

Digenean trematodes of cultured grouper (*Epinephelus coioides* and *E. bleekeri*) in Khanh Hoa Province, Vietnam

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ABSTRACT

Groupers are among the most important fish cultured in Vietnam and, recently, diseases due to parasites have led to massive losses for the aquaculture industry. To date, little research has been conducted on parasitic diseases of marine fish in Vietnam. To address this, 156 specimens, collected from January to December 2006, of pond- and cage-cultured *Epinephelus coioides* and *E. bleekeri*, with size ranging from 85 – 500 mm (mean = 286.8 ± 96.4 mm) were examined. Results show that cultured grouper in Khanh Hoa Province, Vietnam were infected with adults of at least 5 digenean species. Three intestinal digeneans (*Proisorhynchus epinepheli*, *P. pacificus*, and *Helicometra fasciata*), one from the stomach (*Erilepturus hamati*), and one from the skin (*Transversotrema patialense*) were identified. Unidentified didymozoids and metacercaria were found in the musculature. *Proisorhynchus pacificus* had the highest prevalence of infection. There was a statistically significant difference between the prevalence of infection in *P. pacificus* in pond- and cage-cultured *E. coioides* (61% (20/33) prevalence in cage-cultured fish; 27% (9/33) in pond-cultured fish). *Proisorhynchus epinepheli* is a first record for *E. bleekeri*; *P. pacificus* is a first record for *E. coioides* in Vietnam; *E. hamati* and *H. fasciata* are first records for *E. coioides* and *E. bleekeri*; *T. patialense* is a first record for Vietnam and for *E. bleekeri*. This is the first time that the Family Didymozoidae has been recorded in grouper in Vietnam, and a first record in *E. bleekeri*. The difference in intensities of infection of

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P. epinepheli and metacercariae in cage- and pond-cultured *E. bleekeri*, of *P. pacificus* and metacercaria in cage- and pond-cultured *E. coioides* were not statistically significant. Data on intensities of infection of the other digeneans were insufficient to be tested for statistical significance.

Key words: grouper, Vietnam, digenetic trematodes, parasites

INTRODUCTION

Groupers are widely cultured in South Europe and Asia, including Vietnam. In Vietnam, they have been used to replace black-tiger shrimp (*Penaeus monodon* Fabricius) and lobster (*Panulirus ornatus* Fabricius, 1789) farming where disease outbreaks have made the culture of species economically unprofitable (Vo *et al.*, 2008a). Grouper culture provides a good source of income to the poor communities in some provinces in northern and central Vietnam (Vo *et al.*, 2008a). However, groupers are also sensitive to various diseases, and loss of grouper due to diseases has been estimated to cost many millions of dollars (Bondad-Reantaso *et al.*, 2005).

In Vietnam, groupers have been found infected with numerous parasites (Vo *et al.*, 2005, 2007a, 2007b, 2008a, 2008b, 2008c) including digenetic trematodes. Such parasites of marine and brackish water fish have been intensively studied in many countries. Previous to the above studies, the only English publication mentioning digeneans of Vietnamese grouper is the checklist of Arthur and Bui (2006).

While the impact of digeneans on their first intermediate hosts such as snails is clear (Muir and Chiba, 2007; Davies and Knowles, 2001), with specific exception of the blood dwelling sanguinicolids and some flesh dwelling didymozoids, their impact on fish has not often been observed, although some studies reported the impact of larval digeneans on fish (Olson and Pierce, 1997; Lauckner, 1984; Bristow, 1990). In order to evaluate the impact of digeneans to fish, it is first essential to know the composition, prevalence and intensity of infection of the digeneans in their hosts. The first aim of this study was to study these attributes in different fish hosts and of the same fish hosts collected from different culture systems. The second aim of this study was to assess if different culture systems cause differential effects to the parasitism of digeneans by comparing the prevalence and intensities of infection of the parasites.

MATERIALS AND METHODS

Collection sites

Epinephelus coioides Hamilton, 1822, and *E. bleekeri* Vaillant, 1878, were collected live in ponds from Cua Be Estuary and Cam Ranh Bay, and from cages in Cua Be Estuary, Nha Trang and Van Phong Bays, Khanh Hoa Province, Vietnam, between January and December, 2006. The ponds had a surface area of 1,500-3,000 m², and were 1-1.5 m in depth. All the ponds were located in tidal zones. The ponds in Cua Be Estuary were fed by

brackish water (with salinity ranging between 10 and 27 ‰), while the ponds in Cam Ranh Bay were fed by marine water (with salinity ranging between 31 and 33 ‰). The fish density in the ponds was about 3-5 fish/m² of the pond bottom areas. The cages used for rearing fish were floating net cages. Each cage had a surface area of approximately 9 m² and was 3-4 m deep and they were often linked together in groups. The cages in Cua Be Estuary were in brackish water (with salinity ranging between 10 and 27 ‰), while the cages in Nha Trang and Van Phong bays were in marine water (with salinity fluctuating between 31 and 33 ‰). Fish density in the cages was from 5-100 fish/m² of the cage bottom area. Fish in the ponds and cages were fed daily with trash fish, snails, bivalves, and crustaceans.

Fish were transported in styrofoam boxes with water from the original environment with aeration, from collection sites to the Research Institute for Aquaculture No.3 in Nha Trang. They were then kept in cement tanks with marine water (salinity between 30-32 ‰) continual flow through before being examined for parasites.

Each fish specimen was identified using Heemstra and Randall (1993) before parasitic examination. The number of each fish species collected from each sampling site, the length, weight (mean +/- standard deviation) are given in Table 1.

Examination for parasites

Fork length and body weight of each fish specimen were measured. Fins were cut and examined under a stereomicroscope for metacercariae. Skin was scraped and placed on glass slide for digeneans. Each fish was then dissected and its internal organs were placed in separate Petri dishes containing saline solution. Each organ was examined using stereomicroscope for parasites. The gut and stomach were dissected and examined by eye and then under a stereomicroscope. The flesh was pressed using glass pieces and observed under a stereomicroscope.

Identification and enumeration of parasites

Fresh specimens of digeneans collected were photographed, fixed, drawn, and measured for identification.

Table 1

Length and weight of cage and pond cultured *E. coioides* and *E. bleeker*. The minimum and maximum values are given in parenthesis.

	Cage cultured fish		Pond cultured fish	
	<i>E. coioides</i> (n=33)	<i>E. bleekeri</i> (n=62)	<i>E. coioides</i> (n=33)	<i>E. bleekeri</i> (n=28)
Mean length (mm)	351.6±41.0 (265.0-400.0)	256.5±86.7 (115.0-380.0)	327.8±110.1 (85.0-500.0)	227.9±76.3 (110.0-335.0)
Mean weight (g)	644.8±253.7 (187.0-1055.0)	309.5±246.4 (20.0-760.0)	709.2±448.5 (6.0-2000.0)	216.8±208.4 (13.6-650.0)

Data analysis

Standard parasitological parameters followed the recommendations in Bush *et al.* (1997). The prevalence of infection of each trematode species collected from cage- and pond-cultured host species was compared using a Chi-square test with Yates correction being employed if 5 or more fish were infected. When less than 5 fish were infected, the Fisher-exact test was used. The intensities of infection of the parasites between cage- and pond-cultured fish of the same species were compared using the Kruskal Wallis test, using a significance level of $\alpha = 0.05$.

RESULTS

The composition and the prevalence of infection of the parasites are given in Table 2. Five digeneans were identified to the species level. Adult trematodes include three species found in the intestines (*Proisorhynchus epinepheli* Yamaguti, 1939; *P. pacificus* Manter, 1940; and *Helicometra fasciata* Rudolphi, 1819), one species found in the stomach (*Erilepturus hamati* (Yamaguti, 1934) Manter, 1947), and one species found in the skin (*Transversotrema patialense* Soparkar, 1924). An unidentified didymozoid in the flesh and unidentified metacercaria types in the fins were also found.

Table 2

Prevalences (P) and intensities (I) of infection of trematodes of cage- and pond-cultured *E. coioides* and *E. bleekeri*. The minimum and maximum values are given in parenthesis; *only 1 fish infected.

Parasites	Cage cultured fish				Pond cultured fish			
	<i>E. coioides</i>		<i>E. bleekeri</i>		<i>E. coioides</i>		<i>E. bleekeri</i>	
	P (%)	I	P (%)	I	P (%)	I	P (%)	I
<i>Proisorhynchus epinepheli</i>	0.0		6.4	30.8 ±44.1 (1; 95)	0.0		21.4	111.8 ±175.7 (1; 463)
<i>Proisorhynchus pacificus</i>	60.6	9.8 ±11.3 (1;39)	0.0		27.3	4.8 ±3.2 (2; 11)	0.0	
<i>Erilepturus hamati</i>	0.0		3.2	1.0 ±0.0 (1; 1)	3.0	1*	0.0	
<i>Helicometra fasciata</i>	3.0	2*	1.6	1*	0.0		7.1	1.5 ±0.7 (1; 2)
<i>Transversotrema patialense</i>	0.0		3.2	1.0 ±0.0 (1; 1)	0.0		0.0	
Didymozoidae	3.0	2*	1.6	1*	3.0	5*	0.0	
Metacercariae (in the fins)	6.1	1.3 ±0.6 (1; 2)	2.4	5.0 ±6.4 (1; 20)	9.1	3.7 ±3.8 (1; 8)	25.0	22.3 ±35.8 (1; 101)

A brief description of the parasites collected are shown below:

1. *Proisorhynchus epinepheli*: body (1,150 – 1,750) x (350 – 750) μm ; rhynchus (270 – 350) x (260 – 330) μm ; gonads near midbody; testis slightly diagonal; vitellerium with 20-23 follicles, forming an arch between the rhynchus and stomach. This species was found in both cage- and pond-cultured *E. bleekeri*.
2. *Proisorhynchus pacificus*: body (1,100 – 2,850) x (360 – 1250) μm ; rhynchus (220 – 400) x (200 – 410) μm ; gonads near midbody; testis tandem; vitellerium with 18 – 24 follicles, forming 2 lateral lines from front testis up to near the rhynchus. This species was found in both cage- and pond-cultured *E. coioides*.
3. *Erilepturus hamati*: body (1,100 – 2,525) x (340 – 1,000) μm ; ecsoma present. This ecsoma can protrude or withdrawn into the body; ventral sucker much larger than oral sucker; ovary posterior to testis; vitellarium ribbon-shaped. This species was found in cage-cultured *E. bleekeri* and pond-cultured *E. coioides*.
4. *Helicometra fasciata*: body 2.605 x 1.000 μm ; ventral sucker much larger than oral sucker; egg with long tail; ovary lobes anterior to testis; two testes tandem. This species was found in cage-cultured *E. coioides* and in pond-cultured *E. bleekeri*.
5. *Transversotrema patialense*: body flattened dorsal-ventrally (480 – 1,000) x (310,0 – 692,0) μm ; no oral sucker, ventral sucker small, rounded; testis big with 6-8 lobes; one ovary; eye-spots present; found under the scales. This species was found in cage-cultured *E. bleekeri*.
6. Unidentified didymozoid parasite: encapsulated; the capsulate is yellowish found in the flesh and operculum; body slender and whitish; ventral sucker large, in anterior half body. This parasite was found in cage-cultured *E. coioides* and *E. bleekeri* and pond-cultured *E. coioides*.
7. Unidentified metacercariae: the cysts were round or oval; some visible to naked eye; some are dark. Cysts were found in the fins of both *E. coioides* and *E. bleekeri* from both the cage- and pond-culture systems.

The highest (60.6%) and second highest prevalence of infection (27.3%) occurred among *P. pacificus* in cage- and in pond-cultured *E. coioides*, respectively. The prevalence of infection of other digeneans ranged between 1.6% (unidentified didymozoid in cage-cultured *E. bleekeri*) and 25.0% (unidentified metacercariae in pond-cultured *E. bleekeri*).

In each culture system, where both *E. coioides* and *E. bleekeri* were infected with the same digenean, *E. coioides* usually had the higher prevalence of infection. An exception was the prevalence of infection of the metacercaria in pond-cultured *E. coioides* and *E. bleekeri* where numbers were higher in the latter species.

The results of the comparison of the prevalence of each digenean in each fish species from cage- and pond-cultured system are given in Table 3. Results showed that only the prevalence of infection of cage- and pond-cultured *E. coioides* infected with *P. pacificus* were statistically significantly different ($P = 0.01$). In the other cases, the infection levels were either not sufficient to be compared or not statistically different.

Table 3

Comparison of the prevalence of trematode infected fish of the same species from cage and pond system.

Parasites	<i>E. coioides</i>			<i>E. bleekeri</i>		
	Cage cultured fish	Pond cultured fish	P value	Cage cultured fish	Pond cultured fish	P value
<i>Proisorhynchus epinepheli</i>	0/33	0/33	-	4/62	6/28	0.06
<i>Proisorhynchus pacificus</i>	20/33	9/33	0.01	0/62	0/28	-
<i>Erilepturus hamati</i>	0/33	1/33	1.00	2/62	0/28	1.00
<i>Helicometra fasciata</i>	1/33	0/33	1.00	1/62	2/28	0.22
<i>Transversotrema patialense</i>	0/33	0/33	-	2/62	0/28	1.00
Dydimozoidae	1/33	1/33	1.00	1/62	0/28	1.0
Metacercaria (in the fins)	2/33	3/33	1.00	15/62	7/28	0.85

The results of comparison of the intensities of infection of the digeneans in fish of the same species collected from cages and ponds are given in Table 4. This analysis showed that intensities of infection of the digeneans were not significantly different between cage- and pond-cultured hosts.

Table 4

Comparison of the intensities of digeneans in each fish species cultured in cage and pond systems (in the brackets are min and max of the respective values).

Parasites	<i>E. coioides</i>			<i>E. bleekeri</i>		
	Cage cultured fish	Pond cultured fish	P value	Cage cultured fish	Pond cultured fish	P value
<i>Proisorhynchus epinepheli</i>				30.8 ±44.1 (1; 95)	111.8 ±175.7 (1; 463)	0.67
<i>Proisorhynchus pacificus</i>	9.8±11.3 (1; 39)	4.8±3.2 (2; 11)	0.53			
Metacercaria (in the fins)	1.3±0.6 (1; 2)	3.7±3.8 (1; 8)	0.57	5.0±6.4 (1; 20)	22.3±35.8 (1; 101)	0.14

DISCUSSION

Digenean trematodes parasitise a wide-range of fish hosts, and are found in fresh, marine and brackish water fish and many species are found in numerous fish host species. They have been reported in 62 out of 159 species of groupers belonging to the sub-family Epinephelinae. Ninety percent of these host-parasite combinations have been reported only once or twice, and, no reports have suggested that groupers are free from trematode infections (Cribb *et al.*, 2002). This suggests that digenean trematodes in groupers have not been studied sufficiently.

In Vietnam, Arthur and Bui (2006) firstly gathered reports of digeneans in Vietnamese grouper. These authors mentioned digeneans parasitizing *Epinephelus moara* Bloch, 1793, *E. merra* Bloch, 1793, *E. sexfasciatus* Valenciennes, 1828 and *E. tauvina* Forsskäll, 1775. The cases reported by Arthur and Bui (2006) all occurred in the northern Vietnam, where *E. coioides* and *E. bleekeri* have not been reported as fish of natural distribution or in culture. Therefore, this study is the first to report digenean trematodes in *E. coioides* and *E. bleekeri* in Vietnam.

Groupers belonging to Family Serranidae have the fifth largest record of parasitic trematodes. The epinephelins ranks as the 10th largest number of host records for trematodes, harboring at least 147 trematode species (Cribb *et al.*, 2002). This ranking may be higher if trematodes in grouper had received more study. Sixteen species of trematodes are restricted to, and shared among, epinephelins (Cribb *et al.*, 2002). Leong and Wong (1990) found 3, 3, and 2 species of digeneans in cage-cultured *E. malabaricus* Bloch and Schneider, 1801 in Malaysia, Thailand and the Philippines, respectively. This study found 5 species, 1 family and an unidentified metacercaria in cultured *E. coioides* and *E. bleekeri*. Only *P. pacificus* was found by Leong and Wong (1990) and in the present study. This may be due to different fish host species examined.

The individual parasites identified in this study are discussed below:

1. ***Proisorhynchus epinepheli***: This is a first record for this parasite in *E. bleekeri*, and it was not found in *E. coioides*. Arthur and Bui (2006) reported it from *E. tauvina*, *E. bruneus* Bloch, 1793, *E. sexfasciatus*, *E. merra* in Vietnam. While Cribb *et al.* (2002) listed 7 different hosts for this parasite, including *E. akaara* Temminck and Schlegel, 1842, *E. chlorostigma* Valenciennes, 1828, *E. cyanopodus* Richardson, 1846, *E. diacanthus* Valenciennes, 1828, *E. merra*, *E. quernus* Seale, 1901, *E. undulosus* Quoy and Gaimard, 1824. In addition, it was also found from *E. areolatus* Forsskäll, 1775 (Nahhas, Sey and Nakahara, 2006).
2. ***Proisorhynchus pacificus***: This is the first record for Vietnam and for *E. coioides*. This species had the highest prevalence of infection among the digeneans in this study, infesting up to 60.6% and 27.3% of cage and pond cultured *E. coioides*, respectively. This is still lower than the prevalence of infection of 97.2% found by Leong and Wong (1988) in cage cultured *E. malabaricus* in Malaysia. The mean intensities of infection found in this study were also much lower, being 9.8% and 4.8% in cage cultured and pond cultured *E. coioides*, while Leong and Wong (1988) found 81.2% in cage cultured *E. malabaricus*. Chinabut *et al.* (1996) reported it from cultured *E. coioides* in Thailand. Cribb *et al.* (2002) present records for *E. malabaricus* and *E. tauvina*. Both *E. malabaricus* and *E. tauvina* are often misidentified with *E. coioides* (Heemstra and Randall, 1993).
3. ***Erilepturus hamati***: This species has been found in different fish species from various localities. It also has many synonyms; Bray *et al.* (1993) reported 25 synonyms of this species. It was reported in *E. malabaricus* in the Philippines (Arthur and Lumanlan-Mayo, 1997). Velasquez (1962) found this species in *Lates calcarifer* and described it as a new species, *Lecithochirium neopacificum*. Leong and Wong (1988) reported a

- prevalence of infection of 15.7% of *E. malabaricus* and Chinabut *et al.* (1996) found this species in *E. coioides* in Thailand. This is the first record for this species in Vietnam, the first record for this parasite on *E. bleekeri*, and the first record on *E. coioides*.
4. ***Helicometra fasciata***: This is the first record for *E. coioides* and *E. bleekeri*. Arthur and Bui (2006) recorded it from *E. sexfasciatus* in Vietnam. Cribb *et al.* (2002) reported it from *Epinephelus fasciatus* Forsskäll, 1775, *E. merra*, and *E. quoyanus* Valenciennes, 1830.
 5. ***Transversotrema patialense***: This is the first record for Vietnam and for *E. bleekeri*. Cribb, Bray and Barker (1992) reviewed the family Transversotrematidae and formally synonymised *T. koliensis*, *T. laruei*, *T. chackai*, *T. soparkari* with *T. patialense*. *Transversotrema koliensis* was originally described as *Cercaria koliensis* (Olivier, 1947). *Transversotrema laruei* was described by Velasquez (1958) from *Lates calcarifer* Bloch, 1790 in the Philippines, the fish being collected in brackish water. *Transversotrema chackai* was described by Mohandas (1973) from fresh water fish collected in the Chackai Canal in India. *Transversotrema soparkari* was described by Pandey (1971) from fish collected from India. *Transversotrema patialense* was described by Soparkar (1924) from India. Thus, *T. patialense* is found on fish either from fresh or/and brackish waters. In this study, this species was found in two cage cultured *E. bleekeri* from Cua Be Estuary, which is a brackish water area.
 6. **Didymozoidae**: This is the first record from grouper in Vietnam, and the first record for *E. bleekeri*. In Vietnam, 5 species including *Didymozoon polymorphis* Oshmarin and Mamaev, 1963, *Monilicaecum ventricosum* Yamaguti, 1942, *Multubovarium amphibolum* Mamaev, 1970, *Neomatanematobothrioides rachycentri* (Parukhin, 1969) Yamaguti, 1971 and *Torticaecum fenestratum* (Linton, 1907) Yamaguti, 1942 including some unidentified species belonging to this family have been reported from marine fish collected from Tonkin Gulf (Arthur and Bui, 2006). Globally, 7 genera of this family have been reported from epinepheline hosts, of which *Allonematobothrium* is most restricted to epinephelines with 5 out of 6 species being found only in this fish group (Cribb *et al.*, 2002). Cruz-Lacierda *et al.* (2001) reported *Gonapodasmius epinepheli* in *E. coioides*.
 7. **Metacercariae**: Larval trematodes have been studied intensively in many countries. Metacercariae have been reported from brackish and marine waters in some countries in Asia, particularly from South Korea (Joo, 1984; Seo *et al.*, 1984; Chai and Lee, 2002). In Vietnam, Arthur and Bui (2006) reported records of digenean metacercariae found in some marine fish species such as *Drepane punctata*, *Ephippus orbis*, *Gerres filamentosus*. Vo *et al.* (2008b) reported a new record of the metacercaria of *Pygidiopsis suma*. In that paper, the authors also presented new records for *Heterophyopsis continua* and *Procerovum varium* metacercariae, while adult *H. continua* were reported by Nguyen (2000) from the sea gull (*Larus genei*).

Host-specificity has been studied for different parasites from different hosts and some studies have been conducted on the host-specificity of digenean trematode larvae; their hosts are usually mollusks (Möller and Anders, 1986). Generally, there are few references concerning fish host specificity of metacercariae or adult digeneans. Most of parasites

found in these studies seem to be able to parasitize different fish species. *Proisorhynchus epinepheli* is found on many fish species (Cribb *et al.*, 2002; Arthur and Bui, 2006; Nahhas, Sey and Nakahara, 2006). *Erilepturus hamati* is found on different fish genera (Velasquez, 1962; Arthur and Lumanlan-Mayo, 1997; Bray *et al.*, 1993). In the present study, it was found in both fish hosts. *Helicometra fasciata* is found in numerous species of *Epinephelus* (Arthur and Bui, 2006; Cribb *et al.* 2002). It was found in both hosts in this study with similar prevalence of infection. *Transversotrema patialense* is found in different fish species in both fresh and brackish waters (Soparkar, 1924; Olivier, 1947; Velasquez, 1958; Pandey, 1971; Mohandas, 1973). *Epinephelus bleekeri* is a new host record for this digenean. Didymozoidae and metacercariae were not identified to species level. Many species of different genera belonging to didymozoidae have been reported as parasites of different fish species, thus, making it difficult to discuss if these parasites are specific to any host.

In this study, the only evidence of host specificity is with *P. pacificus*. However, this species has also been reported from *E. malabaricus*, *E. tauvina*, and *E. coioides* by different authors and from *E. coioides* in this study. As referred to earlier, there has often been confusion in identifying these host species. In this study, due to the limited number of specimens used, it is not possible to confirm host specificity of this species and further investigation is needed to clarify this.

Muir and Chiba (2007) and Davies and Knowles (2001) reviewed the impacts of digenean trematode larvae on snails and bivalves. The most serious impact is the reduction of reproduction capability, including castration (Lauckner, 1984; Crew and Esch, 1986). They may also affect the burrowing ability of bivalves (Lauckner, 1984). Serious impact of digeneans on human and other animals has been confirmed by many studies (Hong *et al.*, 1996; Chai, Murrell and Lymbery, 2005; Sohn and Chai, 2005). It has also been confirmed that the two fish hosts in this study carry metacercaria of zoonotic trematodes (Vo *et al.*, 2008b). While adult digeneans found in the intestines of fish do not normally cause problems in fish (Schäperclaus, 1986), Lauckner (1984) indicated that a single metacercaria of digenean trematode is sufficient to kill a fish larva. Ogawa, Nakatsugawa and Yasuzaki (2004) reported a case of gross pathology caused by infection with digenean trematode metacercaria in cyprinid fish (*Zacco platypus* Temminck & Schlegel, 1846 and *Squalidus chankaensis chankaensis* Dybowski, 1872) in Japan; bucephalids were believed to be the disease causing agents. Olson and Pierce (1997) reported cartilage proliferation phenomenon in steelhead trout (*Oncorhynchus mykiss* Walbaum, 1792) collected from Willamette River, United States of America; members of the families Heterophyidae and Cryptogonimidae were suggested to be the agents. Blazer and Gratzek (1985) reported another case of proliferation in gill cartilage of fish infected with metacercariae of a digenean trematode. Bristow (1990) found immature final stage digeneans associated with mortalities in “postage stamp” larvae of Atlantic halibut *Hippoglossus hippoglossus* Linnaeus, 1758. To date, there is no chemotherapy to control metacercaria stage of digeneans in fish (Post, 1987). The impact of intestinal digeneans to fish has not been studied intensively, although such parasites are very common in most fish populations.

Environmental effects of many parasitic diseases are well recognized (Liang *et al.*, 2007) and parasites have been used as markers of biosystem health and stress (Marcogliese, 2005; Dzikowski, Paperna and Diamant, 2003). Landsberg *et al.* (1998) showed that parasites of fish are more sensitive to environmental stress than are the fish themselves. These authors described the response of specific parasites to particular environmental factors, for example, the response of monogeneans to temperature, nematodes to contaminants and protists to low dissolved oxygen. The parasite community may also be affected by the presence and/or relative abundance of benthic macro-invertebrates, many of which are intermediate hosts in the digenean lifecycle (Möller and Anders, 1986). In this study, fish in ponds and cages are living in two different environmental systems. The fish density in ponds was much lower than that in cages, the depth of the cage was usually 3-4 meters, while that of the ponds was 1.0-1.5 meters, the surface area of the ponds was much larger than that of the cages. These differences may contribute to different parasite burdens of *P. pacificus* on cage- and pond-reared *E. coioides*. Other than *P. pacificus*, there was no difference in the prevalence of infection of the other parasites between the two culture systems; this may be due to the relatively small sample size. The life cycles of these parasites are complicated; the prevalence of infection may be affected by the biology of the intermediate host rather than environmental factors.

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