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**Embryonation and efficacy of treatments on
monogenean gill flukes infecting silver kob
(*Argyrosomus inodorus*)**

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Declaration

I hereby declare that all the work presented in this thesis is my own, except where otherwise stated in the text. This thesis has not been submitted for a degree at any other university.

Signed by candidate

31/5/2005

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Date

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1. Abstract

Cultured fish are commonly afflicted by disease caused by monogenean flukes, resulting in significant production losses. *In vitro* trials were conducted to identify an effective treatment against the monogenean flukes *Calceostoma* sp. and *Diplectanum* sp. infecting the silver kob *Argyrosomus inodorus*. Treatments included praziquantel, formalin, freshwater, hydrogen peroxide and copper sulphate. Freshwater was found to be 100% effective on both species of flukes, whereas praziquantel was more effective at a higher concentration. The other treatments were not effective against the flukes and are not recommended for use. The influence of temperature on larval hatching was investigated to determine an appropriate time for follow-up treatments to be administered. Temperature did not influence the larval embryonation period of *Calceostoma* sp. ($r = -0.22$), but had an effect on that of *Diplectanum* sp. ($r = -0.87$). It appears that eggs of *Diplectanum* sp. hatch spontaneously, whereas a cue such as light periodicity or mechanical disturbance may be responsible for the hatching of *Calceostoma* sp. eggs. Follow-up treatments should be administered after 7-12 days to prevent reinfection of *Calceostoma* sp. *Diplectanum* sp. should be treated weekly at high temperatures of around 19 °C, and every three to five days at lower temperatures. A general overview of the genera *Calceostoma* and *Diplectanum* is included to facilitate identification of the monogenean flukes infecting silver kob.

2. Introduction

Mariculture has become more intensive over the last two decades largely due to the introduction of new technologies, greater understanding of the farmed fishes' biology, an increased demand for fish products and an improvement in water quality in farming systems (De Voe 1994). Development in aquaculture has put greater emphasis on the problems posed by parasites and the negative impact they have on the productivity of aquaculture facilities in general (Scholtz 1999). Intensive culture systems expose captive fish to a variety of stressors that may include overcrowding, oxygen deficiency, handling injury and poor water quality. Stress can result in the fish becoming more susceptible to disease, as the confined spaces allow the concentrations of pathogens to be higher than in the natural environment, as well as reducing the natural spatial arrangement between parasite and host and thus increasing the host's chances of infection. It is estimated that 10-20% of fish mortalities in culture facilities are the result of parasite related disease (Bauer *et al.* 1981).

Little is known about the diseases and parasites that infect fish, as compared to those that infect land animals such as sheep and pigs (Whittington *et al.* 2001). Commercial production of fish in tanks or sea-cages has been negatively affected particularly by parasitic monogenean flukes. Flukes are ectoparasites frequently found on the gills, skin or fins of fishes (Reed *et al.* 1996). They have caused worldwide havoc in intensive culture facilities primarily due to their ability to reproduce rapidly. They are

hermaphroditic, have a direct life-cycle and shed their eggs straight into the water column, where upon hatching the free-swimming larvae infect a new host directly (Chisholm & Whittington 2002). Flukes attach themselves to their hosts by means of hooks, suckers or clamps and are frequently host-specific (Barnes *et al.* 1993; Reed *et al.* 1996). They feed on epidermal cells, mucus or blood, and in heavily infected fish this leads to emaciation, lethargy and anaemia. The worms rarely cause death to the host directly in nature, but rather through secondary infection, as a result of bacteria and fungi associated with them (Grimes *et al.* 1985). Gill epithelial hyperplasia, excess mucous production and mechanical damage to the gills, causing respiratory difficulties in fish, have been reported as a result of monogenean flukes in captivity (Sharp *et al.* 2001). It is thus of utmost importance to control monogeneans in culture facilities to prevent disease outbreaks (Buchmann 1998).

Diseases caused by monogeneans have resulted in the need for trials to be conducted to assess the effects of anthelmintics on the flukes (Buchmann 1997). Although fish farming contributes more than 10% of fish produced worldwide no anthelmintics have been developed to specifically combat monogenean flukes in aquaculture (Buchmann 1998). Monogeneans are difficult to eradicate, as the majority of treatments used are not 100% effective, merely reducing parasite numbers. The direct life-cycle allows for the recovery of parasite numbers in a matter of days. Furthermore, some treatments that may be effective on the parasite may be harmful to the host or the environment and thus cannot be used. Hence the importance of

identifying a chemical/treatment that is safe to use on the host and can effectively eliminate the monogeneans to control disease outbreaks in culture environments.

Treatments used in culture facilities must effectively control monogenean flukes to ensure reduced losses in fish, while permitting satisfactory growth and an increase in productivity (Buchmann 1998). In order for treatments to be effective they must be administered at the time of monogenean larval hatching to prevent further infestation. Very little is known of the biology of the gill flukes, therefore it is necessary to investigate the incubation time of the eggs to ensure that treatments administered during a hatching period eradicate the larvae before they infect a new host (Cecchini *et al.* 1998). Infections by gill monogeneans have been observed to recur several weeks after fish have been treated. Ineffective dosages or eggs left in the culture system have been identified as potential causes of this reinfection (Christholm & Whittington 2002). A single bath treatment has no effect on the eggs of monogeneans; therefore a second treatment is required to eradicate the new generation of parasites. Embryonation studies are necessary to determine the exact time when to control larvae just hatched from eggs, but before they start to produce eggs themselves (Cecchini *et al.* 1998). Treating parasites in culture systems can be costly and in order to develop an optimal control strategy for the management of a parasite, information on its biology is necessary (Whittington *et al.* 2001). A generic diagnosis must be conducted to correctly identify the monogenean to be treated, as each species of monogenean reacts differently to a specific chemical treatment. An integrated

approach to parasite management, where parasite biology is combined with chemotherapy, ensures treatments are administered when necessary and hence reduce treatment costs by minimising the use of chemicals.

Gill flukes of the genera *Calceostoma* van Beneden 1852, and *Diplectanum* Diesing, 1858 (Fig. 1) have been identified in large numbers on silver kob (*Argyrosomus inodorus* Griffiths & Heemstra, 1995) (Christison *et al.* submitted). Flukes of the same genera were identified on the closely related species *Argyrosomus japonicus* Lacepède, 1802 (previously misidentified as *A. hololepidotus*) off the coast of Western Australia (Williams 1989; Griffiths & Heemstra 1995). Brood stock of silver kob are obtained from the wild and then introduced into culture systems, bringing parasites with them that have the potential to reproduce rapidly, thus increasing the risk of disease outbreaks in the system. The commercial rearing of kob may be at risk of monogenean epizootics that could affect productivity and profitability. Wild-caught kob need to be examined and compared with captive kob for the prevalence and abundance of both *Diplectanum* sp. and *Calceostoma* sp. such a study would provide an indication of the potential risk these parasites pose on kob culture.

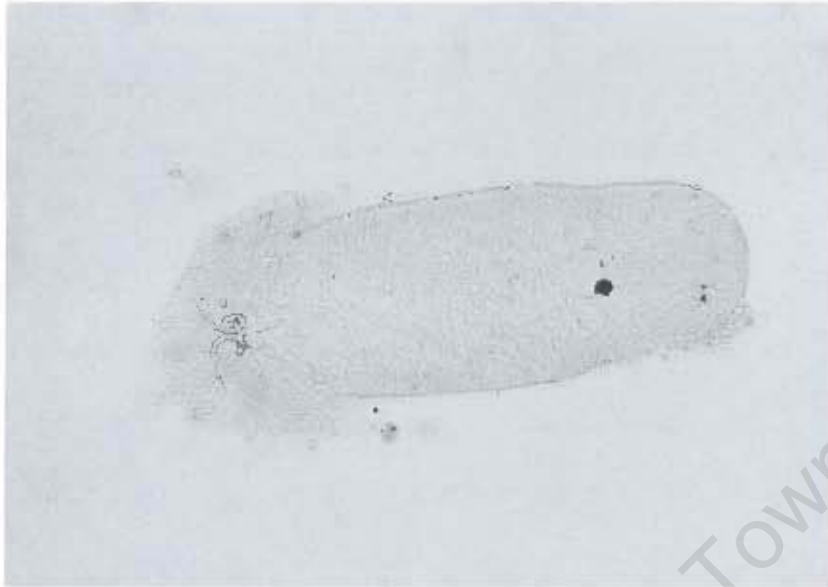


Fig. 1a Adult *Calceostoma* sp. monogenean fluke which infects the gills of silver kob (*Argyrosomus inodorus*), (Christison).

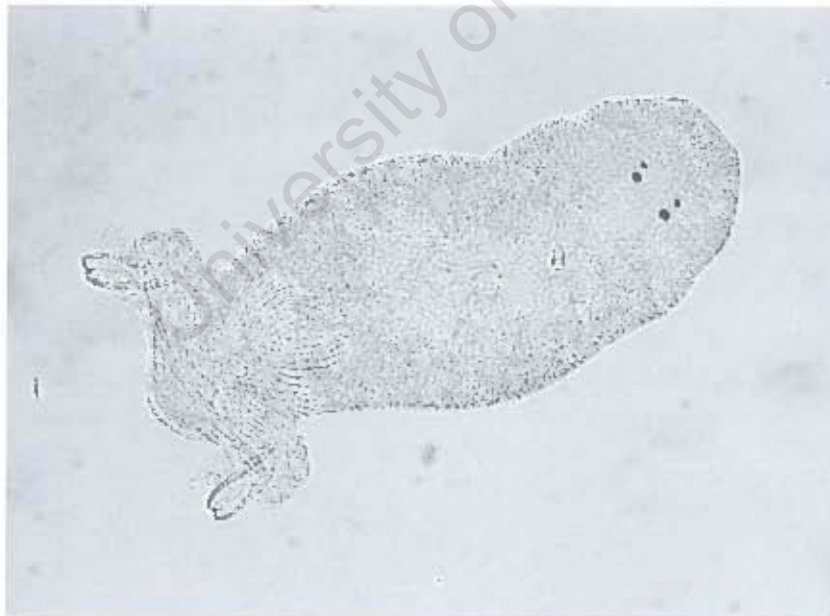


Fig. 1b *Diplectanum* sp. adult with notable eyespots and distinctive attachment organs to infect gills of the silver kob (*Argyrosomus inodorus*), (Christison).

The silver kob (Fig. 2) is a medium sized sciaenid reaching a maximum mass of 34 kg, and is an important recreational and commercial line fish (Thorpe 1990; Griffiths & Heemstra 1995) throughout its distribution, which extends from northern Namibia to the Kei River in South Africa. It is most common between Cape Point and the Kei River, but due to overexploitation numbers have declined in recent years (Griffiths 1997). Culture of a closely related species, *A. japonicus* (dusky kob), occurs in Australia and this species is a potential aquaculture candidate in South Africa, along with *A. inodorus*. Kob are considered ideal for aquaculture as they display fast growth rates, good food conversion ratios and a preference to form schools, allowing them to adjust to a captive environment (Griffiths 1995; Searle 1998; Aquaculture SA 2003). Any farming operation does, however, run the risk of being plagued by disease causing low growth rates, mortalities and an overall loss in productivity. The culture of kob is no exception in this regard.

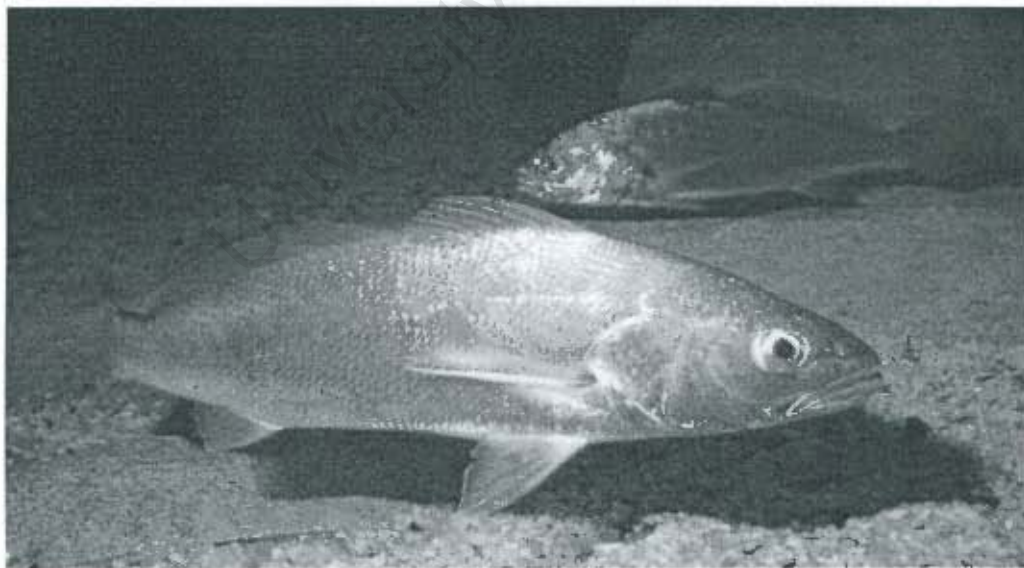


Fig. 2 The silver kob (*Argyrosomus inodorus*) a potential aquaculture species in southern Africa, (Griffiths).

Before the kob can be treated to remove monogeneans, drugs must be tested for efficacy. Efficacy of a drug is tested in trials to compare treated with untreated groups. There is at present no drug being used to treat monogenean parasites on *A. inodorus*, therefore it is necessary to identify a drug that could effectively remove gill flukes from this species. Once a drug has been identified, *in vivo* treatment trials can be conducted to determine the effects of the drug on the host. Treatments including formalin, praziquantel, hydrogen peroxide, freshwater, copper sulphate and trichlorfon have all proved effective at eradicating some species of monogeneans (Thoney & Hargis 1991; Francis-Floyd 1996; Poynton *et al.* 1997; Hirazawa *et al.* 2000; Rajendran *et al.* 2000) and they are legally used internationally or are under review for use (Table 1). Drugs used to treat fish must be accepted internationally as well as locally as many aquaculture products are exported.

Table 1: Examples of drugs either approved or under investigation for approval for treatment of monogenean parasites infecting various aquaculture species. A – drugs approved, UI – drugs under investigation (Schnick *et al.* 1999).

Drug	Country / Continent				
	Asia	Australia	Europe	Canada	USA
Formalin	UI	UI		A	A
Hydrogen peroxide	A (Japan)	UI	A	A	UI
Praziquantel			A	UI	UI
Copper sulphate	UI				UI
Trichlorfon	A (Japan)				UI

The optimal timing of each treatment also needs to be established; hence the necessity to conduct embryonation studies on the eggs of both *Calceostoma* sp. and *Diplectanum* sp. This project aimed to determine the embryonation period of *Calceostoma* sp. and *Diplectanum* sp. and to identify

which treatments will effectively eradicate monogenean flukes from the silver kob.

3. Materials and methods

Generic diagnosis

Specimens of *Calceostoma* sp. and *Diplectanum* sp. from the gills of silver kob (*Argyrosomus inodorus*) caught in Gansbaai by hook and line were used to determine the generic diagnoses of the two species. Drawings were made using a camera lucida. A Leica DMLS microscope with JVC colour video camera (TK – 128OE) was used to take photographs of whole mounts of the flukes.

Prevalence and abundance of *Calceostoma* sp. and *Diplectanum* sp. on wild-caught silver kob

Sub-samples of silver kob brood stock were euthanased to determine the prevalence and abundance of monogenean parasites on the gills of wild-caught kob. The gill arches were removed to count those on the left side of the gill arch only. Thirteen fish were killed directly after capture whilst another sample of five fish was kept in captivity for 30-days before being examined for the presence of flukes. Prevalence is usually expressed as a percentage and is defined as the number of individuals of a host species infected with a particular parasite species divided by number of hosts examined. The abundance of a parasite is determined by the total number of individuals of a particular parasite species in a sample of hosts divided by total number of

individuals of the host species (uninfected plus infected) in a sample (Margolis *et al.* 1982).

Source of parasites for *in vitro* trials and egg hatching experiments

Ten one-year-old wild-caught juveniles of *Argyrosomus inodorus* were transported from the Irvin and Johnson farm (Danger Point, Gansbaai) to the Seapoint Research Aquarium (Cape Town), and kept in a 7.07 m³ holding tank several weeks prior to the commencement of treatment trials and egg hatching experiments, to ensure high parasite numbers on the gills. Two weeks prior to the start of the experimental work one specimen of *A. inodorus* was sacrificed to determine if both *Calceostoma* sp. and *Diplectanum* sp. infected the fish.

***In vitro* treatment trials on adult and juvenile worms**

One fish was sacrificed per treatment trial and the gills removed. Dissections were carried out between 07H00 and 08H00. Once removed, the second and third gill arches on both the left and right side of the fish were allocated randomly to a treatment or control group. Three treatments and a control group were allocated per fish. Three replicates of each treatment were conducted, a single treatment was tested on three separate gill arches from three separate fish. The treatments chosen for the *in vitro* trials included praziquantel, copper sulphate, formalin, hydrogen peroxide and freshwater at the concentrations shown in Table 2. Each drug was dissolved in filtered seawater to obtain the required concentration. Praziquantel does not readily

dissolve in water and therefore had to be made into a fine paste before adding it to the seawater.

The gill arches were placed in Petri dishes (diameter 85 mm) containing 50 ml solution of seawater and a treatment drug. Parasite-infected gills immersed in 50 ml untreated seawater acted as a control group. The effect of the treatment on the gill fluke's behaviour was recorded on an hourly basis for 12 h (adapted from Hirazawa *et al.* 2000). The flukes were observed under a dissecting microscope and both the number of living and dead individuals present on the gills counted in the first hour, thereafter the number of dead individuals were counted and removed each hour. Parasitic worms were assumed dead if there was no response to prodding with a fine needle (Chisholm & Whittington 2002).

Table 2: Chemical treatments used in the *in vitro* trials to test their efficacy on monogenean flukes of the silver kob (*Argyrosomus inodorus*).

Treatment	Dosage
1. Control	None
2. Praziquantel	10 mg/l **
3. Praziquantel	20 mg/l
4. Praziquantel	40 mg/l
5. Hydrogen peroxide	30 mg/l***
6. Freshwater	50 ml
7. Copper sulphate	0.2 mg/l***
8. Formalin (37% formaldehyde)	100 mg/l***
9. Formalin (37% formaldehyde)	200 mg/l

** Buchmann 1993

*** Thoney & Hargis 1991

Collection and incubation of eggs from adult worms

Eggs collected from adult worms were required for life-cycle studies, to determine time of egg hatching, so that follow up treatments could be

administered to eradicate new generations of worms before they infected new hosts. *Calceostoma* sp. adults were removed from the outer excised gill arches using hypodermic needles and placed in Petri dishes containing filtered seawater, where they were left to lay eggs over a 24 h period. The diplectanids were left on the gills to fall off naturally and subsequently lay eggs. The eggs were counted before being immersed in filtered seawater at 12, 15, 17 and 19 °C in small fish tanks, the temperatures chosen reflecting the range of water temperatures off the Cape Peninsula. Partial water changes were conducted on a daily basis, as full immersion of the Petri dish was not possible, as some of the eggs had a tendency to float, eggs from the first batches of eggs were lost because of this. The light regime used was 12 h light and 12 h dark.

Egg development was monitored daily until the last sign of hatching occurred.

The following data were recorded (Chisholm & Whittington 2000):

- Temperature
- Incubation time (days)
- Number of eggs hatched

Statistical Analysis

Data were analysed statistically using a 5% significance level, in order to compare the results of the treatment groups and those of the control. ANOVA was utilised to compare treatments for the final mortality at the end of the 12 h period for *Calceostoma* sp. as the data was homoscedastic, whereas final mortality data for *Diplectanum* sp. was neither normally distributed nor homoscedastic therefore Kruskal-Wallis (ANOVA by Ranks) was used.

Kolmogorov-Smirnov tests were conducted to compare the mortality rate per hour of each treatment to the control. STATISTICA version 7 was used for all statistical analysis. Data were expressed as percentage mortality.

4. Results

Generic diagnosis of the genera *Calceostoma* and *Diplectanum*

Class: Monogenea (van Beneden, 1858)

Subclass: Polyonchoinea (Bychowsky, 1937)

Order: Dactylogyridea (Bychowsky, 1937)

Suborder: Calceostomatinea (Parona & Perugia, 1890)

Family: Calceostomatidae (Parona & Perugia, 1890)

Subfamily: Calceostominae (Parona & Perugia, 1890)

Genus: *Calceostoma* van Beneden, 1852

The genus *Calceostoma* infects the skin and gills of fish over a large geographical distribution including the waters off Australia and southern Africa (Williams 1989). The body of *Calceostoma* is divided into distinct regions: cephalic region, trunk, peduncle and haptor. The tegument is described as being thin and smooth. A thin, flexible cup-shaped haptor is characteristic of this genus, with one ventral and dorsal pair of anchors attached to a "T" shaped median bar. Up to 14 marginal hooks may also be present on the haptor (Kritsky *et al.* 1978; Williams 1989). Figure 3 illustrates these features

in *Calceostoma* sp., and a summary of the generic diagnosis of the genus *Calceostoma* van Beneden, 1852 appears in Table 3.

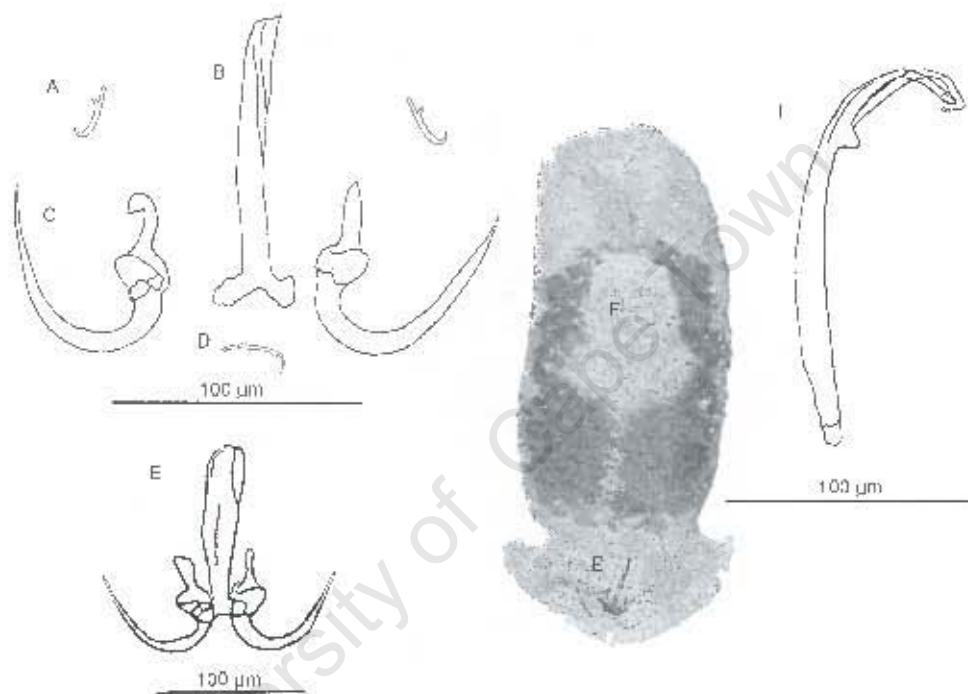


Fig 3. A-F Microscope projection drawings of *Calceostoma* sp. van Beneden, 1852 from the gills of silver kob (*Argyrosomus inodorus*). A – dorsal hamulus, B – median bar, C – ventral hamulus, D – marginal hook, E – hamular complex of median bar and ventral hamuli, F – copulatory organ. Photograph of *Calceostoma* sp. whole-mount not to scale.

Class: Monogenea (van Beneden, 1858)
Subclass: Polychocinea (Bychowsky, 1937)
Order: Dactylogyroidea (Bychowsky, 1937)
Suborder: Dactylogyroinea (Bychowsky, 1937)
Family: Diplectanidae (Monticelli, 1903)
Subfamily: Diplectaninae (Monticelli, 1903)

Genus: *Diplectanum* Diesing, 1958 *sensu* Oliver, 1968

Diplectanum is the largest genus in the family Diplectanidae comprising approximately 80 sp., of which 67 % are found in tropical/subtropical seas (Hayward 1996). Gill tissue of both marine and freshwater teleosts of the order Perciformes is parasitised by this genus (Santos *et al.* 2002). Generally this genus is associated with the inclusion of a ventral and dorsal squamodisc, comprising continuous rows of rodlets. Three transverse bars join up the two pairs of haptorial hooks and the copulatory organ consists of a sclerotised tube (Hayward 1996; Santos *et al.* 2002). Figure 4 illustrates these features in *Diplectanum* sp., and the generic diagnosis of genus *Diplectanum* Diesing, 1958 is summarised in Table 3.

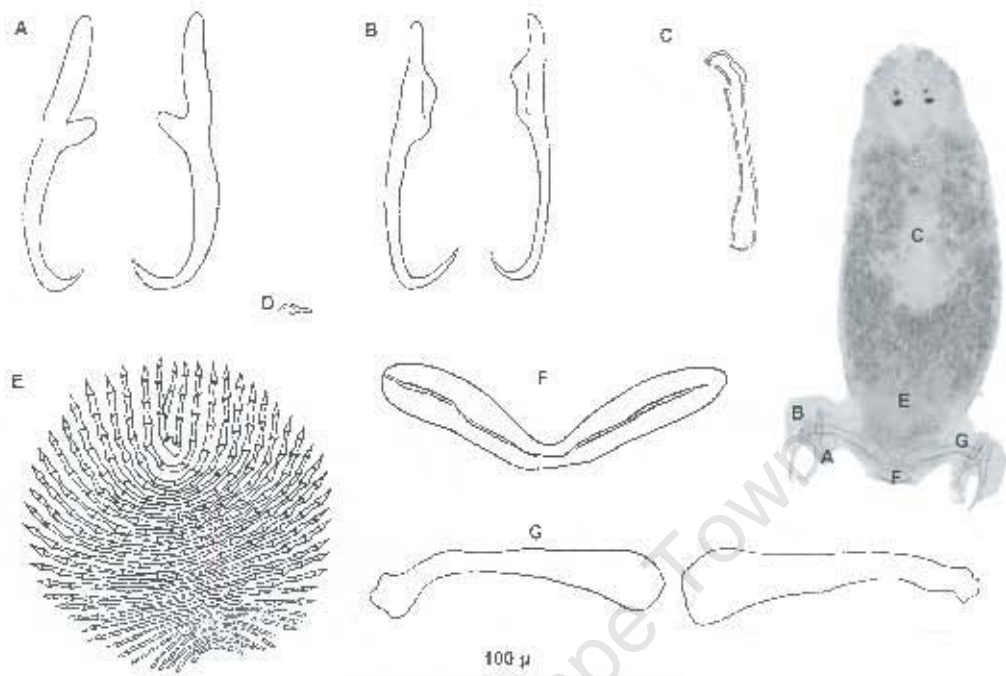


Fig 4. A-H Microscope projection drawings of *Diplectanum* sp. Diesing, 1858 from the gills of silver kob (*Argyrosomus inodorus*). A – ventral hamuli, B – dorsal hamuli, C – "J" shaped copulatory organ, D – marginal hook, E – squamodisc made up of concentric rings of rod lets, F- ventral transverse bar with lateral groove, G – dorsal lateral bars. Photograph of *Diplectanum* sp. whole-mount not to scale.

Table 3: A comparison of the generic diagnosis of genus *Calceostoma* van Beneden, 1852 and genus *Diplectanum* Diesing, 1858, two species of monogeneans that infect the silver kob (*Argyrosomus inodorus*) (Kritsky *et al.* 1978; Obiekezie 1988; Williams 1989; Hayward 1996; Santos *et al.* 2002).

	<i>Calceostoma</i> van Beneden, 1852	<i>Diplectanum</i> Diesing, 1858
Body	<ul style="list-style-type: none"> • Elongate • Thin and smooth tegument • Anterior end expanded to form a hood-like lappet 	<ul style="list-style-type: none"> • Elongate • Dorsoventrally flattened
Haptor	<ul style="list-style-type: none"> • Cup-shaped with a "frilly" margin • One pair dorsal hamuli greatly reduced in size (Fig. 3 A) • 14 marginal hooks (Fig. 3 D) • One pair ventral hamuli with sclerotised median bar ("T" shaped) (Fig. 3 E) 	<ul style="list-style-type: none"> • Wider than body • Two pairs lateral hamuli - dorsal and ventral which differ in size and shape (Fig. 4 A, B) • 14 marginal hooks (Fig. 4 D) • Oval dorsal and ventral squamodisc of concentric rows of rodlets (Fig. 4 E) • median bar with conspicuous lateral groove (Fig. 4 F) • Lateral bars proximal ends are swollen whilst the distal ends taper and articulate with the hamuli (Fig. 4 G)
Female reproductive organ	<ul style="list-style-type: none"> • Ovary lobed • Oviduct elongate 	<ul style="list-style-type: none"> • Ovary simple
Male reproductive organ	<ul style="list-style-type: none"> • Elongate, sclerotised penis with a solid base and hollow central section (Fig. 3 F) 	<ul style="list-style-type: none"> • Penis stylet "J" shaped, tubular and sclerified (Fig. 4 C)
General	<ul style="list-style-type: none"> • Large well developed head organs/cephalic glands at anterior extremity • Pharynx glandular and muscular • Two pairs of small eyespots 	<ul style="list-style-type: none"> • Well developed head organs at the anterior extremity • Two pairs eye spots • Pharynx spherical and muscular

Prevalence and abundance of *Calceostoma* sp. and *Diplectanum* sp. infecting the gills of *Argyrosomus inodorus*

Diplectanum sp. and *Calceostoma* sp. were found on newly caught silver kob (*Argyrosomus inodorus*). *Diplectanum* sp. had a prevalence of 100%, whereas *Calceostoma* sp. prevalence was 38.5 % (Seven out of the 13 fish examined were *Calceostoma* sp. free). The prevalence of *Calceostoma* sp. increased to 100 % when kob were in captivity (Fig. 5). The parasite abundance (mean number of parasite individuals per host examined) on wild-caught kob was 46.4 for *Diplectanum* sp. and 37.3 for *Calceostoma* sp. The abundance of *Diplectanum* sp. greatly increased to 301.6 over a 30-day period in captivity. The abundance of *Calceostoma* sp. on five kob was lower at 18 individuals per host examined (Fig. 5).

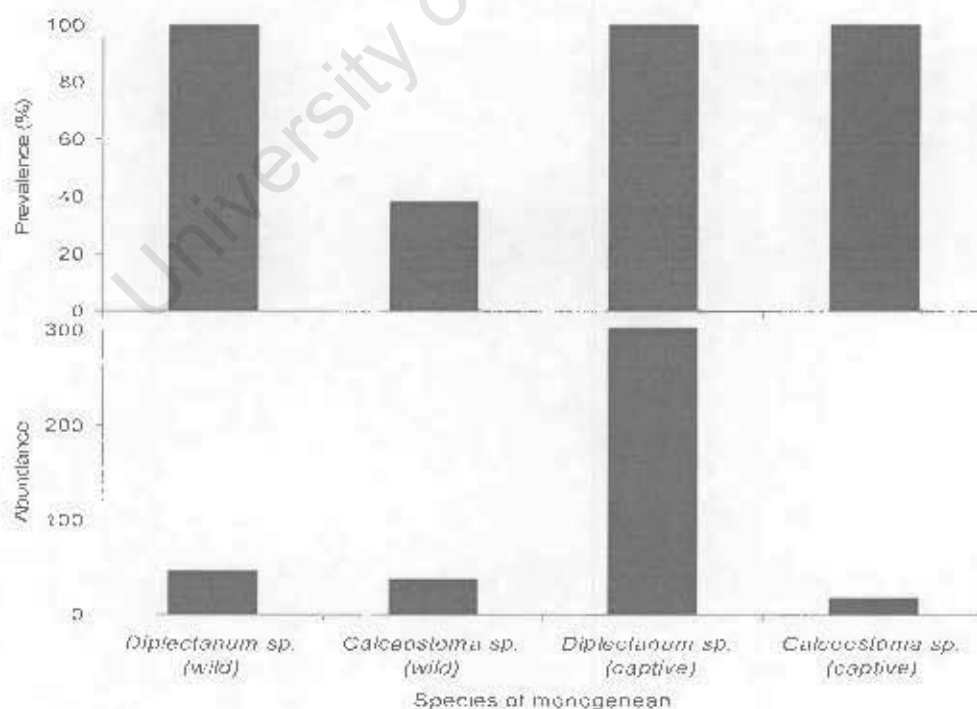


Fig. 5 The prevalence and abundance of *Calceostoma* sp. and *Diplectanum* sp. from wild-caught silver kob in Gansbaai.

In vitro treatment trials

Calceostoma sp.

The cumulative mean percentages of dead worms for eight treatments and the control over the 12 h period are shown in Fig. 6. Freshwater and praziquantel 40 mg/l were 100 % effective at eradicating *Calceostoma* sp. Freshwater was effective after just one hour, whereas praziquantel 40 mg/l was 100 % effective after six hours of exposure to the treatments (Fig. 6). All other treatments displayed a similar trend to that of the control where there was a gradual decline in the number of live worms over 12 h (Fig. 6). Praziquantel at lower concentrations was less effective at removing the worms: praziquantel 20 mg/l was only 100 % after 11 hours whilst praziquantel 10 mg/l only eradicated 90% of the worms present (Fig. 6). Only the efficacy of the freshwater and praziquantel 40 mg/l treatments differed significantly to the control at one-hour intervals over the full 12 h period (Table 4: Kolmogorov-Smirnov, $p < 0.05$). All concentrations of praziquantel had an immediate effect on *Calceostoma* sp. The worms fell off the gills in the first hour and appeared distorted in shape due to what appeared to be tegument damage. Similarly worms exposed to freshwater were immediately affected by the treatment, they fell off the gills in the first hour and curled up. On death the worms appeared black in colouration. No *Calceostoma* sp. eggs were observed in the Petri dishes of praziquantel and freshwater treatments.

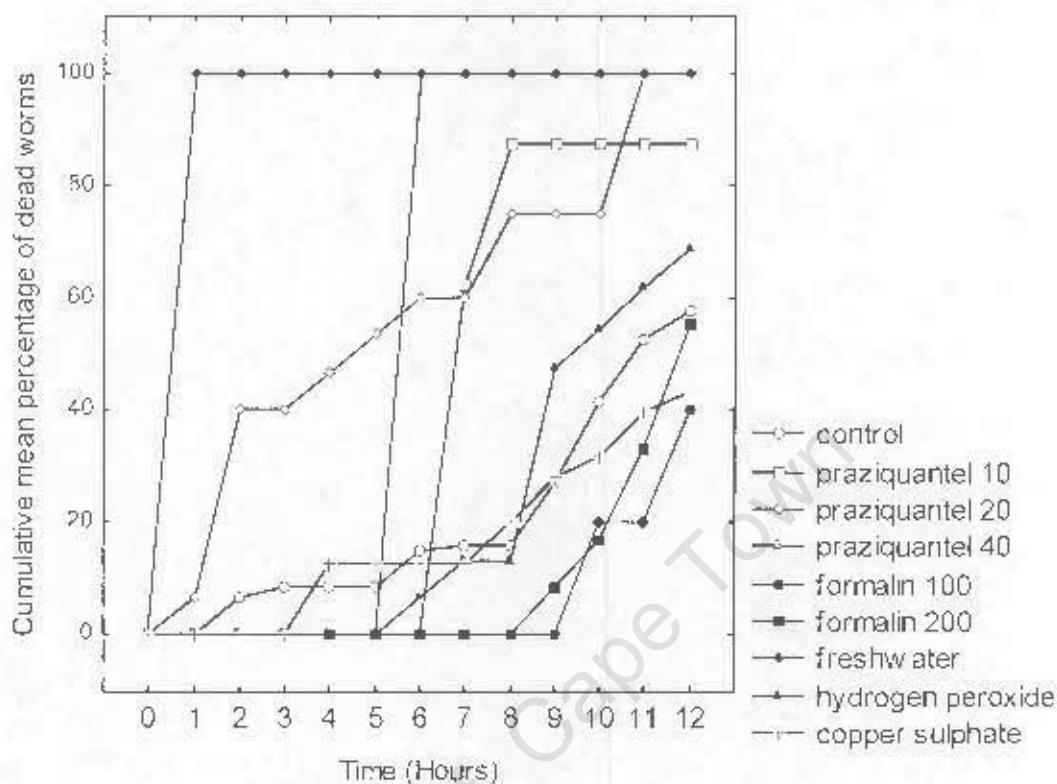


Fig. 6 Cumulative mean percentage of dead *Calcoostoma* sp. after exposure to various treatments *in vitro* over a 12 hour period.

Formalin, hydrogen peroxide and copper sulphate were not effective at completely eradicating *Calcoostoma* sp. from the gills of the kob (Fig. 6). The treatments were found to be ineffective against the flukes (Table 4) and they continued to lay eggs as a result. Formalin took longest to affect the flukes, only after eight hours did the worms start dying off, whereas mortalities in the control were observed from hour 2. Formalin 100 mg/l and copper sulphate were the least effective at eradicating *Calcoostoma* sp. adults, removing approximately 40 % of the worms over the 12 h period (Fig. 6).

Table 4: The results of Kolmogorov-Smirnov test comparing the cumulative mean percentage of dead worms per hour over a 12 hour period in the individual treatments to the control for *Calceostoma* sp. Treatments were considered effective against the worms if the p value was less than 0,05 as compared to the control.

Treatment	Concentration (mg/l)	P value
1. Praziquantel	10	N.S
2. Praziquantel	20	N.S
3. Praziquantel	40	<0.05
4. Formalin	100	N.S
5. Formalin	200	N.S
6. Freshwater	-	<0.05
7. Hydrogen peroxide	30	N.S
8. Copper sulphate	0.2	N.S

There were no statistically significant differences between mortality of *Calceostoma* sp. in the treatments and the control at the end of the 12-hour period (Fig. 7; One-Way ANOVA, $H_{8,N=22}$, $F= 1.7912$, $p>0.05$). Praziquantel at 20 mg/l and 40 mg/l and freshwater appeared to be the most effective at eradicating adult *Calceostoma* sp. from the gills, as 100% mortality was observed, whereas only 60% of the worms in the control died (Fig. 7).

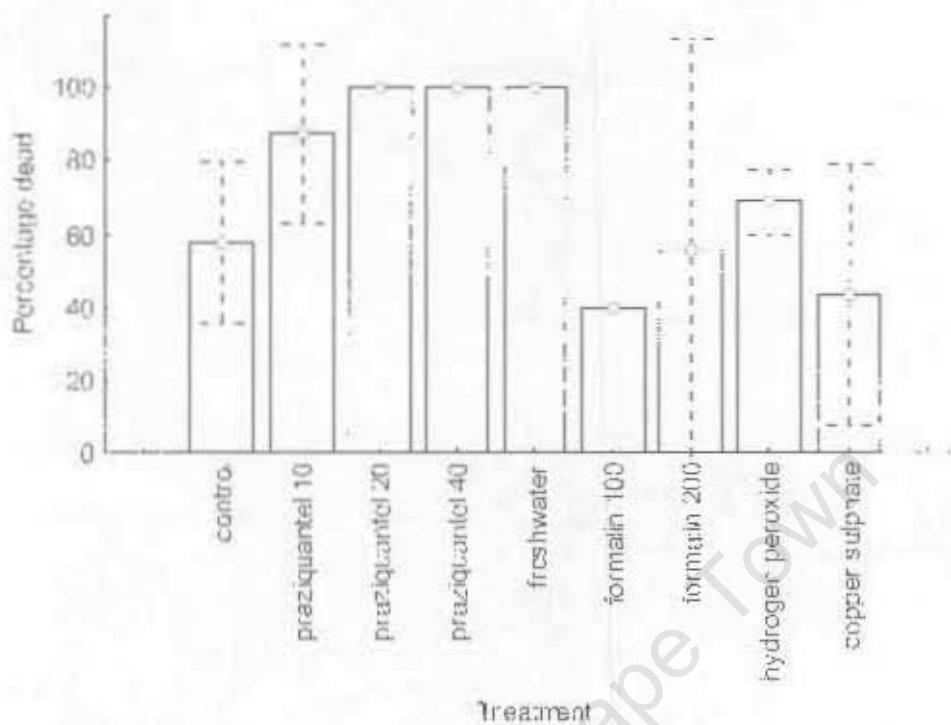


Fig. 7 Percentage of *Calceostoma* sp. dead after 12 hour exposure to various treatments. Two standard error bars were used to indicate whether a treatment differed significantly from the control in its ability to effectively remove flukes from the gills of *Argyrosomus inodorus*.

Diplectanum sp.

The cumulative mean percentages of dead worms for eight treatments and the control over the 12 h period are shown in Fig. 8. In the 12 hour period the freshwater treatment eradicated *Diplectanum* sp. in five hours, and praziquantel 40 mg/l was effective after 10 hours. Praziquantel 10 and 20 mg/l were 90 and 80 % effective at eliminating the worms from the gills respectively (Fig. 8).

Praziquantel caused *Diplectanum* sp. to curl up and become very inactive as soon as they were exposed to the drug. The flukes displayed small jerky movements for hours before their death. Tegument damage was

observed to occur causing the flukes to release from the gills after the first hour of the experiment. The adult worms did not appear to lay eggs in the presence of the treatment. Freshwater also had an immediate effect on the flukes causing them to curl up and turn black in colouration. Once again no eggs appeared to have been laid in the dish.

Formalin caused 45-65 % mortality of the worms in the 12 h period (Fig. 8) proving to be ineffective at eradicating *Diplectanum* sp. The worms were highly active on and off the gills for up to eight hours and eggs were found in the Petri dish at the end of the experiment. *Diplectanum* sp. worms were highly active in the presence of hydrogen peroxide for over eight hours into the experiment. Only 37 % of the worms died over the 12 h period (Fig. 8) and many eggs laid by the adults were found in the Petri dish. Conversely, copper sulphate removed over 70 % of *Diplectanum* sp. flukes, they started falling off the gills after two hours of the experiment, but were active for seven hours before they started to die off (Fig. 8).

The results of the Kolmogorov-Smirnov test show that there were no significant differences between the mortality rate over time between the control and individual treatments (Kolmogorov-Smirnov, $p > 0.05$).

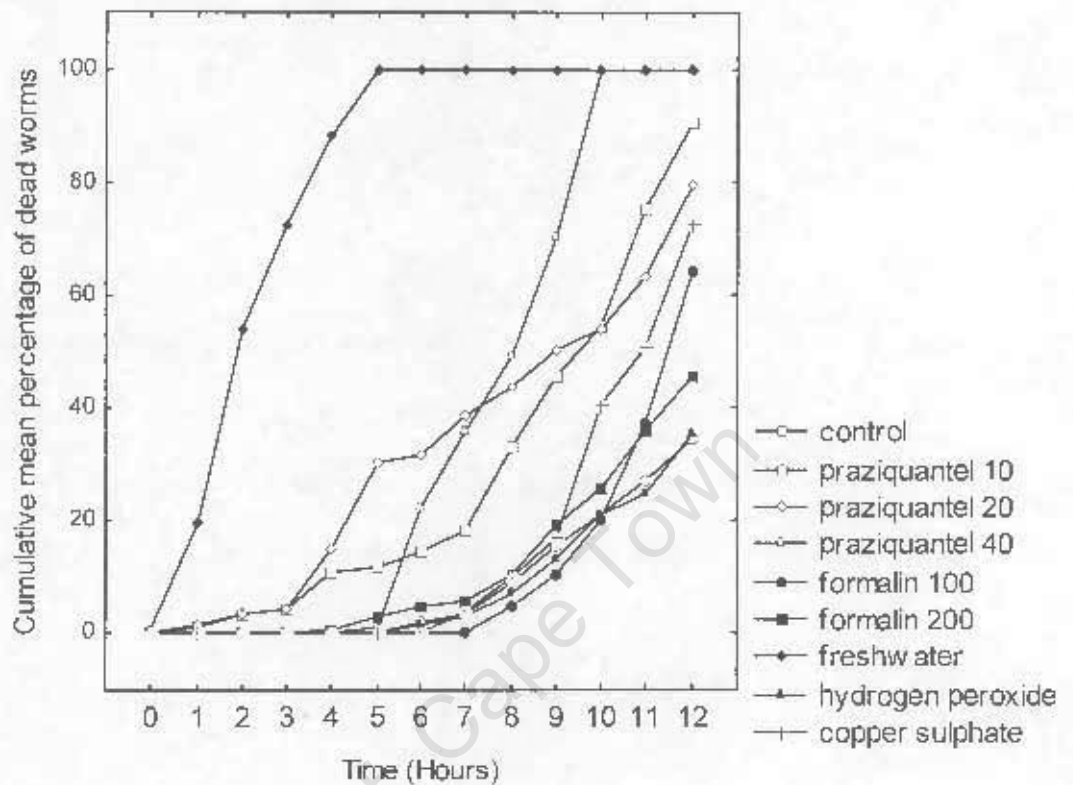


Fig. 8 Cumulative mean percentage of dead *Diplectanum* sp. after exposure to various treatments *in vitro* over a 12 hour period.

However, the Kruskal-Wallis test (ANOVA by Ranks) found a significant difference between the treatments and the control at the end of the experiment (Kruskal-Wallis, $H_8, N=23, F= 16.59, p<0.05$). A lack of overlap of standard errors bars on figure 9 shows significant differences between the total percentage of dead *Diplectanum* sp. worms in freshwater, copper sulphate, praziquantel 10 and 40 mg/l from the control.

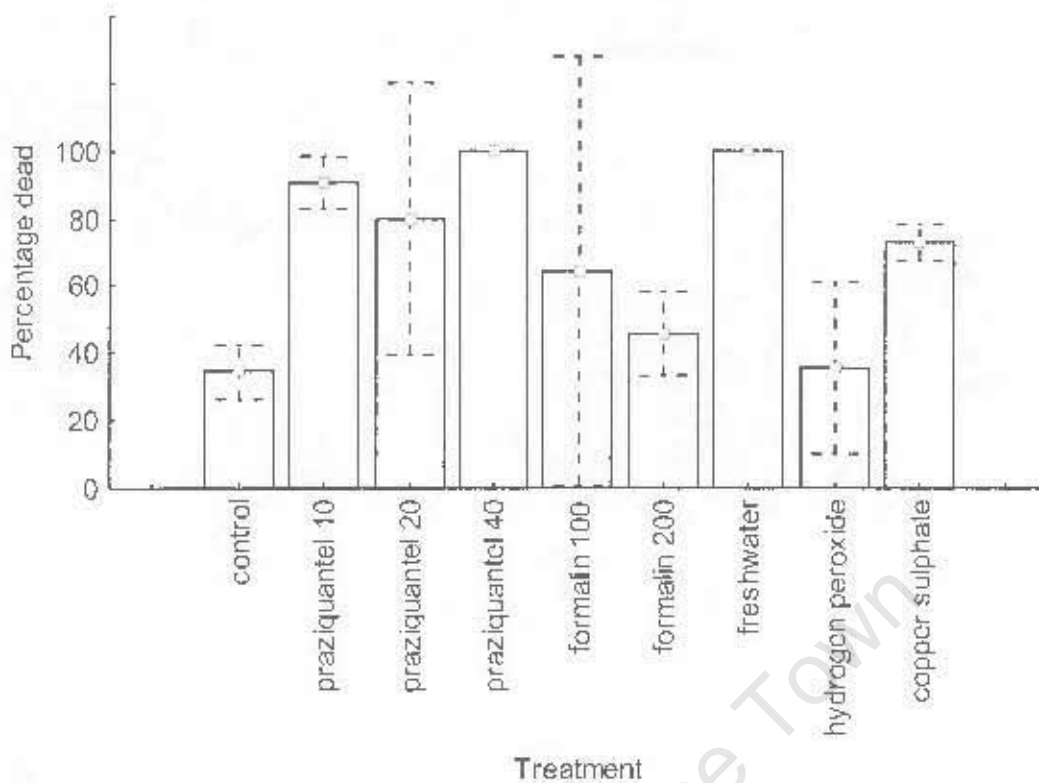


Fig. 9 Percentage of *Diplectanum* sp. dead after 12 hour exposure to various treatments. Two standard error bars were used to indicate whether a treatment differed significantly from the control in its ability to effectively remove flukes from the gills of *Argyrosomus inodorus*.

Egg hatching

The effect of temperature on the development time of *Calceostoma* sp. eggs is shown in Fig. 10. No statistically significant correlation was found between temperature and embryonation period ($r = -0.22, p > 0.05$). Period of development varied between 5 –14 days amongst the various temperatures. Time of egg hatching appeared to be random with no definite pattern evident. This indicates that hatching may not be spontaneous, but rather influenced by a cue. Of the eggs incubated 92-100 % hatched in most batches. There was, however, no hatching observed in two separate batches of eggs, one at 15 °C and another at 17 °C.

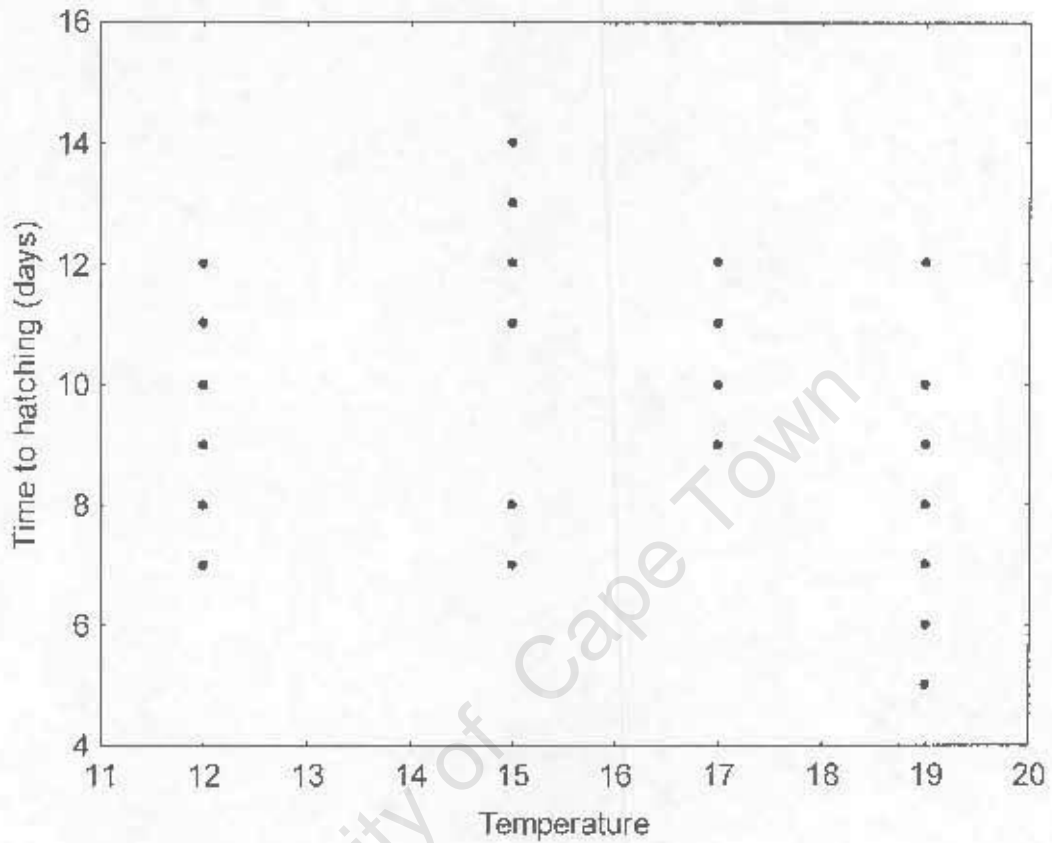


Fig. 10 Day to hatching of *Calceostoma* sp. eggs under the influence of influence of temperature ($r = -0.2219$). Day hatched = $12.612 - 0.1903 \times \text{temperature}$.

The embryonation period of *Diplectanum* sp. eggs was related to temperature ($r = -0.87$, $p < 0.05$), although only two temperatures were tested. At 19 °C hatching started on day three, whereas at 12 °C hatching only occurred from day seven (Fig. 11). Hatching did occur over a number of consecutive days and a 100 % hatching success was observed, indicating that spontaneous hatching had taken place.

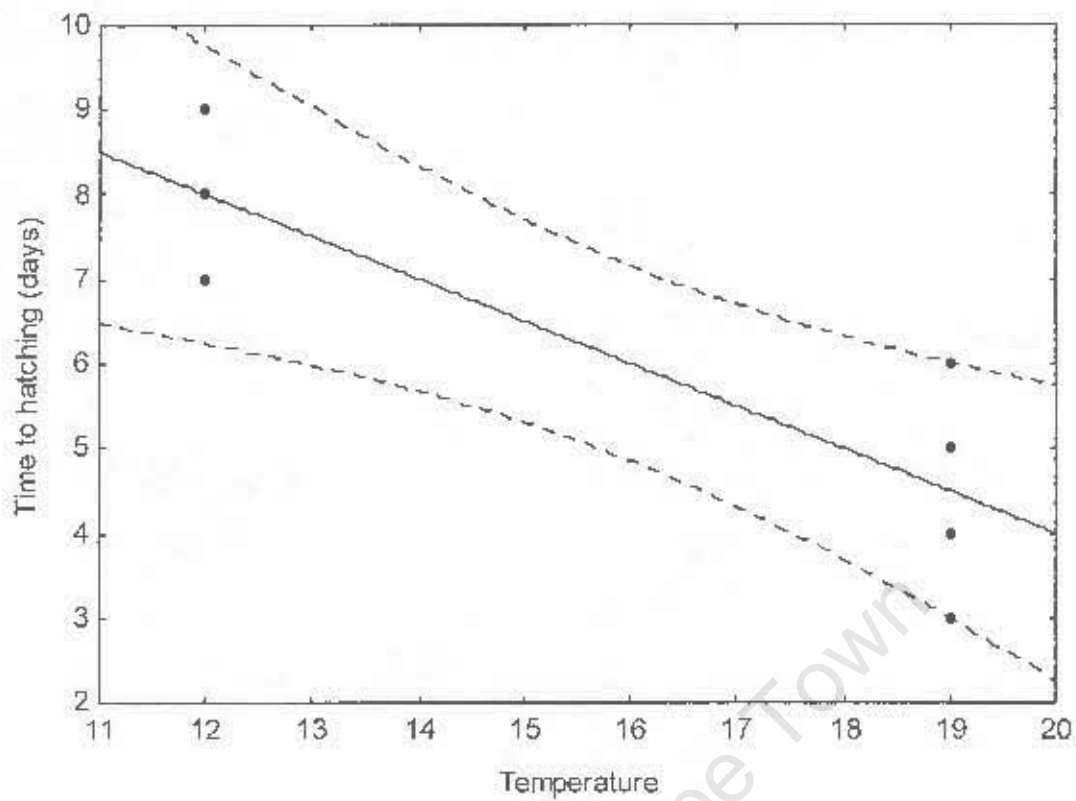


Fig. 11 Day to hatching of *Diplectanum* sp. eggs under the influence of influence of temperature ($r = -0.8860$). Day hatched = $14 - 0.5 \times \text{temperature}$.

5. Discussion

Correctly identifying the monogenean parasites that infect silver kob is essential to finding a solution to combat them in a culture environment. Species or genera of monogeneans respond differently to various treatments (Noga 2000), therefore a correct generic diagnosis is necessary. *Calceostoma* sp. and *Diplectanum* sp. were identified on silver kob (*Argyrosomus inodorus*) from the wild. *Diplectanum* sp. and *Calceostoma* sp. occur naturally on kob in nature, but it is in captivity that their ability to reproduce rapidly poses a threat to kob culture in South Africa on a commercial basis. *Calceostoma* sp. are not as abundant on the gills of kob as *Diplectanum* sp. but are highly reproductive (Christison *et al.* submitted). The potentially high *Calceostoma* sp. abundance on captive fish is not evident in this study as only five fish were available for examination after the 30-day period. *Diplectanum* sp. ability to rapidly reproduce caused a heavy infestation on the host's gills (an average of 301.6 worms per host). The high prevalence of both *Calceostoma* sp. and *Diplectanum* sp. was due to amongst other stressors in captivity, the reduction of the spatial distance between hosts. Culture environments provide ideal conditions for monogenean parasites to increase in numbers, this can lead to disease as well as death to a large number of hosts (Thoney & Hargis 1991; Whittington *et al.* 2001). Identification of effective drug treatments, along with studies of the parasites' life cycles, transmission and epizootiology (Scholtz 1999) are necessary to provide effective management procedures to control monogenean infestations. Losses in aquaculture facilities affect productivity and increase running costs, therefore information that permits the

control of parasites is necessary for the productive culture of any aquaculture species.

In vitro pre-treatment trials revealed that freshwater treatments as well as praziquantel at a higher concentration had a negative effect on both *Calceostoma* sp. and *Diplectanum* sp. Freshwater eradicated both species of fluke within a matter of hours, proving it to be an effective bath treatment. Freshwater has been noted to be effective against a similar species of gill fluke, *Diplectanum latesi* that infects seabass, *in vitro* freshwater treatments killed the parasite in 10-20 minutes (Rajendran *et al.* 2000). Freshwater or low salinity water treatments should be considered for the removal of monogenean parasites on kob as it prevents egg laying by adult worms, does not involve using harmful chemicals and is thus environmentally friendly (Rajendran *et al.* 2000). Freshwater baths are however, stressful to some species of fish (Thoney & Hargis 1991), therefore *in vivo* treatment trials have to be administered to the kob in order to assess the host's response to the treatment before it can be used on a commercial basis.

Praziquantel is regarded as a safe and effective treatment to use on fish against monogeneans (Thoney & Hargis 1991). Worms exposed to the drug were immediately affected by it, the diplectanids curled up instantly, and the calceostomatids became distorted in shape. The drug effect was immediate on the worms at all concentrations tested. Worms fell off the gills almost immediately and were inactive, appearing dead except for jerks caused by muscle spasms. The effects of praziquantel at a cellular level have

been described for many species of monogeneans, severe contractions and irreversible vacuolisation of the tegument have been observed by Buchmann (1993) and Sharp *et al.* (2004). The damage to worms prevented egg laying by both calceostomatids and diplectanids suggesting that praziquantel may be an effective drug at controlling the monogenean populations by preventing reinfection, therefore follow up treatments of praziquantel may be effective at eradicating the flukes altogether. Similarly, adult *Benedonia seriolae* were prevented from laying eggs in the presence of praziquantel, proving it an effective anthelmintic (Sharp *et al.* 2004).

The temperature of the seawater was approximately 12 °C throughout the experiment, which may have had an effect on the efficacy of praziquantel. Praziquantel is greatly influenced by temperature, appearing to be less effective at lower temperatures (Buchmann 1998). Therefore it may be necessary to expose the worms to praziquantel at higher temperatures to eradicate the flukes more rapidly. On the other hand, as an alternative, lower concentrations of praziquantel can be administered over a longer duration, as the length of exposure can have a greater effect on the worms than a higher concentration over a short period. Lower drug concentrations would be economically favourable, as praziquantel is very costly to administer (Table 5) (Sharp *et al.* 2004). Thoney and Hargis (1991) suggest that long exposures to praziquantel are more effective than the concentration used, this supports the results obtained. Praziquantel at lower concentrations started removing the flukes at later stages in the experiment than at the start. Long-term exposure to a drug under culture conditions can, however, have negative effects on the

host, as no water exchange occurs during treatment to maintain drug concentration and this can lead to poor water quality and cause the host to stress.

Table 5: Cost of a drug treatment in a 7cm³ tank at concentrations tested in the *in vitro* trials (Medico Lab; Merck Laboratory supplies pers. comm. 2005)

Drug	Concentration (mg/l)	Unit Cost	Total cost per tank (R)
Praziquantel	10	R 218.88/ 100g	153.22
Praziquantel	20	R 218.88/ 100g	306.43
Praziquantel	40	R 218.88/ 100g	612.86
Hydrogen peroxide	30	R 8.40/ 100ml	176.40
Copper sulphate	0.2	R 6.20/ 100g	0.087
Formalin	100	R 7.44/ 100ml	52.08
Formalin	200	R 7.44/ 100ml	104.16

If the length of exposure to a drug is not long enough, or a drug is ineffective against a monogenean, there is the possibility of anthelmintic resistance developing in fish farms. *Pseudodactylogyrus* strains infecting eels in Dutch commercial farms became resistant to mebendazole following extensive use of benzimidazoles, resulting in large losses in production (Kamstra *et al.* 1994). To avoid anthelmintic resistance in flukes under culture conditions, an alternation of treatments is recommended (Buchmann 1998). For example alternating between praziquantel and treatments of freshwater or formalin.

Formalin treatments have been used successfully to control gill flukes on many other species of fish, but were very unsuccessful at removing both *Diplectanum* sp. and *Calcoostoma* sp. from the gills of silver kob. The ineffectiveness of the treatment may have been due to the use of 37% formaldehyde and not formalin. Confusion frequently occurs between formalin

and formaldehyde in dosages used. The Formaldehyde Solution BP is available as a 34-38% solution with methyl alcohol to act as a stabilising agent to prevent polymerisation, this solution is often referred to as 'formalin' (Scott 1993). Therefore when calculating dosages confusion occurs as to whether to dilute formalin or formaldehyde. Formalin has been noted to be an effective controlling agent, but did not eradicate *Calceostoma* sp. and *Diplectanum* sp. in previous trials conducted by Christison (submitted).

Eggs laid by adult worms in the presence of formalin have been observed for other species of monogeneans. *Benedenia seriolae* and *Zeukapta seriolae* detached from their host and continued to lay viable eggs in concentrations of 250 and 400 mg/l (Sharp *et al.* 2004). As *Calceostoma* sp. and *Diplectanum* sp. lay in the presence of formalin it is not recommended as an effective treatment to eradicate flukes. Eggs are resistant to chemical treatments and will hatch within a matter of days to reinfect the kob. Furthermore the continuous use of formalin for follow up treatments could prove costly (Table 5) and potentially environmentally damaging (Hirazawa *et al.* 2000).

There was a negligible difference between the percentage of worms that died in the presence of hydrogen peroxide and in the control, therefore hydrogen peroxide is not effective at eradicating monogenean infections on the silver kob. The worms laid a large number of eggs into the water before they died, as in formalin treatments, so the chances of reinfection are high. The efficacy of a chemical is not only dependent on the dosage used and/or

the time of exposure, but also on the species of monogenean (Sharp *et al.* 2004). The diplectanids were not susceptible to hydrogen peroxide and hence it had little effect on them. Conversely the majority of calceostomatids were removed by hydrogen peroxide. The possibility of reinfection in this case is still high, due to the laying of treatment-resistant eggs. Christison (submitted) observed hydrogen peroxide to be highly effective at removing *Calceostoma* sp. from the gills of the silver kob. The concentration used was 0,6 ml/l whereas the concentration used in this experiment was equivalent to 0.3 ml/l, thus hydrogen peroxide may be more effective at controlling *Calceostoma* sp. populations in cultured kob at higher concentrations.

Although copper sulphate did not eradicate monogenean flukes on the gills of kob it proved to be more effective at removing *Diplectanum* sp. than *Calceostoma* sp. The ineffectiveness of copper sulphate at removing the flukes entirely may be due to the fact that it is a slow acting agent, which is more effective on the oncomiracidia than on adult worms (Thoney & Hargis 1991). Gill and skin fluke infections on captive lemon sharks have been successfully treated with copper sulphate at a concentration of 0.25 ppm for a period of 85 days (Poynton *et al.* 1997). A continuous treatment ensures that new generations of the parasite are destroyed before infecting a new host. Copper sulphate may, however, be impractical in treating large numbers of kob as it is difficult to maintain the required concentration of copper sulphate in the tank for the duration of treatment. Copper sulphate absorbs to filter media and organic particles very rapidly hence greatly reducing its concentration (Thoney & Hargis 1991).

Monogeneans have proven difficult to eradicate in past experiments. Of approximately 35 compounds tested for their effects on a number of monogenean species very few have an acceptable antiparasitic efficacy without displaying side effects to the fish they are intending to treat (Table 6). The effects of a specific drug vary considerably from parasite to parasite and its toxicity on the host varies, hence the importance of *in vivo* trials to be conducted before any drug is chosen for treatment on a large scale (Buchmann 1998).

Identifying and developing an effective antiparasitic drug can be very costly, and after identifying a drug its use may be restricted due to environmental concerns. There is a growing trend to restrict chemical use in the aquaculture industry, therefore forcing farmers to integrate management strategies of which drug use is a small component. In order to obtain an integrative management approach to farming an understanding of the parasite's biology and infection dynamics is required (Ernst *et al.* 2002).

Not only is it important to know what to treat, but also when to treat kob with monogenean infections. The egg hatching experiments revealed that temperature did not have an effect on the embryonation period of *Calceostoma* sp. eggs. Hatching occurred at all temperatures within 7 - 12 days, indicating that this would be the best time to treat the host to prevent reinfection. Monogenean egg hatching may be spontaneous, or a variety of stimuli could be responsible. Previous studies have revealed that light

Table 6: Drugs tested for effects on monogenean flukes in past studies. +: parasitocidal effect recorded, -: no parasitocidal effect recorded (Buchmann 1997; Buchmann 1998), (Appendix1).

Drug	No. of species tested	No. of host species tested	Effect
Oxibendazole	2	2	+/-
Thiabendazole	2	2	+/-
Albendazole	3	3	+/-
Flubendazole	2	2	+
Mebendazole	6	5	+/-
Parbendazole	2	2	+/-
Fenbendazole	2	2	+/-
Triclabendazole	2	2	+
Luxabendazole	1	1	+
Netobimin	1	1	-
Febantel	1	1	+
Metrifonate	11	9	+/-
Dichlorvos	1	1	+
Pyrantel	1	1	+
Morantel	1	1	+
Ivermectin	1	1	+/-
Nicosamide	3	4	+
Closantel	1	1	+
Rafoxanide	1	1	+
Phenolsulfonphthalein	1	1	-
Chlorsulon	1	1	+
Toltrazuril	10	2	+/-
Praziquantel	7	7	+/-
Levamisole	4	5	+/-
Niclopholan	1	1	+
Bithionol	2	2	+/-
Nitroscanate	1	1	+
Piperazine	2	2	+/-
Bunamidine	2	2	-
Copper sulphate	2	2	+
Formaldehyde	9	8	+
Hydrogen peroxide			+
Potassium manganate/bichomate	2	2	+/-
Sodium chloride	2	2	+
Ammonia	2	1	+
Ammonium hydroxide	1	1	+

periodicity or intensity, temperature, mechanical disturbance and secretions from the host skin are possible cues for hatching (Ernst & Whittington 1996; Chisholm & Whittington 2000). The experiments were conducted in a semi-controlled environment, therefore there are one of two cues that could be responsible for *Calceostoma* sp. egg hatching, namely light periodicity and mechanical disturbance. Patterns influenced by light periodicity have been observed for the monogeneans *Benedenia lutjani* and *B. rohdei* from the skin and gills of *Lutjanus carponotatus* (Ernst & Whittington 1996). Monogenean parasites from the shovelnose ray (*Rhinobatos typus*) display true circadian rhythms, as eggs incubated in 24 h dark or 24 h light continued to hatch at the same time as those under conditions of natural illumination (Chisholm & Whittington 2000). The cue responsible for egg hatching of the monogenean *Diclidophora luscae* is mechanical disturbance by means of a jet of water or prodding with a needle (Whittington & Kearn 1988). Further studies on *Calceostoma* sp. embryonation would have to be conducted to confirm the cue required for egg hatching.

There are a number of factors that could have resulted in two egg batches of *Calceostoma* sp. not hatching, such as the condition, age and sexual maturity of the parasite, which may have affected the eggs viability. Parasites often lay non-viable eggs if they are damaged after removal from the host or as time increases off the host they lay fewer eggs that are frequently malformed. Bacterial infections are common and thus cannot be ruled out as a possible factor that may have prevented egg hatching in this case (Ernst & Whittington 1996; Sharp *et al.* 2004).

A correlation was found between *Diplectanum* sp. larval development and temperature. Therefore, water temperature is a factor to be taken into account to determine time interval between the first and follow-up treatments. The influence of temperature on embryonation has been observed for another diplectanid *Diplectanum aequans*, a parasite of the sea bass (*Dicentrarchus labrax*). At 30 °C spontaneous hatching began after two days, whereas at 15 °C hatching began on day seven (Cecchini *et al.* 1998). A regression curve of temperature verses time can be used to determine the best time to apply follow-up treatments. Only two temperatures were studied in this experiment due to the limited number of fish available for the trials and although there was a large number of diplectanids on the gills of the fish very few eggs were laid that could be collected for egg hatching experiments. The experiment indicates that treatments should be administered on day's three to five at 19 °C, or on day's seven to nine at a lower temperature of 12 °C. Further studies on this species at differing temperatures would be advantageous to demonstrate a stronger relationship between temperature and time to spontaneous hatch.

Eggs of both species from several batches were observed to hatch over a number of consecutive days. Hatching patterns have often been linked to host behaviour so as to increase the chances of the larvae being successful at finding a new host (Chisholm & Whittington 2000). The oncomiridia of *Diplozoon homoion gracile* hatch and infect the southern barbel at night, when the hosts are inactive (Macdonald & Jones 1978).

Unfortunately little is known about the behaviour of silver kob in the wild, to predict whether the larval hatching of the monogenea infecting this species is linked to the host's behaviour. Silver kob do, however, school well in captivity during the hours of daylight. If this is true in the wild, then it may give us an indication that hatching during daylight hours is advantageous to the flukes, as their chances of finding a new host are greatly increased when host numbers are high.

Monogeneans have in the past proved difficult to eradicate from culture systems, therefore the best way to control them is to prevent their introduction into culture facilities. A sub-sample of new fish should be taken to assess if monogeneans are present and if so which species. In order to do this a wet mount of the gills should be made and observed under a microscope. Monogeneans move by means of caterpillar-like movements and are thus easy to identify (Noga 2000). Kob considered for culture should then be quarantined and treated with chemicals. A period of isolation can help prevent the spread of contagious diseases and parasites (Vaughan 2004). Treating and thus controlling monogenean populations on a small scale is easier than trying to treat fish in large numbers. A quarantine period allows for the observation of the hosts progress and the ability to control the concentration of drugs administered more effectively. The quarantine period should consist of both a primary and secondary treatment to eradicate monogeneans before the kob enters the culture environment (Vaughan 2004). The primary treatment will remove adult worms and a secondary treatment removes immature worms subsequently hatched from eggs present in the system

during primary treatment. The secondary treatment is essential to disrupt the parasites life-cycle, therefore it must be timed appropriately to ensure removal of the new generation of worms before reinfection occurs (Sharp *et al.* 2004). If prophylactic treatments fail the fish should be destroyed, as prevention of introducing a monogenean into a culture facility is the most effective way at controlling infestations (Thoney & Hargis 1991).

In order to eradicate *Calceostoma* sp. and *Diplectanum* sp. from kob freshwater treatments are recommended. Freshwater treatments should be administered at intervals of 7-12 days to eradicate *Calceostoma* sp. When treating *Diplectanum* sp. temperature must be taken into account. For higher temperatures around 19 °C treatments should be administered every three – five days whereas, treatments at cooler temperatures should be conducted weekly. Although freshwater has been shown to be effective at removing monogeneans infecting silver kob, it may not be as effective on those infecting dusky kob (*Argyrosomus japonicus*). The dusky kob is a euryhaline species spending much of its life in turbid estuaries (Griffiths 1995), therefore the monogeneans may be more resilient to low salinity treatments (Thoney & Hargis 1991). Praziquantel is an effective agent of eradication that could be utilised as an alternative; there is however the risk of drug resistance on farms if praziquantel is used on an on going basis, thus another controlling agent could be used intermittently. Christison (submitted) found formalin to be effective at controlling both *Calceostoma* sp. and *Diplectanum* sp. Alternating treatments of the two chemicals may prove effective at controlling the monogenean populations on both silver and dusky kob.

In order to find an appropriate treatment for flukes infecting the silver kob further treatment trials (*in vitro* and *in vivo*) and egg hatching experiments will have to be conducted. Although freshwater and praziquantel treatments appear suitable, they may have a negative impact on the host rendering them unsuitable treatments for use. A time of larval hatching has been estimated but further studies may help pinpoint a more accurate time to administer follow-up treatments.

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8. Appendix

Appendix 1: A list of drugs tested to eradicate monogenean flukes. (+ : some parasitological effect recorded; - : no parasitological effect recorded), (Buchmann 1997)

Parasite	Host	Drug and effect
<i>Benedeniella posterocolpa</i>	<i>Rhinoptera bonasus</i>	Metrifonate (+) Praziquantel (+) Copper sulphate (+)
<i>Dermophthirius</i> sp.	<i>Negaprion brevirostris</i>	Formaldehyde (+) Metrifonate (+)
<i>Gyrodactylus aculeati</i>	<i>Gasterosteus aculeatus</i>	Praziquantel (+) Niclosamide (+)
<i>Diplozoon paradoxum</i>	<i>Abramis brama</i>	Levamisole-HCl (+) Metrifonate (+)
	<i>Squalius cephalus</i>	
<i>Gyrodactylus</i> spp.	<i>Oncorhynchus mykiss</i>	Oxibendazole (-) Thiabendazole(+) Albendazole (-) Oxfendazole (+) Flubendazole (+) Mebendazole (-) Parbenazole (-) Fenbendazole (-) Triclabendazole (+) Ivermectin (-/+)
		Closantel (+) Netobimin (-) Febantel (+) Praziquantel (+) Niclofolan (+) Bithionol (+) Trichlorfon (+) Levamisole-HCl (+) Piperazine (-/+) Nitroscanate (+) Albendazole (-) Levamisole (-) Mebendazole (-) Praziquantel Toltrazuril (-) Potassium bichromate (-) Ammonium hydroxide (+) Toltrazuril (+)
<i>Ancylo-discoides vistulensis</i>	<i>Silurus glanis</i>	
<i>Dactylogyrus vastator</i> , <i>D. extensus</i> , <i>D. cornu</i> , <i>Diplozoon homoion</i> , <i>D. paradoxum</i> , <i>Pseudodactylogyrus bini</i> , <i>P. anguillae</i> , <i>Gyrodactylus arcuatus</i>		
<i>Pseudodactylogyrus anguillae</i>	<i>Anguilla anguilla</i>	Metrifonate (+) Dichlorvos (+) Metrifonate (+) Formaldehyde (+) Sodium chloride (+) Bunamidine (-) Praziquantel (+) Levamisole (-) Praziquantel (+) Formaldehyde (+) Mebendazole (+) Metrifonate (+) Sodium chloride (+) Bithionol (-) Ammonia (+) Albendazole (+) Fenbendazole (+) Flubendazole (+) Luxabendazole (+) Mebendazole (+) Oxibendazole (+) Parbendazole (+) Thiabendazole (-) Triclabendazole (+) Niclosamide (+) Toltrazuril (-) Rafoxanide (+) Ivermectin (-) Pyrantel (+) Morantel (+) Albendazole (+) Mebendazole (+) Triclabendazole (+) Metrifonate (+) Ammonia (+) Formaldehyde (+) Sodium chloride (+) Potassium permanganate (+) Praziquantel (+)
<i>Pseudodactylogyrus bini</i>	<i>Anguilla anguilla</i>	
<i>Pseudodactylogyrus anguillae</i>	<i>Anguilla anguilla</i>	
<i>P. bini</i>		
<i>Pseudodactylogyrus</i> spp.		
<i>Dactylogyrus vastator</i>	<i>Cyprinus carpio</i>	Metrifonate (+)
<i>Dactylogyrus vastator</i>	<i>Carassius auratus</i>	Mebendazole (+) Metrifonate (-)
<i>Gyrodactylus elegans</i>	<i>Carassius auratus</i>	
<i>Gyrodactylus derjevini</i>	<i>Carassius auratus</i>	Formaldehyde (+)
<i>Gyrodactylus wagneri</i>	<i>Notemigonus crysoleucas</i>	Formaldehyde (+)
<i>Gyrodactylus cyclopteri</i>	<i>Cyclopterus lumpus</i>	Formaldehyde (+)
<i>Gyrodactylus sprostonae</i>	<i>Cyprinus carpio</i>	Copper sulphate (+)

