Occurrence of a Monogenean Gill Parasite *Pseudorhabdosynochus epinepheli* on Red Spotted Grouper *Epinephelus akaara* and Its Experimental Treatment by Hydrogen Peroxide Bathing

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ABSTRACT—Since 1997, infection of a monogenean gill parasite *Pseudorhabdosynochus epinepheli* has been occurring in red spotted grouper *Epinephelus akaara* at the Kagawa Prefectural Sea-Farming Center, Japan. The efficacy of hydrogen peroxide (H₂O₂) bathing was examined *in vivo*. Bathing in a 700 ppm H₂O₂ solution for 45 min at 10 or 15°C, or for 15 min at 20 or 25°C, with infected fish of 230–2100 g in body weight, resulted in 100% mortality of *P. epinepheli* on the gills. Mortality occurred in fish (550–1730 g) bathed for 30 min or longer at 25°C, but not in fish bathed for at least 60 min at 10, 15 or 20°C. Results indicate that chemotherapy with H₂O₂ has a high potential as an effective treatment for *P. epinepheli* infection in red spotted grouper.

Key words: *Pseudorhabdosynochus epinepheli*, Monogenea, hydrogen peroxide, *Epinephelus akaara*, red spotted grouper

The red spotted grouper *Epinephelus akaara* belonging to the order Perciformes is distributed mainly in coastal waters off southwestern Japan and the southern Korean Peninsula¹⁾. In Japan, seed production for stock enhancement and aquaculture has been developing in a number of hatcheries in southwestern regions, including Kagawa Prefecture, owing to its high commercial value. Since 1997, infection of a monogenean *Pseudorhabdosynochus epinepheli* on the gills of red spotted grouper has been occurring at the Kagawa Prefectural Sea-Farming Center. Bathing in hydrogen peroxide (H₂O₂) is an effective treatment for monogenean infections, such as *Heterobothrium okamotoi* and

Neobenedenia girellae in tiger puffer *Takifugu rubripes*, *Benedenia seriola* in *Seriola* spp., and *Bivagina tai* in red sea bream *Pagrus major*²⁾. A H₂O₂ bath medicine (Marine Sour SP-30; Katayama Kagaku Kogyo Kenkyujo, Japan) is commercially available for treatment of these parasitic diseases in Japan*. In this paper, we describe the occurrence of *P. epinepheli* in red spotted grouper at the Kagawa Prefectural Sea-Farming Center and *in vivo* experiments on the efficacy of H₂O₂ bathing on the gill parasite.

Materials and Methods

Red spotted groupers (230–2100 g in body weight (BW)), naturally infected with *P. epinepheli*, were obtained from the Seto Inland Sea of Japan and reared at the Kagawa Prefectural Sea-Farming Center for 1 year or longer. Prior to use all experimental fish were individually tagged.

The efficacy of bathing in 700 ppm H₂O₂ against *P. epinepheli* was investigated at four different temperatures (10, 15, 20 and 25°C). Twenty fish were bathed in 500 L of seawater containing 700 ppm H₂O₂, which was prepared with a H₂O₂ chemical (35% solution; Kemira-Ube Ltd., Japan), with gentle aeration at the appropriate temperature. Five fish were transferred to new tanks at 15-min intervals up to 60 min and maintained in 500 L of seawater without H₂O₂ at the same temperatures. Three h after transfer of treated fish, a piece of the gill filaments was excised from the outermost gill arch of each fish and examined microscopically. Live or dead worms on the gills were counted separately: swollen non-motile worms were considered to be dead based on our preliminary experiments (data not shown). Two control groups of five fish were bathed in seawater without H₂O₂ for 60 min at 10 or 20°C and gill filaments examined at 3 h post-treatment, as in the experimental groups. Abnormalities and mortality of experimental and control fish were recorded during treatment and the 3-h follow-up. Surviving fish in each group were reared in land-based tanks with a flow-through seawater system at above 15°C with feeding, and clinical signs and occurrence of infectious diseases monitored for 2 wk.

Results and Discussion

Occurrences of *P. epinepheli* in the grouper at the Kagawa Prefecture Sea-Farming Center

The occurrence of *P. epinepheli* was first found on the gills of brood stocks of red spotted grouper (approximately 500–1000 g BW), which had been reared in land-based tanks supplied with heated water (15°C) at the

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Fig. 1. Light micrograph of the gill of a red spotted grouper infected with *Pseudorhabdosynochus epinepheli*. Parasites (arrows) attach to the gill filaments by hooks. Scale bar = 0.2 mm.

Kagawa Prefecture Sea-Farming Center in the winter of 1997. Since then, this parasite has been occurring on adult and juvenile groupers at the same hatchery almost throughout the year, where water temperatures ranged from about 10 to 25°C. It has caused moderate mortalities in the juveniles.

Infected fish commonly show operculum-rubbing against tank walls leading to abrasion and hemorrhage on the skin of the operculum or body surface followed by occasional secondary infection with *Vibrio* spp. Heavily infected fish become lethargic and float near the water surface or remain on the bottom. They exhibit a whitish slime over the entire body surface, plus pale gills and internal organs due to diminished appetite. The gills or body surface of these weakened fish are occasionally infected with other ectoparasites, such as *Benedenia* sp. and *Trichodina* sp. In spawning seasons, egg production from weakened brood stocks due to heavy infection is very low.

In an outbreak in 1999, *P. epinepheli* was noticed in fish a few days after being introduced to the hatchery from the Inland Sea, suggesting that wild-origin brood stock is a possible source of *P. epinepheli* infection. The parasite attached to the gill filaments by hooks at densities of 5–500 individuals per 100 gill filaments (Fig. 1). Intensity of infection tends to be low at low water temperatures (around 10°C) but increases gradually from late spring to summer when water temperatures range from about 15 to 25°C.

Treatments of P. epinepheli infection by H₂O₂ bathing

The effect of H₂O₂ bathing on *P. epinepheli* and host fish is shown in Table 1. Treatments at 10 and 15°C showed a similar effect. Briefly, 100% mortality of

worms occurred in the experimental groups bathed for 45 and 60 min, but not in those bathed for 15 and 30 min. No treated fish died during or after treatments. On the other hand, treatments at 20 and 25°C showed a 100% mortality of the parasite after a 15-min bath as well as in longer bathing. The number of worms on the gills at 3 h post-treatment at 25°C was much lower than at other temperatures. Note that the intensity of infection showed little difference within the experimental groups before treatments (data not shown). Detachment of dead worms appears to occur relatively quickly with bathing at 25°C, although detachment of a small number of dead worms might also occur with powerless treatment at 10°C for 15 or 30 min, or at 15°C for 15 or 30 min. Because it is desirable that a single treatment kills all worms on the gills of adult groupers to maintain good husbandry without handling of spawners during the spawning season, efficacy was evaluated only by mortality of worms remaining on the gills after treatments in the present study.

In bathing treatment at 20°C for 60 min, some fish became inactive after 45 min, followed by lying on the bottom, but no mortality occurred during or after treatment. In contrast, toxicity of the H₂O₂ against the host fish was noticeable at 25°C. Treatment at 25°C resulted in mortalities of 60, 100 and 100% after bathing for 30, 45 and 60 min, respectively. Dead fish (550–1730 g BW) commonly displayed petechia and brown discoloration of the gills, indicating damages due to H₂O₂ exposure.

In the control groups, all examined worms were active at 3 h post-treatment and no abnormalities or mortality were recorded in the host fish. In fish surviving the treatments, the characteristic sign of infected fish, oper-

Table 1. Results of treatments of *Pseudorhabdosynochus epinepheli* on red spotted grouper by 700 ppm hydrogen peroxide bathing

Bathing water temp. (°C)	Bathing period (min)	Fish No.	Percent mortality of		Bathing water temp. (°C)	Bathing period (min)	Fish No.	Percent mortality of	
			Worms* ¹	Host fish				Worms	Host fish
10	15	1	33 (1/3)* ²	0 (0/5)* ³	20	15	1	100 (13/13)	0 (0/5)
		2	33 (1/3)				2	100 (2/2)	
		3	40 (6/15)				3	100 (7/7)	
		4	0 (0/1)				4	100 (18/18)	
		5	0 (0/2)				5	100 (17/17)	
	30	1	47 (8/17)	0 (0/5)		30	1	100 (3/3)	0 (0/5)
		2	100 (5/5)				2	100 (4/4)	
		3	46 (6/13)				3	100 (15/15)	
		4	20 (1/5)				4	100 (3/3)	
		5	17 (1/6)				5	100 (4/4)	
	45	1	100 (6/6)	0 (0/5)		45	1	100 (3/3)	0 (0/5)
		2	100 (30/30)				2	100 (9/9)	
		3	100 (1/1)				3	100 (1/1)	
		4	100 (11/11)				4	100 (9/9)	
		5	100 (22/22)				5	100 (17/17)	
	60	1	100 (5/5)	0 (0/5)		60	1	100 (26/26)	0 (0/5)
		2	100 (14/14)				2	100 (10/10)	
		3	100 (9/9)				3	100 (3/3)	
		4	100 (13/13)				4	100 (4/4)	
		5	100 (23/23)				5	100 (5/5)	
	Control	1	0 (0/29)	0 (0/5)		Control	1	0 (0/11)	0 (0/5)
		2	0 (0/21)				2	0 (0/12)	
		3	0 (0/11)				3	0 (0/30)	
		4	0 (0/24)				4	0 (0/24)	
		5	0 (0/34)				5	0 (0/35)	
15	15	1	0 (0/16)	0 (0/5)	25	15	1	100 (4/4)	0 (0/5)
		2	0 (0/14)				2	ND* ⁴ (0/0)	
		3	0 (0/30)				3	100 (4/4)	
		4	0 (0/2)				4	100 (2/2)	
		5	0 (0/8)				5	100 (2/2)	
	30	1	94 (16/17)	0 (0/5)		30	1	100 (3/3)	60 (3/5)
		2	100 (16/16)				2	100 (2/2)	
		3	100 (7/7)				3	ND (0/0)	
		4	100 (18/18)				4	ND (0/0)	
		5	91 (20/22)				5	ND (0/0)	
	45	1	100 (27/27)	0 (0/5)		45	1	100 (8/8)	100 (5/5)
		2	100 (17/17)				2	ND (0/0)	
		3	100 (18/18)				3	100 (2/2)	
		4	100 (15/15)				4	100 (2/2)	
		5	100 (50/50)				5	ND (0/0)	
	60	1	100 (33/33)	0 (0/5)		60	1	ND (0/0)	100 (5/5)
		2	100 (47/47)				2	ND (0/0)	
		3	100 (5/5)				3	100 (1/1)	
		4	100 (35/35)				4	100 (1/1)	
		5	100 (154/154)				5	ND (0/0)	

*¹ The mortality was determined by the microscopic examinations of worms on the gills which were excised from each treated fish at 3 h post-treatment.

*² Number of dead worms/number of worms on the gills examined.

*³ Number of dead fish/number of experimental fish.

*⁴ Not determined.

culum-rubbing, disappeared. Furthermore, treated fish became active and appetites improved, resulting in good husbandry conditions at 1 wk post-treatments. No clinical signs of disease were evident 2 wk after treatments.

The study shows that the bathing in 700 ppm H₂O₂ for 15 min at 20 or 25°C, or for 45 min at 10 or 15°C is effective in controlling *P. epinepheli* infection in adult red

spotted grouper of 200–2000 g in body weight. However, treatment at 25°C for 30 min and longer can cause mortality of above 60% in the host fish and, thus, requires rapid transfer of treated fish from the H₂O₂ solution at the end of bathing in the field trial. In general, host toxicity of H₂O₂ depends on life stage of the fish³⁾, therefore, further studies are required to clarify the toxic-

ity of the H₂O₂ bathing on juvenile red spotted grouper. In conclusion, chemotherapy with H₂O₂ has a high potential as an effective treatment for *P. epinepheli* infection in red spotted grouper.

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