Parasitol Res (2009) 104:523–532
DOI 10.1007/s00436-008-1226-7

ORIGINAL PAPER

Transmission of fish parasites into grouper mariculture (Serranidae: *Epinephelus coioides* (Hamilton, 1822)) in Lampung Bay, Indonesia

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Received: 22 September 2008 / Accepted: 26 September 2008 / Published online: 15 October 2008

Abstract Differently fed groupers *Epinephelus coioides* from an Indonesian finfish mariculture farm were studied for ecto- and endohelminth parasites. Pellet-fed *E. coioides* were infested with 13 parasite species/taxa of which six had a monoxenous and seven a heteroxenous life cycle. A total of 14 parasite species/taxa were found in the fish that were fed with different trash fish species, four of them with a monoxenous and ten with a heteroxenous life cycle. The use of pellet food significantly reduced the transfer of endohelminths and the number of parasites with a heteroxenous life cycle. Out of ten studied trash fish species, 62 parasite species were isolated (39% ectoparasitic and 61% endoparasitic), four of them also occurring in the cultured *E. coioides* and 14 in different groupers from Balai Budidaya Laut Lampung. The trash fish is held responsible for the transmission of these parasites into the mariculture fish. Endohelminth infestation of pellet fed fish demonstrates that parasite transfer also occurs via organisms that naturally live in, on, and in the surroundings of the net cages. Seventeen recorded invertebrates from the net cages might play an important role as intermediate hosts and hence parasite transmitters. The risk of parasite transfer can be considerably reduced by feeding selected trash fish species with a lower parasite burden, using only trash fish musculature or minimizing the abundance of invertebrates (fouling) on the net cages. These methods can control the endoparasite burden of cultivated fish without medication. The control of ectoparasites requires more elaborate techniques. Once they have succeeded in entering a mariculture farm, it is almost impossible to eliminate them from the system.

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Introduction

With the decline of natural fish stocks, aquaculture has been an expanding business worldwide (Worm et al. 2006; FAO 2007; Nowak 2007). In Indonesia, the development of mariculture started with the systematic expansion of shrimp culture, reaching a production of approximately 140,000 tons in 1992. The outbreak of the white spot virus caused drastic economic losses between 1992 and 1998, when the Indonesian culture of *Penaeus monodon* (tiger prawn) collapsed to an estimated 50,000 tons (Harris 2001). As an alternative and supported by high prices and the proximity to economically strong sale markets such as Hong Kong and Singapore, the culture of coral reef fishes of the genera *Epinephelus*, *Lates*, and *Cromileptes* has been consistently promoted and developed since the late 1980s (Harris 2001). However, the finfish mariculture industry had to accept substantial economic losses caused by fish

diseases and parasites in the late 1980s/early 1990s (e.g., Leong 1997). It is well-known that mariculture development can result in such problems (Koesharyani et al. 2001), leading to mass mortalities, marketing problems, and reduced commercial value (Davy and Graham 1979).

Parasites in finfish mariculture can have monoxenous (single host) or heteroxenous (multiple hosts) life cycles. The infection of the fish can happen either direct or indirect through the infected intermediate host. Under mariculture conditions, a variety of both monoxenous and heteroxenous parasites can occur in high numbers. Especially parasites with direct life cycles such as ciliates, monogeneans and crustaceans (e.g., Copepoda, Isopoda) occur in fish cages under high stocking densities (Grabda 1991; Diamant et al. 1999; Williams and Bunkley-Williams 2000). Such infestations can lead to enormous economic loss due to resulting mass mortalities (e.g., Tucker et al. 2002).

The transmission pathways of parasites to cultured fishes can vary considerably. They are dependent on the parasites life cycle. Fish for stocking, small fish species in the vicinity of the net cages, and trash fish used as feed can introduce parasites into the net cages. However, disinfection and the control of the intermediate hosts can prevent the transfer of fish parasites. Intermediate hosts such as molluscs for digenean parasites can be promoted through uncontrolled bio fouling on the cages. The knowledge of potential sources of parasite larvae is essential in order to develop effective control mechanisms for the mariculture industry (e.g., Velasquez 1976; Williams and Bunkley-Williams 2000).

Lampung province at the southern tip of Sumatra, Indonesia has vast potential for the development of grouper mariculture. Consequently, the number of net cages increased by 50% in the Lampung Bay area since 1999. The present study investigates the occurrence of ecto- and endohelminths in cultivated and differently fed groupers *Epinephelus coioides* from a mariculture farm in Lampung Bay. The comparison of pellet and trash fish fed fish was done in order to identify the potential risk of parasite transfer through the feed. Trash fish species were studied in order to identify potential sources of parasites, and invertebrates on the net cages were collected to reveal possible parasite pathways. Our results provide basic information for the development of preventive parasite control measures for the Indonesian grouper mariculture.

Materials and methods

Experiment and studied material

An experiment with *E. coioides* was carried out to analyze the influence of the feeding methodology on the transmission of parasites. A total of 600 fingerlings were purchased directly from the Balai Budidaya Laut (BBL, National Seafarming Development Centre) hatchery in June 2002 and separated into two groups; group I was fed with pellets (total length=TL 16.0–21.5 cm, total weight=TW 64.2–155.8 g) and group II with trash fish (TL 17.0–23.5 cm, TW 74.7–264.7 g) during the run of the experiment. They were kept in 1×1×1-m square wooden net cages close to the regular net cages of the BBL. Both groups were sampled after 3 months with dissection of 30 specimens of each group.

Ten different trash fish species were studied for metazoan fish parasites: *Sardinella gibbosa* (TL 11.5–14.0 cm, TW 16.6–31.3 g) (Fam. Clupeidae), *Nemipterus furcosus* (TL 11.3–18.1 cm, TW 21.1–75.1 g), *Nemipterus japonicus* (TL 11.0–15.3 cm, TW 18.9–42.6 g) (both Fam. Nemipteridae), *Pentaprion longimanus* (TL 9.2–14.5 cm, TW 14.7–44.1 g) (Fam. Gerreidae), *Gazza minuta* (TL 9.5–14.9 cm, TW 17.3–57.7 g), *Leiognathus stercorarius* (TL 8.8–11.5 cm, TW 9.7–24.5 g) (both Fam. Leiognathidae) (six omnivorous species), *Scolopsis taeniopterus* (TL 9.2–16.2 cm, TW 16.0–67.6 g) (Fam. Nemipteridae), *Upeneus moluccensis* (TL 11.0–15.8 cm, TW 18.7–51.6 g), *Upeneus sulphureus* (TL 9.3–14.5 cm, TW 15.1–48.5 g), *Upeneus vittatus* (TL 8.7–15.2 cm, TW 12.2–57.6 g) (all Fam. Mullidae) (four carnivorous species).

Invertebrates were collected from the net cage bio fouling after regular net exchange, fixed in 10% formalin, and stored for further identification. The organisms were identified to the lowest possible taxon under a dissecting microscope (Leica Wild M3B) or a stereomicroscope (Zeiss Axiostar plus).

Parasitological examination

Smears were taken from the gills and the inner opercula of the living groupers. The fish were examined directly after catch from the net cages. Trash fishes were investigated after they were deep frozen overnight. The total fish lengths (TL, to the nearest 0.1 cm) and total fish weights (TW, to the nearest 0.1 g) were taken. Skin, fins, eyes, gills, and the mouth and gill cavity were studied for ectoparasites. The digestive tract, liver, gall bladder, spleen, kidney, gonads, heart, and swim bladder were separated and transferred into Petri dishes with saline solution. The internal organs were examined under a stereomicroscope, while the gall bladder was removed and studied by using a phase-contrast microscope. Belly flaps and musculature were examined on a candling table.

The isolated parasites were fixed in 4% formalin and preserved in 70% ethanol. Smears from the gills and opercula were stained by using silver nitrate impregnation

after Klein (1926, 1958). The slides were rinsed and covered with 5% silver nitrate solution and impregnated for 30 min in the dark. The $AgNO_3$ was removed, and the slides were covered with distilled water and exposed to ultraviolet light for 40 to 50 min. The smears were dried after exposure and directly studied under the microscope for parasites of the genus *Trichodina*. Acanthocephala were transferred to freshwater until the proboscis everted prior to fixation. For identification purposes, Nematoda and Acanthocephala were dehydrated in a graded ethanol series and transferred to 100% glycerine (Riemann 1988). Digenea, Monogenea, and Cestoda were stained with acetic carmine (Palm 2004), dehydrated, cleared with Eugenol, and mounted in Canada balsam. Parasite identification followed standard identification literature and original descriptions.

Data analyses

The parasitological terms follow Bush et al. (1997): Prevalence (*P*) is the number of infected fish with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined (expressed as a percentage); intensity (of infection, *I*) is the number of individuals of a particular parasite species in a single infected host (expressed as a numerical range); and mean intensity (of infection, m*I*) is the average intensity or the total number of parasites of a particular species found in a sample divided by the number of infected hosts.

Results

Feeding methodology and parasite transmission

In September/October 2002, 3 months after the start of the experiment, 30 specimens of each feeding group (pellets/trash fish) were dissected for parasites. Table 1 illustrates the prevalence and intensity of infestation of pellet and trash fish fed *E. coioides*. The pellet fed *E. coioides* were infested with 13 parasite species/taxa of which six had a monoxenous and seven a heteroxenous life cycle. *Neobenedenia melleni* (Monogenea), *Hysterothylacium* sp. I (Nematoda), Pennellidae gen. et sp. indet., and Gnathiidae gen. et sp. indet. (Crustacea) occurred exclusively in feeding group I. Except for Myxozoa gen. et sp. indet. (*P*=63.3%), the heteroxenous parasites had low prevalence of infestation (<20%), while three monoxenous

Table 1 Prevalence (*P* in %), mean intensity (m*I*), and intensity (*I*) of the parasites isolated from *Epinephelus coioides* fed with pellets and trash fish

	Epinephelus coioides pellets (*n*=30)		*Epinephelus coioides* trash fish (*n*=30)	
	P (%)	m*I* (I)	*P* (%)	m*I* (I)
Monoxenous parasite species/taxa				
Trichodina spp. (P)	3.3	1.0 (1)	10.0	1.3 (1–2)
Benedenia epinepheli (M)	46.7	4.1 (1–11)	10.0	1.0 (1)
Neobenedenia melleni (M)	16.7	1.2 (1–2)	–	–
Capsalidae gen. et sp. indet. (M)	96.7	5.3 (1–15)	56.7	2.3 (1–7)
Pseudorhabdosynochus spp. (M)	100.0	58.8 (5–183)	100.0	37.9 (4–132)
Gnathiidae gen. et sp. indet. (CR)	3.3	1.0 (1)	–	–
Heteroxenous parasite species/taxa				
Microsporea gen. et sp. indet. (MI)	3.3	1.0 (1)	10.0	2.3 (1–5)
Myxozoa gen. et sp. indet. (MY)	63.3	–	43.3	–
Prosorhynchus australis (D)	6.7	1.0 (1)	13.3	1.3 (1–2)
Prosorhynchus luzonicus (D)	3.3	1.0 (1)	66.7	3.6 (1–12)
Prosorhynchus indet. (D)	–	–	6.7	1.0 (1)
Parotobothrium balli (C)	–	–	6.7	1.0 (1)
Scolex pleuronectis (C)	–	–	3.3	1.0 (1)
Hysterothylacium sp. I (N)	3.3	1.0 (1)	–	–
Terranova sp. (N)	–	–	3.3	1.0 (1)
Raphidascaris sp. I (N)	3.3	1.0 (1)	6.7	1.0 (1)
Serrasentis sagittifer (A)	–	–	3.3	2.0 (2)
Pennellidae gen. et sp. indet. (CR)	3.3	1.0 (1)	–	–

n number of the investigated specimens of fish, *A* Acanthocephala, *C* Cestoda, *CR* Crustacea, *D* Digenea, *M* Monogenea, *MI* Microsporea, *MY* Myxozoa, *N* Nematoda, *P* Protozoa

parasite species were above 20%. The monogenean taxon *Pseudorhabdosynochus* spp., comprised of two species, was isolated from all investigated fish and had the highest prevalence of infestation (100%). A total of 14 parasite species/taxa were found in the trash fish group II. Four species had a monoxenous and ten a heteroxenous life cycle. Therefore, less heteroxenous parasites were found in group I compared with group II. Heteroxenous *Prosorhynchus* indet. (Digenea), *Parotobothrium balli*, *Scolex pleuronectis* (Cestoda), *Terranova* sp. (Nematoda), and *Serrasentis sagittifer* (Acanthocephala) were isolated exclusively from feeding group II. With the exception of Myxozoa gen. et sp. indet. (43.3%), Capsalidae gen. et sp. indet. (56.7%), *Pseudorhabdosynochus* spp. (100.0%), and *Prosorhynchus luzonicus* (66.7%), the infestation rates were below 20.0%. Figure 1 illustrates differences in the prevalence of infestation with monoxenous compared to heteroxenous parasites between the two feeding groups.

Fish parasites in trash fish used for grouper mariculture

The ten studied trash fish species were infected with a total of 62 parasite species/taxa: nine Digenea, nine Monogenea, 11 Cestoda, 11 Nematoda, seven Acanthocephala, one Hirudinea, and 14 Crustacea. Tables 2 and 3 illustrate the isolated parasite species and their prevalence and intensities of infestation. A total of 39% (24 species) were ectoparasites and 61% (38 species) endoparasites. *S. gibbosa* was infested with the lowest number (eight) of parasite species, while *N. furcosus* had the highest parasite richness with 25 species (*N. japonicus* 20, *P. longimanus* 15, *G. minuta* 20, *L. stercorarius* 11, *S. taeniopterus* 18, *U. moluccensis* 19, *U. sulphureus* 15, *U. vittatus* 18). Of the 62 recorded parasite species, four were also isolated from cultured *E. coioides* at BBL Lampung (compare Table 4), most of them from the internal organs.

Invertebrates on the net cages as possible parasite transmitters

A total of 19 different organisms were collected from the net cages. According to the known life cycles of marine fish parasites, 17 might play an important role as intermediate host and hence parasite transmitters. These organisms belonged to the Bivalvia (three species), Sipunculidae (one), Polychaeta (Eunicidae one, Nereididae three), Oligochaeta (one), Crustacea (Gammaridea four, Caridea one, Porcellanidae one, Xanthidae one), and Echinodermata (Ophiuroidea one). These invertebrates have been recorded as intermediate hosts in the life cycles of various parasites. They are naturally occurring on the net cages and might be involved in the transmission of some of the isolated parasites.

Discussion

Fish feeding methodology and parasite transmission

It is known that trash fish as feed for cultured groupers can transmit parasites (Leong 1997; Tucker 1999; Sim et al. 2005; Williams and Rimmer 2005). The use of pellets during the feeding experiment should therefore prevent parasite transmission and reveal the real effect of fish feed on the parasite transfer. The present study demonstrates that the number of endoparasite species was lower in pellet fed

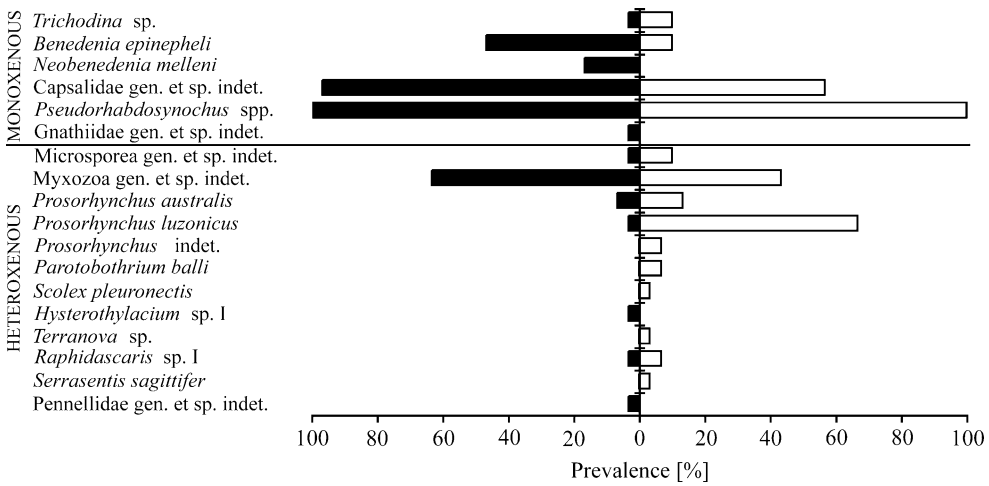

Fig. 1 Prevalences of the isolated monoxenous and heteroxenous parasite species of 30 analyzed *Epinephelus coioides* fed with pellets (*black bars*) and 30 specimens fed with trash fish (*white bars*)

compared with trash fish fed groupers (Fig. 1, Table 1). There was also a significant difference in the burden with heteroxenous parasites, resulting from different feeding methods and the pellet use. However, the pellet fed *E. coioides* was not parasite free, inhabiting six different endoparasite species (Fig. 1). Because the trophic transmission of parasites is limited while feeding manufactured pellets (Nowak 2007), the infested fish must have preyed upon natural intermediate or transport hosts originating from or in the surroundings of the net cages.

The prevalence of infestation was low. Thus, the use of pellet feed is an adequate method to reduce or even prevent transmission of several helminths. As a result of the natural distribution of parasites via organisms living in the vicinity of the mariculture facilities, parasite transmission can not be ruled out completely by using pellets. It is well known in the mariculture business that the existing pellets are often neglected by the cultured fish (Sim et al. 2005), and fish farmers themselves are often reluctant to use more costly feed. Fish parasites might be an indirect measure of artificial food acceptance by fish that is kept in mariculture.

In contrast to endohelminths, the infection with monoxenous parasites did not show any differences between the two feeding groups. Feeding pellets did not have a measurable effect on the monoxenous parasites, represented by ectoparasites only. Due to direct life cycles monogenean parasites such as *Benedenia epinepheli* and *Pseudorhabdoynochus* spp. are not dependent on intermediate hosts. Transfer happens directly from fish to fish, a pathway that is not affected by feed choice. However, indirect effects might be caused by the fitness of the cultivated fish that is influenced not only by the holding facility but also by the offered feed.

Trash fishes as parasite transmitters

Trophic parasite transmission into cultured groupers while feeding trash fish has been a matter of discussion (Tucker 1999; Sim et al. 2005; Williams and Rimmer 2005). However, not a single study provided evidence on the feed-transferred parasite species and the source of infestation. Ten trash fish species from five families that are regularly used at BBL Lampung were examined, resulting in a total of 62 different parasite species/taxa. There were significant differences in the parasite fauna of the studied fishes. The omnivorous species *S. gibbosa* was infested with the lowest number of parasites (eight), while *N. furcosus* (also omnivorous) harbored 25 species. We can assume that feeding *S. gibbosa* can reduce trophic parasite transmission into the cultured grouper, compared with the parasite species rich *N. furcosus*.

Four of the recorded 62 parasite species from trash fish were also detected in the cultured *E. coioides*. A total of 14 species are infecting different grouper species, *Lates calcarifer*, *E. coioides* and *Epinephelus fuscoguttatus* that are regularly cultivated at BBL Lampung (see Rückert 2006; Rückert et al. 2008). Of these, two are ectoparasites (non-transferable) and 12 endoparasites (transferable) (Table 4). *L. stercorarius* (omnivorous) was the only trash fish that did not harbor any of the 12 transferable endoparasites. *S. taeniopterus* (carnivorous) harbored eight, and only two transferable parasite species were isolated from *S. gibbosa*. The latter two species were identified as *Hysterothylacium* sp. I and *Camallanus paracarangis* (both nematodes). It is thus possible that *S. gibbosa* serves as an intermediate or transport host. It is obvious that the importance of the respective trash fish species for the parasite life cycles differs, resulting in a different risk to transfer fish parasites into grouper mariculture.

Except for *Nybelinia indica* (Cestoda), *Hysterothylacium* sp. I, and *Raphidascaris* sp. II (both Nematoda) that were isolated from the fish muscle, the majority of the transferable parasites was found in the internal organs. Six further parasite species (compare Tables 2 and 3) were also recorded from the trash fish muscles; however, they could not be recorded from the cultured groupers. It can be assumed that these species were not transferred successfully, with groupers being the wrong host.

The parasite burden of cultured grouper can be reduced by feeding well-chosen trash fish species in accordance to their parasite load. Another important aspect is feed treatment. Transmission of most parasites can be considerably limited if gutted fish is fed to the cultured fish. Deep freezing of trash fish (24 h) before feeding kills all ectoparasites and prevents transfer into the net cages. The transmission of muscle infesting endoparasites (e.g. *N. indica*, *Hysterothylacium* sp. I, and *Raphidascaris* sp. II), however, is still possible because short-term freezing does not kill robust larval nematodes. Other preventive measures such as heating, shredding, or marinating (Huss 1993) are time consuming and often not applicable in commercial mariculture farming in Indonesia. Consequently, natural feed (trash fish) replacement with manufactured pellets can better control helminth parasite transmission, and also prevent overfishing of trash fish species for the steadily growing mariculture industry.

Can invertebrates on the net cages be responsible for parasite transmission?

It is difficult to control marine life such as net fouling organisms (Nowak 2007). These organisms have been

Table 2 Prevalence (*P* in %), mean intensity (m*I*), and intensity (*I*) of the parasites isolated from the omnivorous trash fish species *Sardinella gibbosa*, *Nemipterus furcosus*, *Nemipterus japonicus*, *Pentaprion longimanus*, *Gazza minuta*, *Leiognathus stercorarius*

Parasite species/taxa	*Sardinella gibbosa* (*n*=30)		*Nemipterus furcosus* (*n*=30)		*Nemipterus japonicus* (*n*=30)		*Pentaprion longimanus* (*n*=30)		*Gazza minuta* (*n*=30)		*Leiognathus stercorarius* (*n*=30)		
	P (%)	m*I* (I)	*P* (%)	m*I* (I)	*P* (%)	m*I* (I)	*P* (%)	m*I* (I)	*P* (%)	m*I* (I)	*P* (%)	m*I* (I)	Site
Prosorhynchinae gen. et sp. indet. (D)					3.3	1.0 (1)							G
Aphanurus sp. (D)	76.7	7.3 (1–22)			3.3	1.0 (1)	3.3	2.0 (2)					G, I, S
Parahemiurus sp. (D)									3.3	1.0 (1)			S
Magnacetabulum leiognathi (D)											3.3	1.0 (1)	S
Neochoanostoma sp. (D)					13.3	1.0 (1)			3.3	1.0 (1)			G, PC, S
Didymozoidae gen. et sp. indet. (D)			3.3	1.0 (1)	3.3	1.0 (1)	23.3	3.9 (1–10)					BC, GO, I, PC, S
Digenea gen. et sp. indet. (D)	3.3	1.0 (1)	3.3	1.0 (1)	6.7	1.0 (1)	10.0	1.0 (1)	3.3	2.0 (2)			I, S
Capsalidae gen. et sp. indet. (M)			30.0	1.3 (1–4)									G
Callydiscoides scolopsidis (M)					93.3	29.7 (1–147)			73.3	3.1 (1–11)	76.7	14.4 (1–39)	G
Lamellodiscus flexuosus (M)					76.7	9.1 (1–51)			96.7	35.1 (2–103)			G
Mazocreaoides prashida (M)	20.0	2.2 (1–4)											G
Microcotyle adacis (M)			23.3	2.0 (1–5)									G
Microcotyle sp. I (M)									30.0	1.8 (1–4)	23.3	2.9 (1–7)	G
Microcotyle indet. (M)			3.3	1.0 (1)			3.3	1.0 (1)			3.3	1.0 (1)	G
Gastrocotyle indica (M)							3.3	2.0 (2)					G
Nybelinia indica (C)			6.7	1.0 (1)									SW
Mixonybelinia southwelli (C)			3.3	1.0 (1)									MU
Kotorella pronosoma (C)			3.3	1.0 (1)									SW
Callitetrarhynchus gracilis (C)									10.0	1.3 (1–2)			BC, L
Scolex pleuronectis (C)			3.3	1.0 (1)					6.7	1.0 (1)			I, S
Cestoda gen. et sp. indet. (C)	3.3	1.0 (1)			3.3	1.0 (1)	3.3	1.0 (1)					I, PC, S
Hysterothylacium sp. I (N)	3.3	1.0 (1)	56.7	2.7 (1–5)	53.3	2.4 (1–13)	30.0	1.7 (1–3)	10.0	1.3 (1–2)			BC, GO, I, L, ME, PC, S, SW
Hysterothylacium sp. II (N)			60.0	2.7 (1–11)	3.3	1.0 (1)							GO, I, ME, PC, S, SW
Raphidascaris sp. I (N)			3.3	1.0 (1)					3.3	1.0 (1)			I, ME
Raphidascaris sp. II (N)									3.3	1.0 (1)			SW
Cucullanus sp. I (N)			16.7	1.0 (1)			3.3	1.0 (1)					I, ME
Cucullanus sp. II (N)							6.7	1.5 (1–2)					I, S
Cucullanus indet. (N)			3.3	1.0 (1)					3.3	1.0 (1)			ME, S
Camallanus paracarangis (N)	16.7	1.0 (1)			6.7	1.0 (1)			30.0	1.2 (1–2)			I, ME, PC, S
Echinocephalus sp. (N)			3.3	1.0 (1)									L
Nematoda gen. et sp. indet. (N)	6.7	1.0 (1)	13.3	1.5 (1–3)	16.7	2.0 (1–5)	10.0	1.0 (1)	3.3	1.0 (1)			GO, I, L, ME, PC, S, SW
Serrasentis sagittifer (A)			3.3	1.0 (1)	3.3	1.0 (1)							ME, S
Gorgorhynchus sp. (A)			13.3	1.0 (1)	6.7	1.5 (1–2)	3.3	1.0 (1)					I, ME, PC
Leptorhynchoides thecatus (A)			16.7	1.6 (1–3)	30.0	1.2 (1–2)	33.3	10.5 (1–32)			6.7	1.0 (1)	G, L, I, PC, S
Palisentis sp. (A)									6.7	1.0 (1)			I

Acanthocephala gen. et sp. indet. (A)			3.3	1.0 (1)	3.3	1.0 (1)					3.3	1.0 (1)	I, ME, S
Holobomolochus nemipteri (CR)			26.7	1.4 (1–3)	13.3	1.3 (1–2)							G
Holobomolochus sp. (CR)			6.7	1.0 (1)			6.7	1.0 (1)			3.3	1.0 (1)	G
Nothobomolochus quadriceros (CR)							3.3	1.0 (1)			6.7	1.0 (1)	G
Pumiliopes squamosus (CR)	63.3	1.6 (1–2)											EC
Bomolochidae gen. et sp. indet. (CR)			10.0	1.0 (1)	6.7	1.0 (1)			6.7	1.0 (1)	3.3	1.0 (1)	G
Parataeniacanthus longicervis (CR)							3.3	1.0 (1)					G
Taeniacanthus sp. I (CR)											10.0	1.0 (1)	G
Caligidae gen. et sp. indet. (CR)			3.3	1.0 (1)	50.0	1.8 (1–3)	3.3	2.0 (2)					G
Proclavellodes pillaii (CR)									56.7	2.4 (1–5)			G
Lernanthropus gazzis (CR)									90.0	2.2 (1–8)	26.7	1.0 (1)	G
Gnathiidae gen. et sp. indet. (CR)			3.3	1.0 (1)	13.3	2.3 (1–4)	13.3	1.0 (1)					G

n number of the investigated specimens of fish, *A* Acanthocephala, *BC* body cavity, *C* Cestoda, *CR* Crustacea, *D* Digenea, *EC* eye cavity, *G* gills, *GO* gonads, *I* intestine, *L* liver, *M* Monogenea, *ME* mesenteries, *MU* muscles, *N* Nematoda, *PC* pyloric caeca, *S* stomach, *SW* stomach wall

widely neglected under the aspect of parasite transmission. However, 19 different invertebrates were collected from a net cage during the present study, with 17 of them playing a possible role as parasite intermediate hosts. Amphipoda are known as intermediate hosts in the life cycles of Digenea (Fam. Opecoelidae: *Podocotyle*), Cestoda, Nematoda (Fam. Cystidicolidae, Anisakidae), and Acanthocephala (Fam. Echinorhynchidae) (e.g., Klimpel et al. 2003, 2006, 2008). Digeneans such as *Podocotyle atomon* and *Allopodocotyle epinepheli* use Amphipoda as second intermediate hosts and might be transferred via the collected amphipods into the cultured fish (Paperna 1995; Klimpel et al. 2003; Rückert 2006). Amphipods might also function as intermediate or transport hosts for cestodes, nematodes (such as *Hysterothylacium* spp., e.g., Klimpel and Rückert 2005; Klimpel et al. 2008), and acanthocephalans (Rohde 1984; Klimpel et al. 2008). It is possible that *Serrasentis sagittifer* and *Gorgorhynchus* sp. are transmitted through amphipods living on the net cages.

Bivalvia are known as intermediate hosts of minor importance for Digenea and Nematoda (Jones et al. 2004). Because digeneans were isolated from the cultured fish as adults and no metacercaria were found (Rückert 2006; Rückert et al. 2008, present study), the collected Bivalvia cannot be responsible for the parasite transmission. Nematodes such as *Echinocephalus sinensis* (Fam. Gnathostomatidae) infest the oyster *Crassostrea gigas* (Rohde 1984). However, this family was not detected in the cultured groupers, and no oysters were found on the net cages. Sipunculidae are known as intermediate hosts for Myxozoa. They harbor the actinosporidian stage (Køie et al. 2004) and might play a role in transmitting the detected Myxozoa into *E. coioides*. Polychaeta can also serve as intermediate hosts for Myxozoa, harboring the actinosporidian stage (Køie 2002; Køie et al. 2004), or for nematodes such as *Hysterothylacium* spp. (Køie 1993; Klimpel 2005; Klimpel and Rückert 2005). *Hysterothylacium* sp. I was detected in the cultured groupers (compare Rückert 2006). Similarly, Oligochaeta have been described as intermediate hosts for Myxozoa (e.g., *Myxobolus cerebralis*) harboring the actinosporidian stage (Williams and Jones 1994).

Ophiuroidea play a role in the life cycles of Digenea (e.g., for the Fam. Zoogonidae) and Nematoda (*Hysterothylacium aduncum*; Bray and Gibson 1980; Klimpel et al. 2003, 2006). As second intermediate hosts for digeneans, they can harbor the metacercarian stage (Williams and Jones 1994; Paperna 1995), while for nematodes, they act as second intermediate host (Køie 1993). Consequently, they might play a role as second intermediate host for *Hysterothylacium* sp. Though Decapoda have been recorded as intermediate hosts for some nematode species, no species with known life cycle could be isolated from the cultured fish species.

Table 3 Prevalence (*P* in %), mean intensity (m*I*), and intensity (*I*) of the parasites isolated from the carnivorous trash fish species *Scolopsis taeniopterus*, *Upeneus moluccensis*, *Upeneus sulphureus*, *Upeneus vittatus*

Parasite species/taxa	*Scolopsis taeniopterus* (*n*=30)		*Upeneus moluccensis* (*n*=30)		*Upeneus sulphureus* (*n*=30)		*Upeneus vittatus* (*n*=30)		
	P (%)	m*I* (I)	*P* (%)	m*I* (I)	*P* (%)	m*I* (I)	*P* (%)	m*I* (I)	Site
Prosorhynchinae gen. et sp. indet. (D)							10.0	1.3 (1–2)	BC, MU
Propycnadenoides philippensis (D)	6.7	2.0 (1–3)							I, PC
Lecithochirium neopacificum (D)	3.3	1.0 (1)							G
Didymozoidae gen. et sp. indet. (D)			13.3	1.3 (1–2)	3.3	1.0 (1)	10.0	1.0 (1)	BC, I, MU
Digenea gen. et sp. indet. (D)	6.7	1.0 (1)			3.3	1.0 (1)			MU, PC
Capsalidae gen. et sp. indet. (M)	3.3	1.0 (1)							G
Callydiscoides scolopsidis (M)	100.0	46.0 (4–243)							G
Tagia otolithis (M)			53.3	4.1 (1–8)	26.7	1.8 (1–4)	3.3	1.0 (1)	G, GC
Nybelinia indica (C)			10.0	1.0 (1)	23.3	1.9 (1–4)	36.7	1.6 (1–4)	BC, L, ME, MU, S, SW
Nybelinia goreensis (C)			3.3	1.0 (1)			3.3	1.0 (1)	MU
Mixonybelinia southwelli (C)			3.3	1.0 (1)	3.3	2.0 (2)	6.7	1.0 (1)	BC, MU
Heteronybelinia minima (C)			6.7	2.0 (2)			6.7	1.5 (1–2)	MU
Symbothriorhynchus tigaminacantha (C)			6.7	2.5 (2–3)					BC, I
Dollfusiella sp. (C)	3.3	3.0 (3)							PC
Scolex pleuronectis (C)	6.7	4.0 (1–7)			3.3	1.0 (1)			I, L, PC
Pseudophyllidea gen. et sp. indet. (C)			6.7	1.0 (1)					I
Cestoda gen. et sp. indet. (C)			3.3	1.0 (1)					I
Hysterothylacium sp. I (N)	63.3	3.8 (1–23)	60.0	2.5 (1–7)	36.7	3.4 (1–9)	3.3	1.0 (1)	BC, GO, I, L, ME, MU, PC, S, SW
Hysterothylacium sp. II (N)			73.3	11.0 (1–35)	23.3	4.3 (1–12)	33.3	5.0 (1–16)	BC, GO, I, L, ME, PC, S, SW
Terranova sp. (N)	6.7	1.0 (1)	3.3	1.0 (1)			3.3	1.0 (1)	BC, I, PC
Raphidascaris sp. I (N)	6.7	1.5 (1–2)			6.7	1.0 (1)	3.3	1.0 (1)	GO, I, MU, PC
Raphidascaris sp. II (N)	3.3	1.0 (1)	20.0	1.5 (1–2)	16.7	1.8 (1–4)	13.3	1.3 (1–2)	BC, GO, I, L, MU, PC, S
Camallanus paracarangis (N)					3.3	1.0 (1)	3.3	1.0 (1)	I
Nematoda gen. et sp. indet. (N)	16.7	1.6 (1–3)	13.3	2.3 (1–6)	6.7	1.5 (1–2)	10.0	1.7 (1–2)	BC, G, GO, I, L, ME, PC, S, SW
Serrasentis sagittifer (A)	13.3	2.5 (1–4)	3.3	1.0 (1)	3.3	1.0 (1)			GO, I, ME, PC, S
Gorgorhynchus sp. (A)	3.3	1.0 (1)					10.0	1.0 (1)	BC, I
Rhadinorhynchus sp. (A)	3.3	1.0 (1)							I
Piscicolidae gen. et sp. indet. (H)							20.0	1.7 (1–4)	G
Holobomolochus nemipteri (CR)	46.7	1.5 (1–3)							G
Parataeniacanthus longicervis (CR)			53.3	3.9 (1–13)	23.3	3.1 (1–10)	53.3	64.6 (6–394)	G
Caligus sp. (CR)					3.3	1.0 (1)	3.3	1.0 (1)	G
Hatschekia longigenitalis (CR)	83.3	8.0 (1–47)							G
Lernanthropus gazzis (CR)			3.3	2.0 (2)					G
Lernanthropus sp. (CR)			10.0	1.7 (1–2)					G
Gnathiidae gen. et sp. indet. (CR)	6.7	1.0 (1)	10.0	3.3 (2–4)					G

n number of the investigated specimens of fish, *A* Acanthocephala, *BC* body cavity, *C* Cestoda, *CR* Crustacea, *D* Digenea, *G* gills, *GC* gill cavity, *GO* gonads, *H* Hirudinea, *I* intestine, *L* liver, *M* Monogenea, *ME* mesenteries, *MU* muscles, *N* Nematoda, *PC* pyloric caeca, *S* stomach, *SW* stomach wall

Table 4 List of parasites isolated from the dissected trash fish species as well as cultured grouper species with regard to their transferability

		Site
Monoxenous parasite species (no transfer, because of pre-treatment)		
Monogenea	Capsalidae gen. et sp. indet.	G
Crustacea	Gnathiidae gen. et sp. indet.	G
Heteroxenous parasite species (transferable)		
Digenea	*Lecithochirium neopacificum*	G
Cestoda	***Nybelinia indica***	BC, L, **MU**, S, SW
	Scolex pleuronectis	I, L, PC
	Pseudophyllidea gen. et sp. indet.	I
Nematoda	***Hysterothylacium* sp. I**	BC, GO, I, L, ME, **MU**, PC, S, SW
	Terranova sp.	I, PC
	Raphidascaris sp. I	GO, I, ME, PC
	***Raphidascaris* sp. II**	BC, GO, I, L, **MU**, PC, S, SW
	Camallanus paracarangis	I, ME, PC, S
	Echinocephalus sp.	L
Acanthocephala	*Serrasentis sagittifer*	GO, I, ME, PC, S
	Gorgorhynchus sp.	I, ME, PC

In bold, parasite species infesting the muscles
BC body cavity, *G* gills, *GO* gonads, *I* intestine, *L* liver, *ME* mesenteries, *MU* muscles, *PC* pyloric caeca, *S* stomach, *SW* stomach wall

Conclusions

The present study demonstrates a diverse number of invertebrates on the net cages that might function as parasite transmitters, necessitating future thorough investigations. The control of bio fouling has already been addressed by scientists since the 1970s, resulting in the use of different materials, management methods, and net cages (Blair and Burgess 1979, Lee et al. 1985; Lai et al. 1993; Hodson et al. 2000; Braithwaite and McEvoy 2004). We could recognize two major pathways that transfer fish parasites into grouper mariculture. Trash fish is held responsible for the transmission of endohelminths, depending on differently infested trash fish species and the use of the whole fish or the musculature without viscera. Endohelminth infestation of pellet fed fish demonstrates that the parasite transfer also occurs via organisms that naturally live in, on, and in the surroundings of the net cages. However, pellet feed in general reduces the infestation of the mariculture fish. The knowledge on transmission pathways, alternate feed use, and preparation methods as well as bio fouling control significantly reduces the risk of parasite transfer into the Indonesian grouper mariculture.

Acknowledgments The authors want to thank Mr. Sudjiharno, head of the National Seafarming Development Centre (Balai Budidaya Laut, BBL Lampung), for the possibility use the facilities at BBL, and Ireny Ohoiulun for her help during the trash fish investigation. Coauthor H.M. wants to thank the King Saud University, Riyadh, Kingdom of Saudi Arabia. This study was supported by the German Academic Exchange Service (DAAD), the Federal Ministry of Education and Research (BMBF Grant No. 03F0391A, SPICE/LOICZ-project), the German Research Council (DFG PA 664/4-1).

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