

Effect of Formalin Treatment on the Monogenean *Pseudorhabdosynochus epinepheli* (Monogenea) and the Fish *Epinephelus coioides**

YANG Ting_bao¹, LIN Li¹, CHEN Yong_zhi¹, LIU Sheng_fa²

(1. Key Laboratory of GD for Improved Reproduction of Economic Animals//

Institute of Aquatic Economic Animals, Sun Yat_sen University, Guangzhou 510275, China;

2. Department of Biology, Xiamen University, Xiamen 361005, China)

Abstract: Prophylactic methods against *Pseudorhabdosynochus epinepheli* (Monogenea) infection on the fish *Epinephelus coioides* were investigated in April 2000. The effect of formalin in seawater on *P. epinepheli* was that the semi_lethal concentration is 250×10^{-6} for 25 min, and 50×10^{-6} for 50 min. The effect of formalin in freshwater on *P. epinepheli* is reported as: the semi_lethal concentration was 83×10^{-6} for 3 min. The semi_lethal time for freshwater alone was 21 min. The toxicity of formalin in seawater to *E. coioides* was assessed: the semi_anesthetic concentration was 940×10^{-6} in 30 min and 645×10^{-6} in 45 min. The toxicity of formalin in freshwater to *E. coioides* was as follows: the semi_anesthetic concentration was $1\,300 \times 10^{-6}$ in 5 min and 500×10^{-6} in 10 min. The semi_lethal formalin concentration to *E. coioides* in seawater was between 600×10^{-6} and $1\,000 \times 10^{-6}$, and in freshwater was between 250×10^{-6} and 500×10^{-6} . The semi_anesthetic and semi_lethal time of *E. coioides* in freshwater were 95 min and 115 min respectively. These results demonstrated that the lethal formalin concentrations for *P. epinepheli* in both seawater and freshwater fell in a safe range for *E. coioides*.

Key words: *Epinephelus coioides*; *Pseudorhabdosynochus epinepheli*; prophylactic methods

CLC number: S941.52⁺1 **Document code:** A **Article ID:** 0529-6579 (2003) 04-0059-05

The grouper, *Epinephelus coioides*, is a highly valued cultured fish on South China coast. Culture started in the 1980s and the annual production has exceeded 2500 metric tons in recent years. The fish are cultured in floating off_shore cages, but many parasite_related disease outbreaks have become a serious problem and often resulted in significant economic loss.

P. epinepheli is frequently found in both diseased juvenile and dead grown fish and the diseased fish is treated by eradicating the worms. This worm is thus considered one of the persistent potential threats to the culture of *E. coioides*. Fishermen in South China usually expel the worms together with other monogeneans by freshwater or formalin dip, methods widely used in Southeast Asia mariculture (Leong, 1997). These methods are often but not always effective because of the inaccurate dosage in practical use and the lack of precise concentration range within which parasite are killed or expelled without any harm done to the fish.

The objective of this study is to investigate the toxicity of formalin in freshwater and seawater to *E. coioides* and to *P. epinepheli* *in vitro*.

1 Materials and Methods

Freshwater formalin solution was prepared in various concentrations, 100×10^{-6} , 150×10^{-6} , 200×10^{-6} , 250×10^{-6} , 500×10^{-6} , $1\,000 \times 10^{-6}$, $1\,500 \times 10^{-6}$ and $2\,000 \times 10^{-6}$; whereas seawater formalin solution was prepared in concentrations of 200×10^{-6} , 400×10^{-6} , 600×10^{-6} , $1\,000 \times 10^{-6}$, $1\,500 \times 10^{-6}$ and $2\,000 \times 10^{-6}$. Fourteen tanks of 5000ml each were filled with 2 000 mL of the prepared solutions respectively and an extra tank was filled with 2 000 mL pure freshwater. Eighty healthy fish of length 26~ 30 mm were selected for the experiment. Each tank had 5 experimental fish put into it and was supplied with aeration. Observation was carried out at 5 minute intervals for one hour. Then, the solution in all tanks was changed into ordinary seawater and the fish were cultured for 5 hours in order to observe the recovery of the fish from anaesthesia. The fish recovering from the subsequent culture were considered to have been anaesthetized, while those not recovering died.

* 收稿日期: 2002- 08- 16

基金项目: 国家 863 资助项目 (2001AA621010); 广东省自然科学基金资助项目 (021681)

作者简介: 杨廷宝 (1963 年生), 男, 教授, E-mail: tingbao123@163.com

P. epinepheli was obtained from a fish of body length 72 cm and body weight 13 000 g, that was close to death because of serious infection of *P. epinepheli*. Fifty active worms were selected for treatment experiment. The treatment time of killing efficacy of formalin solution to *P. epinepheli* in freshwater and seawater was determined according to the practical experience of fishermen and previous fish treatment experiments. In freshwater solution it was less than 10 minutes and it was 30~60 minutes in seawater. Seawater formalin concentrations for parasite dip were 50×10^{-6} , 200×10^{-6} , 300×10^{-6} , and 400×10^{-6} ; freshwater formalin concentration were 50×10^{-6} , 100×10^{-6} , and 200×10^{-6} . Pure freshwater (distilled water) and filtered seawater (pH 8.3 and salinity 33‰) were set as control. Water temperature was 15 °C. Treatments were conducted in 9 petri dishes of 10 cm in diameter, each holding 5 worms. Worms stopping vermiculation and not recovering in pure seawater were regarded as dead. The time when a worm stops vermiculating was recorded.

Reed_Muench method was adopted to calculate semi_anaesthetic and semi_lethal concentration and time (Chen, 1981).

2 Results

2.1 Toxicity of seawater formalin solution treatments to *E. coioides*

Seawater formalin solution showed weaker effect in anaesthetizing fish (Table 1). With 600×10^{-6} of seawater formalin solution dip, the fish was not anaesthetized in 60 minutes. When the solution was as high as $1\,000 \times 10^{-6}$ the fish still showed no sign of anaesthesia until 20 minutes later. Therefore 30 minutes and 45 minutes were chosen to calculate semi_anaesthetic concentration. The results were showed in Table 2 and 3.

The semi_anaesthetic concentration at 45 minutes was calculated as 645×10^{-6} , and that at 30 minutes was worked out as $1\,000 \times 10^{-6}$.

The semi_anaesthetic time of fish in $1\,000 \times 10^{-6}$ solution was 16 minutes with the data in table 4.

2.2 Toxicity of freshwater formalin solution treatment to *E. coioides*

The freshwater formalin solution of concentration less than 250×10^{-6} could not anaesthetize *E. coioides* within 60 min (Tab. 4). The effect of freshwater formalin solution of concentration of 500×10^{-6} on the fish was first observed at 5 minutes. With the increase of treatment time and formalin concentration the toxicity became more and more distinct (Tab. 5). Results of anaesthetization at 5 and 10 minutes were chosen to

Tab. 1 Anaesthetized and died record of *E. coioides* in seawater formalin solution¹⁾

t/ min	c/10 ⁻⁶					
	200	400	600	1000	1500	2000
5	0	0	0	0	0	0
10	0	0	0	0	0	0
15	0	0	0	0	0	1 ^a
20	0	0	0	1 ^a	1 ^a	2 ^a
25	0	0	0	1 ^a	1 ^a	2 ^a
30	0	0	0	1 ^a	1 ^a	3 ^a
35	0	0	0	2 ^a	2 ^a	4 ^a
40	0	0	0	2 ^a	2 ^a	4 ^a
45	0	0	0	3 ^a	4 ^a	5 ^a
50	0	0	0	5 ^a	5 ^a	5 ^a
55	0	0	0	5 ^a	5 ^a	5 ^a
60	0	0	0	5 ^a	5 ^a	5 ^a
Mortality % ^b	0	0	0	100	100	100

1) a: Anaesthetized; b: mortality after moving into ordinary seawater 5 hours late

Tab. 2 Anaesthetic ratio of *E. coioides* in different concentration of seawater formalin solution at 45 min

c/10 ⁻⁶	No. Ana.	No. of Sur.	Acc. ana.	Acc. Sur.	Ana. Ratio
600	0↓	5	0	8	0
1000	3	2	3	3	50
1500	4	1	7	1	87.5
2000	5	0↑	12	0	100

Ana.: anaesthetized; Sur: survived;

Acc.: Accumulated number along the arrow direction

Tab. 3 Anaesthetic ratio of *E. coioides* in $1\,000 \times 10^{-6}$ seawate formalin solution at different time¹⁾

t/min	No. Ana.	No. of Sur.	Acc. ana.	Acc. Sur.	Ana. Ratio
15	0↓	5	0	25	0
20	1	4	1	20	5
25	1	4	2	16	11
30	1	4	3	12	20
35	2	3	5	8	38
40	2	3	7	5	58
45	3	2	10	2	83
50	5	0↑	15	0	100

1) Ana.: anaesthetized; Sur: survived;

Acc.: Accumulated number along the arrow direction

calculate semi_anaesthetic concentration because all fish were anaesthetized in high concentration solution at 10 minutes later. The semi_anaesthetic concentration at 10 minutes was calculated to be 500×10^{-6} and at 5 minutes it was 1300×10^{-6} . The semi_anaesthetic time of the fish treated with $1\,000 \times 10^{-6}$ freshwater formalin solution was 6.5 minutes.

Tab. 4 Anaesthetized and died record of *E. coioides* in freshwater formalin solution¹⁾

t/min	c/10 ⁻⁶							
	100	150	200	250	500	1000	1500	2000
5	0	0	0	0	1 ^a	1 ^a	2 ^a	4 ^a
10	0	0	0	0	2 ^a	3 ^a	5 ^a	5 ^a
15	0	0	0	0	4 ^a	4 ^a	5 ^a	5 ^a
20	0	0	0	0	5 ^a	4 ^a	5 ^a	5 ^a
25	0	0	0	0	5 ^a	5 ^a	5 ^a	5 ^a
30	0	0	0	0	5 ^a	5 ^a	5 ^a	5 ^a
35	0	0	0	0	5 ^a	5 ^a	5 ^a	4 ^a 1 ^b
40	0	0	0	0	5 ^a	5 ^a	5 ^a	2 ^a 3 ^b
45	0	0	0	0	5 ^a	5 ^a	5 ^a	2 ^a 3 ^b
50	0	0	0	0	5 ^a	5 ^a	5 ^a	5 ^b
55	0	0	0	0	5 ^a	5 ^a	1 ^a 4 ^b	5 ^b
60	0	0	0	0	5 ^a	5 ^a	5 ^b	5 ^b
Mortality % ^c	0	0	0	0	100	100	100	100

1) a: Anaesthetized; b: died; c: mortality after moving into ordinary seawater 5 hours late

Tab. 5 Anaesthetic ratio of *E. coioides* in different concentration of freshwater formalin solution at 10 min¹⁾

c/10 ⁻⁶	No. Ana.	No. of Sur.	Acc. ana.	Acc. Sur.	Ana. Ratio
250	04	5	0	10	0
500	2	3	2	5	29
1000	3	2	5	2	71
1500	5	0	10	0	100
2000	5	0†	15	0	100

1) Ana. : anaesthetized; Sur: survived;
Acc. : Accumulated number along the arrow direction

2.3 Toxicity of freshwater treatment to *E. coioides*

E. coioides in freshwater can normally swim for more than one hour although with abnormally opened mouth and gill cavity. Anaesthesia was observed after 2 hours, as shown in slow swimming, sinking to the bottom

Tab. 7 Death record of *P. epinepheli* in seawater, freshwater formalin solution of different concentrations and the pure freshwater along the treatment time

Treatment	t/ min										
	1	3	5	10	15	20	25	30	40	50	60
Filtered Seawater	0	0	0	0	0	0	0	0	0	0	0
50×10 ⁻⁶ in seawater	0	0	0	0	0	0	0	0	0	0	0
200×10 ⁻⁶ in seawater	0	0	0	0	0	0	1	2	2	4	4
300×10 ⁻⁶ in seawater	0	0	0	0	0	0	1	2	2	4	5
400×10 ⁻⁶ in seawater	0	0	0	0	0	3	4	5	5	5	5
Pure Freshwater	0	0	0	0	0	2	2	4	4	4	5
50×10 ⁻⁶ in Freshwater	0	1	2	5	5	5	5	5	5	5	5
100×10 ⁻⁶ in Freshwater	1	2	5	5	5	5	5	5	5	5	5
200×10 ⁻⁶ in Freshwater	2	4	5	5	5	5	5	5	5	5	5

and finally dying with widely opened mouth. No twitching was observed. From the data in table 6 the semi_anaesthetic and semi_lethal times were worked out as 95 minutes and 115 minutes.

Tab. 6 Anaesthetized and died record of *E. coioides* in freshwater

t/min	10	20	30	40	50	60	70	80	90
number	0	0	0	0	0	0	0	0	0
t/min	100	110	120	130	140	150	160	170	180
number	0	0	0	2a	4a	5a	3a2b	1a4b	5b

a: Anaesthetized; b: died; * : mortality after moving into ordinary seawater 5 hours late

2.4 Killing effect of formalin solution treatment of different concentrations to *P. epinepheli* in vitro

The tolerance of *P. epinepheli* to seawater formalin solution was better than to freshwater formalin solution. In seawater formalin solution, worms were not killed in low concentration solution of 50×10⁻⁶ within 60 minutes (Tab.7). Even in 400×10⁻⁶ seawater formalin solution, worms could survive for 15 min. In freshwater formalin solution, worms began to die in 3 minutes and all were killed within 10 minutes in low concentration of 50×10⁻⁶. The semi_lethal concentration of *P. epinepheli* in seawater formalin solution at 25 minutes (250×10⁻⁶) was much higher than that in freshwater formalin solution at 5 minutes (83×10⁻⁶) (Tab. 8).

Freshwater dip anaesthetized *P. epinepheli* in vitro after 10 minutes of treatment and killed all of them within 60 minutes (Tab.9). The semi_lethal time is 21 minutes.

3 Discussion

Many studies have been made of the monogenean parasites on serrenids (Dyer, Williams and Bunkley_ Williams, 1995; Vidal_Martinez et al., 1997)

Tab. 8 Semi-lethal concentration of *P. pinepheli* in different treatment and time

t/ min	Treatment	Death ratio	No. of Death	No. of Survivor	Total death	Total Survivor	Mortality	Semi-lethal concentration
30	50× 10 ⁻⁶ in seawater	0	0	5	0	12	0	232
	200× 10 ⁻⁶ in seawater	40	↓ 2	3	2	7	22	
	300× 10 ⁻⁶ in seawater	40	2	3 ↑	4	4	50	
	400× 10 ⁻⁶ in seawater	80	4	1	8	1	89	
60	50× 10 ⁻⁶ in seawater	0	0	5	0	6	0	75
	200× 10 ⁻⁶ in seawater	80	4	1	4	1	80	
	300× 10 ⁻⁶ in seawater	100	5	0	9	0	100	
	400× 10 ⁻⁶ in seawater	100	5	0	14	0	100	
2	50× 10 ⁻⁶ in freshwater	20	1	5	1	9	10	169
	100× 10 ⁻⁶ in freshwater	40	2	3	3	4	43	
	200× 10 ⁻⁶ in freshwater	80	4	1	7	1	88	
5	50× 10 ⁻⁶ in freshwater	40	2	3	2	3	40	83
	100× 10 ⁻⁶ in freshwater	100	5	0	7	0	100	
	200× 10 ⁻⁶ in freshwater	100	5	0	12	0	100	

Tab 9 Mortality of *P. pinepheli* in freshwater treatment *in vitro*

t/ min	No. of death	No. of survivor	Total death	Total survivor	Mortality
15	0	5	0	14	0
20	2	3	2	9	22
25	2	3	4	6	40
30	4	1	8	3	72
40	4	1	12	2	86
50	4	1	16	1	94
60	5	0	21	0	100

because of the economic importance of these fishes in mariculture in many areas of the world. But few studies were found to investigate the control and prevention of these monogeneans on groupers.

Formalin has remained a popular treatment based on surveys of its use by fish farmers (Speare and Ferguson 1989; Thorburn and Moccia, 1993). Many studies demonstrate that repeated use of formalin has no effect on the gills of juvenile channel catfish, *Ictalurus punctatus* (Rafinesque) (Bodensteiner et al., 1993), on the growth or gill ionic function of Chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), and smolts (Smith et al., 1987), nor does it have any effect on the growth and condition indices of Atlantic salmon, *Salmo salar* L. and rainbow trout (Powell et al., 1996; Speare and MacNair, 1996), or on plasma electrolytes, haematology and post seawater transfer survival of Atlantic salmon smolt (Powell et al., 1996). Treatment of ectoparasitic worms of marine fish with diluted formalin or freshwater has been widely practised for many years.

The toxicity of freshwater to *E. coioides* was that the semi-anaesthetic time was 95 min and the fish could not be anaesthetized in freshwater within 120 min. The fish were not anaesthetized within 60 min in seawater formalin solution of concentration less than 600×10^{-6} , nor were the fish anaesthetized in freshwater formalin solution of concentration less than 250×10^{-6} in 60 min.

The toxicity of freshwater to *P. pinepheli* was that the semi-lethal time was 19 min and all worms were killed within 60 min. In seawater formalin solution of 400×10^{-6} , worms began to die in 20 min and were all dead within 30 min. In freshwater formalin solution of 10×10^{-6} worms were killed within 10 min.

These results suggest that the tolerance of fish to freshwater and formalin solution is much stronger than that of parasite. As for the suitable concentration of formalin solution of seawater, choosing 30 ~ 60 minutes as treatment time, 500×10^{-6} of seawater formalin solution is suggested to be the safe treatment concentration because 400×10^{-6} of seawater formalin solution can kill all worms *in vitro* and the safe seawater formalin solution concentration for fish is 600×10^{-6} . As the safe treatment concentration of freshwater formalin solution to *E. coioides* is 250×10^{-6} in 60 minutes and considering freshwater formalin solution of 50×10^{-6} can kill all worms within 10 min, 150×10^{-6} of freshwater formalin solution was recommended as the safe and effective cure concentration.

References:

- [1] BODENSTEINER L R, SHEEHAN R J, LEWIS W M, et al. Effects of repetitive formalin treatments on channel catfish juveniles [J]. J Aquatic Animal Health, 1993, 5: 59- 63.

- [2] CHEN Q L. Handbook of fish diseases investigation[M]. 2ed ed. Shanghai Science and Technological Literature Press (In Chinese), 1981.
- [3] DYER W G, WILLIAMS E H Jr, BUNKLEY WILLIAMS L. *Pseudorhabdosynochus kritskyi* n sp (Monogenea: Diplectanidae) on gag from the Gulf of Mexico [M]. J Aquatic Animal Health, 1995: 337– 340.
- [4] LEONG T S. Control of parasites in cultured marine finfishes in southeast Asia: an overview [J]. International Journal for Parasitology, 1997, 27: 1177– 1184.
- [5] MORAVEC F, VIDAL MARTINEZ V M, VARGAS VAZQUEZ J, et al. Helminth parasites of *Epinephelus morio* (Pisces: Serranidae) of the Yucatan Peninsula, southeast Mexico [J]. Folia Parasitologica, 1997: 255– 266.
- [6] POWELL M D, SPEARE D J, FULTON A E, et al. Effects of intermittent formalin treatment of Atlantic salmon juveniles on growth, condition factor, plasma electrolytes, and hematocrit in freshwater and after transfer to seawater [J]. J Aquatic Animal Health, 1996, 8: 64– 69.
- [7] SMITH S D, GOULD R W, ZAUGG W S, et al. Safe prerelease disease treatment with formalin for fall Chinook salmon smolts [J]. Progressive Fish culturist, 1987, 49: 96– 99.
- [8] SPEARE D J, FERGUSON H W. Clinical and Pathological features of common gill diseases of cultured salmonids in Ontario [J]. Canadian Veterinary J, 1989, 30: 882– 887.
- [9] SPEARE D J, MACNAIR N. Effects of intermittent exposure to therapeutic levels of formalin on growth characteristics and body condition of juvenile rainbow trout [J]. J Aquatic Animal Health, 1996, 8: 58– 63.
- [10] THORBURN M A, MOCCIA R D. Use of chemotherapeutics on trout farms in Ontario [J]. J Aquatic Animal Health, 1993, 5: 85– 91.
- [11] VIDAL MARTINEZ V M, MENDOZA FRANCO E F. *Pseudorhabdosynochus capurroi* sp n (Monogenea: Diplectanidae) from the gills of *Mycteroperca bonaci* (Pisces: Serranidae) of the Yucatan Peninsula, Mexico [J]. Folia Parasitologica, 1998, 45: 221– 224.

福尔马林处理对石斑拟合片盘虫和斜带石斑鱼的影响

杨廷宝¹, 林 鑫¹, 陈勇智¹, 刘升发²

(1. 中山大学水生经济动物研究所 // 广东经济动物改良育种重点实验室, 广东 广州 510275;
2. 厦门大学生物系, 福建 厦门 361005)

摘 要: 2000年4月研究了斜带石斑 *Epinephelus coioides* 寄生拟合片盘虫 *Pseudorhabdosynochus epinepheli* 的防治方法。海水福尔马林对拟合片盘虫的作用表现为: 25 min 处理的半致死浓度为 250×10^{-6} , 50 min 处理的半致死浓度为 50×10^{-6} 。淡水福尔马林的效果为: 3 min 的半致死浓度为 83×10^{-6} , 纯淡水处理的半致死时间为 21 min。海水福尔马林对斜带石斑的毒性表现为: 30 min 和 45 min 的半致死浓度分别为 940×10^{-6} 和 645×10^{-6} 。淡水福尔马林对斜带石斑的毒性表现为: 5 min 和 10 min 的半致死浓度分别为 1300×10^{-6} 和 500×10^{-6} 。福尔马林处理海水中的斜带石斑半致死浓度介于 $600 \times 10^{-6} \sim 1000 \times 10^{-6}$, 淡水处理对斜带石斑半致死浓度介于 $95 \times 10^{-6} \sim 115 \times 10^{-6}$ 。淡水中斜带石斑的半麻醉和半致死时间分别为 95 min 和 115 min。以上结果表明: 海水和淡水福尔马林对石斑拟合片盘虫的致死浓度, 处于斜带石斑的安全范围之内。

关键词: 斜带石斑; 石斑拟合片盘; 防治方法

中图分类号: S941.52⁺1 **文献标识码:** A **文章编号:** 0529-6579 (2003) 04-0059-05