), and can, according to our results, be used on larvae and juveniles of *C. gariepinus* younger than 20 d. Experience, however, showed that for larvae and juveniles of *C. gariepinus* up to this age, treatment with 100 mg/ℓ malachite green for periods exceeding 10 s should be avoided.

It is important to point out that the development of the suprabranchial membrane and epibranchial organ may to some extent be responsible for an increase in mortality recorded amongst the 20-day old *C. gariepinus* juveniles when subjected to 200 mg/ℓ formalin, compared to the 12-day old juveniles exposed for the same period to this concentration of formalin.

It is doubtful that the decline in the survival rate of the 4-day old larvae and 12-day old *C. gariepinus* juveniles, exposed for 90 min to 200 mg/ℓ formalin, can be attributed to any physiological and/or morphological effects associated with respiratory metamorphosis, as this process has not yet commenced at this stage.

In the attemps to determine any short-term after-effects of malachite green and formalin treatment on the larvae and juveniles of both species, the survivors of the dip treatments were kept under observation for a number of days following treatment. It was, however, not possible to draw any meaningful conclusions from the growth results obtained because of the varied densities under which the surviving juveniles were kept. The lower densities inevitably resulted in a faster rate of growth in those aquariums containing the smaller numbers of specimens which originally survived the treatments.

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