Parasite diversity as an indicator of environmental change? An example from tropical grouper (*Epinephelus fuscoguttatus*) mariculture in Indonesia

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SUMMARY

Fish parasites are used to monitor long-term change in finfish grouper mariculture in Indonesia. A total of 210 *Epinephelus fuscoguttatus* were sampled in six consecutive years between 2003/04 and 2008/09 and examined for parasites. The fish were obtained from floating net cages of a commercially run mariculture facility that opened in 2001. The fauna was species rich, consisting of ten ecto- and 18 endoparasite species. The ectoparasite diversity and composition was relatively stable, with the monogeneans *Pseudorhabdosynochus* spp. (83–100% prevalence, Berger-Parker Index of 0·82–0·97) being the predominant taxon. Tetraphyllidean larvae *Scolex pleuronectis* and the nematodes *Terranova* sp. and *Raphidascaris* sp. 1 were highly abundant in 2003/04–2005/06 (max. prevalence *S. pleuronectis* 40%, *Terranova* sp. 57%, *Raphidascaris* sp. 1 100%), and drastically reduced until 2008/09. These parasites together with the prevalence of *Trichodina* spp., ecto-/endoparasite ratio and endoparasite diversity illustrate a significant change in holding conditions over the years. This can be either referred to a definite change in management methods such as feed use and fish treatment, or a possible transition of a relatively undisturbed marine environment into a more affected habitat. By visualizing all parameters within a single diagram, we demonstrate that fish parasites are useful bioindicators to monitor long-term change in Indonesian grouper mariculture. This also indicates that groupers can be used to monitor environmental change in the wild. Further taxonomic and systematic efforts in less sampled regions significantly contributes to this new application, supporting fish culture and environmental impact monitoring also in other tropical marine habitats.

Key words: Applied parasitology, biodiversity, bioindicator, environment, *Epinephelus fuscoguttatus*, finfish mariculture, Indonesia, long-term change, taxonomy, aquaculture management.

INTRODUCTION

Aquatic tropical ecosystems are among the most vulnerable systems on earth and face increasing anthropogenic stress in terms of pollution and environmental degradation. About 2·75 bn people are expected to live within 60 miles of a coastline in 2025, living from or indirectly using the delicate coastal environments. It is obvious that an extensive use by various different stakeholders negatively influences the long-term perspective and sustainability of these ecosystems. This also has effects on local communities such as fishermen that directly depend on the natural resources along the coast.

Finfish mariculture is one of the activities that provide alternative income and new employment opportunities. The area used for the culture of finfish is extended year by year. Especially Indonesia with more than 17,500 islands has high potential to intensify fish production. Grouper (*Epinephelus* spp.) mariculture has increased from a production of 6,552 to in 2004 to 8,800 to in the year 2008, with a target of 30,000 to or a 340% increase for 2009 or in the forthcoming years (DJPB, 2009). However, the establishment of open water net cages close to the coast and often in the vicinity of corals reefs with high biodiversity can negatively affect the coastal environment and contributes to environmental degradation (e.g. Shuanglin *et al*. 2000). This might be caused by the activity itself due to regular feeding, fish treatment, boat traffic and anthropogenic waste. Nonetheless, fish mariculture requires good water quality and can become affected through activities in the surroundings, such as extensive fisheries or general habitat destruction.

Marine environments and environmental change can be studied either directly by a regular monitoring of water quality parameters or indirectly by using bioindicators (compare Palm and Rückert, 2009). These indicators react sensitively to specific environmental conditions or change, leading to a wide range of applications (indication of e.g. trace metals,

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organochlorines and radionuclides (*Dreissena polymorpha*, Mersch *et al*. 1992) or heavy metals (*Corbicula fluminea*, Graney *et al*. 1983)). Fish parasites have been successfully applied as biological indicators (e.g. Lafferty, 1997; Marcogliese and Cone, 1997; Overstreet, 1997; Marcogliese, 2005; Vidal-Martínez *et al*. 2010; Klimpel and Palm, 2011; Palm, 2011), especially to describe environmental conditions (water quality, MacKenzie *et al*. 1995, Galli *et al*. 2001; pollution, Sures, 2003, Sures and Reimann, 2003, Rückert *et al*. 2009*a*) or environmental stress (Khan and Thulin, 1991; Landsberg *et al*. 1998). Ectocommensals with direct life cycles such as trichodinid ciliates favour high bacterial load and can indicate polluted waters (Palm and Dobberstein, 1999; Ogut and Palm, 2005; Palm and Rückert, 2009; Rückert *et al*. 2009*a*). This contrasts with many endoparasites with complex life cycles that favour stable and non-polluted waters, where the full range of their required hosts is present (Khan and Thulin, 1991; Yeomans *et al*. 1997; Diamant *et al*. 1999; Lafferty *et al*. 2008*a*). Sasal *et al*. (2007) utilized the parasite community of apogonid fish to detect anthropogenic influences on two coral reef lagoons in New Caledonia. Lafferty *et al*. (2008*b*) proposed that the sampling of larval cestodes in teleosts is a convenient method for assessing spatial variation in shark distribution, and that the lower parasitism at Kiritimati Island compared to Palmyra Atoll resulted from a simplified food web due to over fishing. Low biodiversity could impair parasite transmission by reducing the availability of hosts required by parasites with complex life cycles. Rückert *et al*. (2009*a*) applied parasite parameters to describe the environmental situation in Segara Anakan lagoon, an extensively used mangrove ecosystem in Indonesia, and Rückert *et al*. (2009*b*) compared free living *Epinephelus fuscoguttatus* with specimens from mariculture. Palm and Rückert (2009) developed a method to visualize environmental differences between habitats, among them a grouper mariculture farm in Indonesia.

The present study applies the method by Palm and Rückert (2009) to monitor the parasite community of groupers from a mariculture facility in the Thousand Islands, a marine national park in Indonesia. Using six different parasite metrics from *Epinephelus fuscoguttatus* presented in a single figure, a significant change in parasite composition and abundance is recorded over six consecutive years. For the first time, fish parasites are used to monitor long-term change in holding conditions within a commercially run tropical finfish mariculture farm. This also suggests groupers to be used as biomarkers to monitor environmental change in the wild, requiring more detailed information on the parasite systematics and especially taxonomy. Possible reasons for the observed parasite community structures in the sampled cultured fish are discussed.

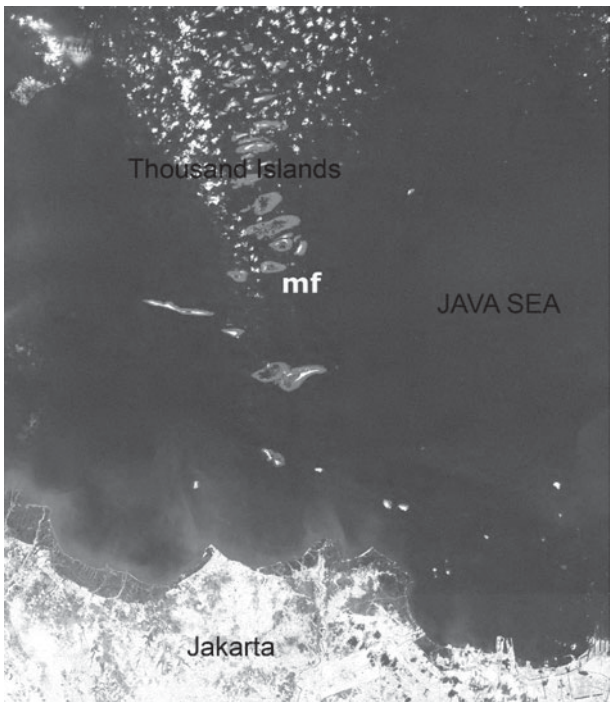

Fig. 1. Area of investigation in the Thousand Islands (Pulau Seribu), Indonesia. mf = mariculture farm.

MATERIAL AND METHODS

Fish samples and parasitological examination

Samples were taken within the framework of the project 'Science for the Protection of Indonesian Coastal Marine Ecosystems' during a single dry season (July/August 2006) and 5 rainy seasons between 2003/04 and 2008/09 (November 2003, January/February 2005, February 2006, March 2007/08–2008/09). A total of 210 *Epinephelus fuscoguttatus* (Hamilton) was studied from a mariculture facility in the utilization zone of the Thousand Islands (Pulau Seribu, Jakarta Bay, northern coast of Java), a marine national park with a salinity of around 32 (Fig. 1). All groupers were randomly taken from the surviving subsample of about 200 groupers that were cultivated for the present study, and originated from the same construction of 8 connected grouper net cages in the periphery of the farm.

The fish were examined directly after catch. Smears were taken from the gills, surface and the inner opercula of the living fish. The skin, fins, eyes, gills, mouth- and gill cavity were studied for ectoparasites. The inner organs such as the digestive tract, liver, gall bladder, spleen, kidneys, gonads, heart and swim bladder were separated and transferred into saline solution. While the internal organs were examined under a stereomicroscope, the gall bladder was removed and studied by using a phase-contrast microscope. Belly flaps and musculature were examined on a candling table. During examination, the following measurements were taken: total fish length (L_T, to the nearest 1·0 cm), total weight (W_T and slaughter weight (Ws) to the nearest 1·0 g) (Table 1).

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Table 1. Sampling periods, number (n) of dissected specimens, mean length and mean weight (range in parentheses) of *Epinephelus fuscoguttatus*, sampled in consecutive years from the rainy season 2003/04 until 2008/09 in the Thousand Islands. n number, L_T = total length, W_T = total weight, W_S = slaughter weight

Season	n	L_T [cm]	W_T [g]	W_S [g]
Rainy season 2003/04	35	26·9 23·5–33·0	429·9 272–743	393·3 254–683
Rainy season 2004/05	35	27·8 24·0–31·0	490·9 370–700	453·9 300–655
Rainy season 2005/06	35	27·0 24·0–31·0	387·1 495–559	353·3 282–498
Dry season 2006	35	26·4 24·5–30·0	397·8 292–585	362·3 264–533
Rainy season 2007/08	35	27·8 24·5–29·7	387·6 283–466	336·3 253–412
Rainy season 2008/09	35	26·7 23·9–28·9	373·3 304–477	324·5 262–414

Isolated parasites were fixed in 4% formalin and preserved in 70% ethanol. The smears from the gills, surface 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 minutes in the dark. The $AgNO_3$ was removed and the slides were covered with distilled water and exposed to ultraviolet light for 40–50 minutes. The smears were dried after exposure. For identification purposes, Nematoda were dehydrated in a gradated ethanol series and transferred to 100% glycerine (Riemann, 1988). Digenea, Monogenea and Cestoda were stained with acetic carmine, dehydrated, cleared with Eugenol and mounted in Canada balsam. Parasite identification followed standard parasite literature and original descriptions.

Parasitological parameters

The parasitological terms (prevalence, intensity and mean intensity) followed Bush *et al*. (1997). The Berger Parker Index characterizes the dominance of a respective parasite species within the sample $BP = N_{max}/N$, with N_{max} being the number of specimens of the most dominant species and N the total number of collected parasites within the sample. The diversity of the metazoan endoparasite fauna of each fish species was determined by using the Shannon–Wiener diversity index (H') and the evenness index (E) of Pielou (Magurran, 1988). The evenness was not considered a separate bioindicator within the present study. Myxozoan and microsporean parasites were not considered because it was not possible to calculate the intensity. The ratio of ecto- to endoparasites was calculated [Ec/En ratio (R) = No. of ectoparasite species/No. of endoparasite species], with trichodinid ciliates treated as present or absent. Species groups (higher taxa such as Nematoda indet.) that could not be further identified and might represent other recorded taxa were omitted from the calculations.

Statistical analyses were used to determine a significant change throughout the years. Chi-square test was used to compare each year with another and a combined analysis over 6 years for all parameters showing parasite prevalences and ecto/endoparasite ratio. Trend analyses according to Neumann were performed in order to demonstrate a significant change of the Ec/En ratio (R) and the H' diversity index.

Development of the mariculture farm

The sampled grouper mariculture combined with milkfish (*Chanos chanos*) culture started with the building activities on the site and experimental culture in 2001. For the construction, the former reef flat off Pulau Pramuka was elevated with sand sacks appr. 0·5 m above the highest sea level providing space of 250 × 70 m for building space and on land-based culture activities.

The development of the mariculture farm in terms of its size and production was monitored over the whole period (see Table 2). The following culture methods were used. Net cage sizes for *E. fuscoguttatus*: 3 × 5 × 1·5 m (< 10 cm), 4 × 4 × 4 m (> 10 cm) from 2001–2004, 3 × 3 × 3 m from 2004–2009; mesh size 0·64 cm (< 10 cm), 1·91 cm (> 10 cm); for *Chanos chanos*: net cage size 14 m in diameter and 5 m deep; net change: 1–2 times per month in *E. fuscoguttatus*. *E. fuscoguttatus* was fed 5–6% of the body weight twice a day with trash fish without head and organs. *Chanos chanos* was fed 3% of the body weight 4–5 times a day with pellet food (Luxindo 585, Misumafeed, Growbest, Comfeed, Pokpand). Additional treatment for *E. fuscoguttatus* was vitamin C and multivitamin food enrichment 1–2 times a week, freshwater bath once or twice per month, and sorting every second month. Mortality rate over the years has been low, appr. 10% per batch, antibiotic treatment occasionally. The fish was cultured for 9–12 months (*E.f.*) and 5–6 months (*C.c.*) before harvesting.

RESULTS

A total of 28 different parasite species/taxa were collected from *Epinephelus fuscoguttatus* out of the net cages from the Thousand Islands (Table 3). Beside new findings of not further identified higher taxa such as Gnathiidae gen. et sp. indet., Penellidae gen. et sp. indet. (Crustacea), unidentified Microsporea, Didymozoidae gen. et sp. indet., Sanguinicolidae gen. et sp. indet. (Digenea) and a not further identified trypanorhynch cestode, 5 new host and locality records (*Trichodina* spp./Ciliata; *Neobenedenia*

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Table 2. Development of the sampled mariculture farm. Not all net cages are filled with fish throughout the year. Bacterial infections were typically observed from October–November, dry season rainy season, and from April–May, rainy season into dry season

Year	2001	2002	2003	2003/4	2004/5	2005/6	2006	2007/8	2008/9
Net cages (n total)	6	6	7	31	31	42	42	44	41
Net cages (*E. fuscoguttatus*, >10 cm)	4–6	4–6	5–7	5–7	5–7	7–8	7–8	7–10	12–18
Fish number per net cage (*E.f.*)	150	150	200	200	200	300	300	400	400
Feed (kg/day)	0,81	0,81	1,26	1,26	1,26	2,16	2,16	3,6	6,48
Production based on the year 2008/9 (%)	1	1	5	5	10	20	20	50	100
Net cages (*C. chanos*)	0	0	1	12	12	46	46	23*	20*
Fish number per net cage (*C.c.* * 1000)	0	0	12	30–35	30–35	20–25	20–25	20–25	20–25
Feed (kg/day*1000)	0	0	0·075	0·5–1	0·5–1	3·5–4	7–8	4–4·5	3
Production based on the year 2006 (%)	0	0	1	10	10	50	100	60	40
Bacterial infection*	+++	+++	++	++	++	++	++	+	+

melleni/Monogenea; *Alcirona* sp./Crustacea; *Zeylanicobdella arugamensis*/Hirudinea; *Lecithochirium neopacificum*/Digenea) could be established. *Prosorhynchus australis* of Rückert *et al.* (2009*b*) and Palm and Rückert (2009) and *Prosorhynchus* cf. *crucibulum* of Palm and Rückert (2009) were treated as *Prosorhynchus* sp. 1 and 2 according to Bray and Palm (2009). Most species rich were groupers in 2004/05 (17 taxa), followed by 2005/06 (16 taxa) and 2003/04 (15 taxa). From the rainy season 2007/08 to 2008/09 the parasite richness decreased from 11 to 8. The parasite fauna changed from year to year, with highest parasite diversity in the first year of sampling in the second year of production after the establishment of the mariculture farm, and lowest biodiversity in the final year of sampling in 2008/09. The ectoparasite diversity and composition was relatively stable, with the monogeneans *Pseudorhabdosynochus* spp. (83–100% prevalence, Berger-Parker Index of 0·82–0·97) being the predominant taxon. *Pseudorhabdosynochus* spp. collected in 2003/04–2006 represents a combination of the two species *P. epinepheli* and *P. lantauensis*. In 2007/08 and 2008/09 only *P. lantauensis* was found.

To analyse the change in parasite composition during the years, the ecological parameters prevalence of the trichodinid ciliates, endo- and ectoparasite ratio and endoparasite diversity H' as suggested by Palm and Rückert (2009) together with the prevalence of infection with the nematodes *Terranova* sp. (larvae) and *Raphidascaris* sp. 1 (adults) as well as the tetraphyllidean cestode larvae *Scolex pleuronectis* (according to Lafferty *et al.* 2008*b*) were considered as given below.

Trichodinid ciliates, ratio of ecto-/endoparasites and metazoan endoparasite diversity (Shannon-Wiener Index)

Trichodinid ciliates were rare on the mariculture fish, and only a single fish each was found to be infected with a low density in each of the years 2003, 2008 and 2009. All other sampled groupers were trichodinid free. No significant change in trichodinid infestation was observed (Table 4).

The species richness ranged from eight to 17 species in 2008/09 and 2004/05 respectively. Lowest species richness was observed in the final year of sampling. The number of ecto- and endoparasite species differed. Highest ectoparasite richness occurred in 2003/04, 2005/06 and 2006, when five ectoparasite species were recorded. Lowest ectoparasite richness was found in 2004/05 and within the last two sampling periods in 2007/08 and 2008/09 (four ectoparasite species). The number of endoparasite species ranged from four to 13 in 2008/09 and 2004/05 respectively. The ecto-/endoparasite ratios calculated using the numbers of ectoparasite species vs. the numbers of endoparasite species ranged from 0·3 to 1·0, with a higher number of endoparasites compared to the number of ectoparasites except for the last sample in 2008/09. The increase of the ecto-/endoparasite ratio was not significant.

The endoparasite diversity of *Epinephelus fuscoguttatus* ranged from 0·10–1·63, with a significant trend ($p < 99$) in the data set. The highest endoparasite diversity (1·63) was recorded in 2003/04, followed by the sampling during 2004/05 (1·58) and 0·93 during dry season 2006 (Table 4). The lowest endoparasite diversity was calculated from the last sampling period in 2008/09 (0·10). Consequently, the endoparasite diversity decreased in the sampled groupers over the whole sampling periode (Table 3).

Prevalence of infection of metazoan parasites

The prevalence of infestation of the larval nematode *Terranova* sp., the adult *Raphidascaris* sp. 1 as well as larval tetraphyllidean cestode *Scolex pleuronectis* changed significantly during the sampling period. The most significant change was observed for both nematodes ($P < 00·1$), followed by the cestode larvae

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Table 3. Prevalence (*P*), intensity (*I*) and mean intensity (*Im*) of the collected parasite species from *Epinephelus fuscoguttatus*, sampled in consecutive years from the rainy season 2003/04 until 2008/09 in the Thousand Islands. n.c. = not calculated, * = published in Palm and Rückert (2009)

Parasite species/ -taxa		Rainy season 03/04		Rainy season 04/05*		Rainy season 05/06		Dry season 06		Rainy season 07/08		Rainy season 08/09	
		P [%]	*I* m (*I*)	*P* [%]	*I* m (*I*)	*P* [%]	*I* m (*I*)	*P* [%]	*I* m (*I*)	*P* [%]	*I* m (*I*)	*P* [%]	*I* m (*I*)
Ectoparasites	*Trichodina* sp. (P)	3	1·0 (1)							3	2·0 (2)	3	1·0 (1)
	Benedenia epinepheli (Mo)			87	5·2 (1–14)	89	2·7 (1–9)	14	2·2 (1–4)	40	4·5 (1–24)	20	2·4 (1–6)
	Neobenedenia melleni (Mo)							6	1·0 (1)				
	Capsalidae gen. et sp. indet. (Mo)			90	6·0 (1–35)	86	3·3 (1–8)	51	2·3 (1–8)				
	Pseudorhabdosynochus spp. (Mo)	100	158·2 (30–377)	100	344·4 (116–1006)	100	87·4 (41–179)	100	195·2 (46–363)	83	29·2 (1–205)	100	30·5 (1–186)
	Zeylanicobdella arugamensis (H)											17	1·5 (1–2)
	Alcirona sp. (Cr)	6	1·0 (1)										
	Penellidae gen et sp. indet. (Cr)	6	1·0 (1)			6	2·5 (14)						
	Gnathiidae gen et sp. indet. (Cr)									3	1·0 (1)		
	Microsporea (Mi)					3	n.c.						
	Myxosporea gen. et sp. indet. (My)	20	n.c.	69	n.c.	71	n.c.	69	n.c.				
	Prosorhynchus sp. 1 (D)	43	8·7 (1–42)	3	1·0 (1)	11	4·0 (1–13)					3	1·0 (1)
	Prosorhynchus sp. 2 (D)	49	10·1 (1–57)	3	1·0 (1)	14	3·6 (1–9)	3	2·0 (2)	6	1·5 (1–2)		
	Prosorhynchus indet.	9	3·0 (1–7)										
	Enenteridae gen. et sp. indet. (D)	51	2·7 (1–7)	3	2·0 (2)								
	Lecithochirium magnaporum (D)			23	1·1 (1–2)	9	1·0 (1)	6	1·0 (1)	11	1·5 (1–3)	6	1·0 (1)
	Lecithochirium neopacificum (D)							6	1·0 (1)				
	Didymozoidae gen. et sp. indet. (D)							3	1·0 (1)	14	1·2 (1–2)		
	Allopodocotyle epinepheli (D)	29	3·0 (1–7)	3	1·0 (1)					3	1·0 (1)		
	Sanguinicolidae gen. et sp. indet. (D)					29	4·6 (1–24)						
	Parotobothrium balli (C)			23	1·0 (1)			3	1·0 (1)				
Endoparasites	Trypanorhyncha gen. et sp. indet. (C)					3	1·0 (1)						
	Scolex pleuronectis (C)	26	1·7 (1–4)	40	3·9 (1–18)	23	3·1 (1–11)	14	3·6 (1–9)	11	4·3 (1–9)	3	6·0 (6)
	Hysterothylacium sp. (N)			6	1·0 (1)	3	1·0 (1)	9	1·3 (1–2)				
	Terranova sp. (N)	57	2·6 (1–9)	26	1·7 (1–4)	29	1·2 (1–2)	6	1·0 (1)				
	Raphidascaris sp. 1 (N)	83	4·1 (1–8)	63	3·0 (1–6)	100	8·7 (1–24)	77	4·0 (1–15)	31	1·7 (14)	29	1·5 (1–3)
	Raphidascaris sp. 2 (N)			3	1·0 (1)								
	Camallanus carangis (N)	3	1·0 (1)	11	1·0 (1)			9	1·0 (1)	3	1·0 (1)		

Table 4. Parasitological metrics from the studied *Epinephelus fuscoguttatus* used as biological indicators to monitor long-term change. Prevalence (*P*), dry season (DS), rainy season (RS).

Parameter	RS 03/04	RS 04/05	RS 05/06	DS 06	RS 07/08	RS 08/09
Trichodinids (*P*)	2·9	0	0	0	2·9	2·9
Shannon-Wiener index (*H'*)	1·63	1·58	0·81	0·93	0·24	0·1
Ec/En ratio (*R*)	0·5	0·3	0·5	0·5	0·6	1
Terranova sp. (*P*)	57	26	29	6	0	0
Raphidascaris sp. (*P*)	83	63	100	77	31	29
Scolex pleuronectis (*P*)	26	40	23	14	11	3
Berger-Parker index (*BP*)	0·89	0·96	0·82	0·97	0·88	0·95

($P<1$). The elasmobranch parasitic *Terranova* sp. that utilizes fish as intermediate hosts could be isolated during the sampling periods 2003/04 until 2006, and was absent during the last two sampling periods in 2007/08 and 2008/09. The prevalence of infestation decreased from 57%–6% in 2003/04 and 2006 (Table 3).

The fish-parasitic nematode *Raphidascaris* sp. 1 that utilizes invertebrates as first and small fish as second intermediate hosts was recorded during all sampling periods in high numbers. The prevalence of infestation ranged from 29% to 100%. The highest prevalence (100%) was observed in 2005/06, followed by a prevalence of 83% in 2003/04, and decreased to 29% in 2008/09 (Table 3).

The elasmobranch-parasitic tetraphyllidean larvae (*Scolex pleuronectis*) were isolated from the sampled fish during all sampling periods. The highest prevalence of 40% was observed in 2004/05, followed by a prevalence of 26% in 2003/04, and decreased continuously from 2005/06 (23%) until 2008/09 with a final prevalence of infestation of 3% (Table 3).

Visual integration of the six parasite bioindicators

To visualize all six utilized parasite bioindicators, the values obtained for the different samplings were transferred onto a positive (natural)–negative (non-natural) axis as given in Fig. 2. Following Palm and Rückert (2009), theoretical threshold values are used to distinguish between natural and non-natural conditions in which natural conditions refer to parasite infections comparable to those that occur in free living *E. fucoguttatus* from a natural, healthy environment (see Rückert *et al.* 2009*c*). A 50% prevalence of trichodinid ciliates was chosen as a threshold for non-natural holding conditions, in the middle of a possible range between 0–100%, indicating an increasing bacterial load in the surrounding water body. The threshold value 1·0 for the ecto-/endoparasite ratio represents an equal number of ecto- and endoparasites, where the higher numbers of endoparasites indicate the typical conditions of free living fish. The endoparasite diversity threshold of 1·25 is in the middle of its range (0–2·5), and low endoparasite diversity refers to non-natural conditions compared to free living predatory *E. fuscoguttatus*. Most drastic changes in parasite prevalences and intensities were recorded for the nematodes *Terranova* sp. and *Raphidascaris* sp. 1 and unidentified tetraphyllidean cestodes/*Scolex pleuronectis* (Table 3, Fig. 3). The threshold value was set on 50% prevalence of infestation, though the choice of the threshold for these metrics is still arbitrary, and does not yet allow justified judgement for decision makers. This value will change after more comparative data on the most common fish parasite infestation of groupers in Indonesian coastal waters become available.

The six chosen bioindicators are visualized within a star graph according to Bell and Morse (2003) and Palm and Rückert (2009), with the positive values (natural conditions of free living groupers) oriented towards the outer tips of the axes within the star graph. The resulting star graphs are given in Fig. 3 for the five sampling periods during the rainy and a single sample taken during the dry season over the years from 2003–2009.

Development of the mariculture farm

The mariculture farm sampled developed from a test phase (2001–2002) with the sole production of *E. fuscoguttatus* into an increased production mainly based on *Chanos chanos* (2003–2006), and a phase with reduced milkfish production (Table 2). The production of the groupers increased steadily though at a smaller scale compared to the milkfish. The number of net cages and the feed use intensified with fish production, increasing the total size of the finfish mariculture. The total production (%) over the years on bases of the year with the highest production respectively is given in Table 2.

DISCUSSION

Fish parasites are useful biological indicators to monitor environmental change (Vidal-Martínez *et al.* 2010). They have been used to indicate e.g. bacterial biomass (Palm and Dobberstein, 1999; Palm and Rückert, 2009), heavy metals (Sures and Siddall, 2003) or environmental stress (Landsberg *et al.* 1998) (for reviews on parasitological bioindicators

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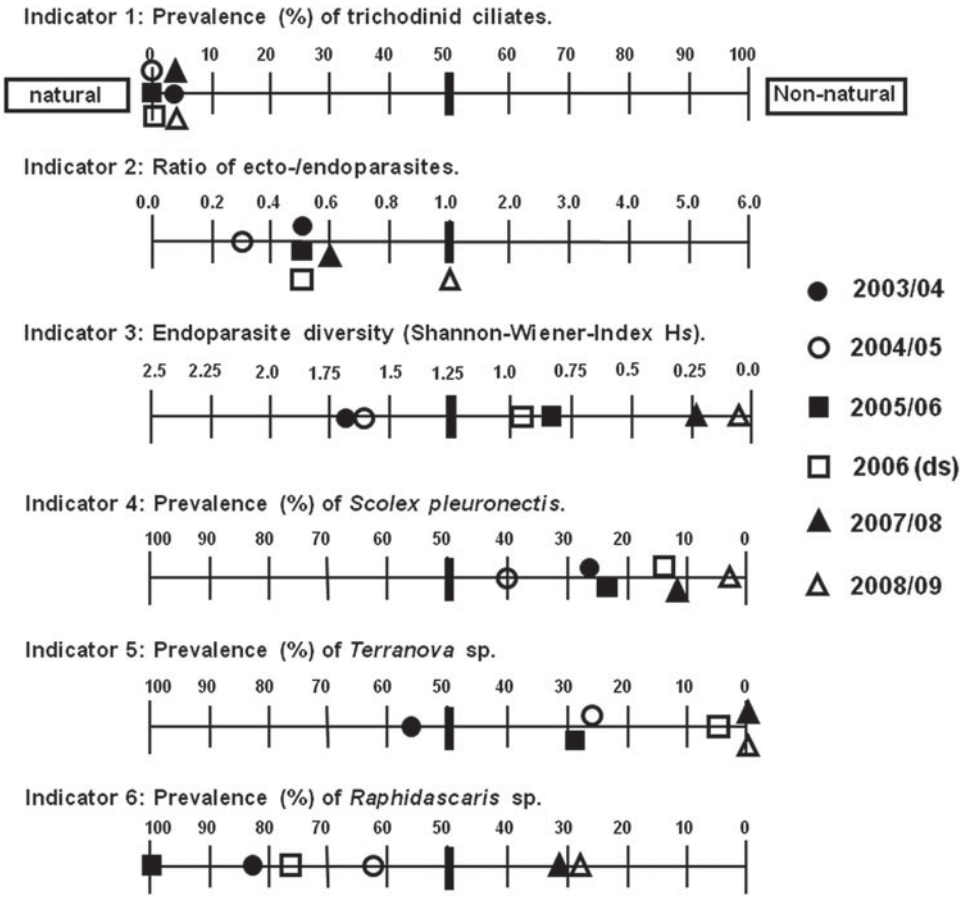

Fig. 2. Indicator transfer onto a positive-negative axis. The markings give the data points for the studied fish between 2003/04 and 2008/09. The black bars show theoretical threshold values for the holding conditions. The chosen indicators are explained in the text below. ● 2003/04, ○ 2004/05, ■ 2005/06, □ 2006, ▲ 2007/08, △ 2008/09, dry season (ds).

see Lafferty, 1997; Marcogliese and Cone, 1997; Overstreet, 1997; Williams and MacKenzie, 2003; Marcogliese, 2005). Sasal *et al*. (2007) utilized fish parasites to detect anthropogenic influences (urban and industrial pollution), and Lafferty *et al*. (2008*b*) proposed them being a convenient method for assessing spatial variation in their final host distribution. Because their occurrence is dependent on the composition of the food web, a low biodiversity could impair parasite transmission by reducing the availability of hosts required by parasites with complex life cycles (Lafferty *et al*. 2008*b*). Consequently, heteroxenous fish parasites with complex life cycles are generally useful tools to indicate food web relationships in unaffected marine habitats (e.g. Palm, 1999; Palm *et al*. 2007; Klimpel *et al*. 2006), and thus reflect the status of the marine food web that might be affected through environmental change.

The present study analysed the fish parasite fauna of groupers from a mariculture facility in a marine national park in Indonesia. The samples were carried out in 6 consecutive years, using fish of a similar size range and from the same net cages. By using the methodology of Bell and Morse (2003) and Palm and Rückert (2009), we applied (1) the prevalence of trichodinid ciliates to indicate bacterial load (Palm and Dobberstein, 1999; Ogut and Palm, 2005; Palm and Rückert, 2009); (2) the ratio of ecto- versus endoparasites to indicate the natural parasite composition of free living *E. fuscoguttatus* in Indonesia (where the endoparasite richness is higher than the ectoparasite richness (Vidal-Martínez *et al*. 1998; Jakob and Palm, 2006; Rückert *et al*. 2009*a*)); (3) the endohelminth parasite diversity to indicate parasite transfer into the open water netcage mariculture (Rückert *et al*. 2009*a*); (4) the prevalence of tetraphyllidean cestodes as common parasites of elasmobranch final hosts (Lafferty *et al*. 2008*b*), and the prevalence of the nematodes *Terranova* sp. (5) and *Raphidascaris* sp. 1 (6). Four of the chosen bioindicators demonstrated significant change over the years, where the metazoan prevalence of infection decreased significantly together with a significant trend towards reduced endoparasite diversity. The Ec/En ratio remained relatively stable in the first four sampling periods, but increased in the last two years. The ectoparasitic trichodinid ciliates were only recorded in 2003/04 and 2007/08 and 2008/09 at a low prevalence of 2·9%.

According to our data, the holding conditions of the cultured *Epinephelus fuscoguttatus* must have changed significantly since the beginning of our sampling in 2004 until 2009. Since the holding conditions in terms of cage usage, food enrichment,

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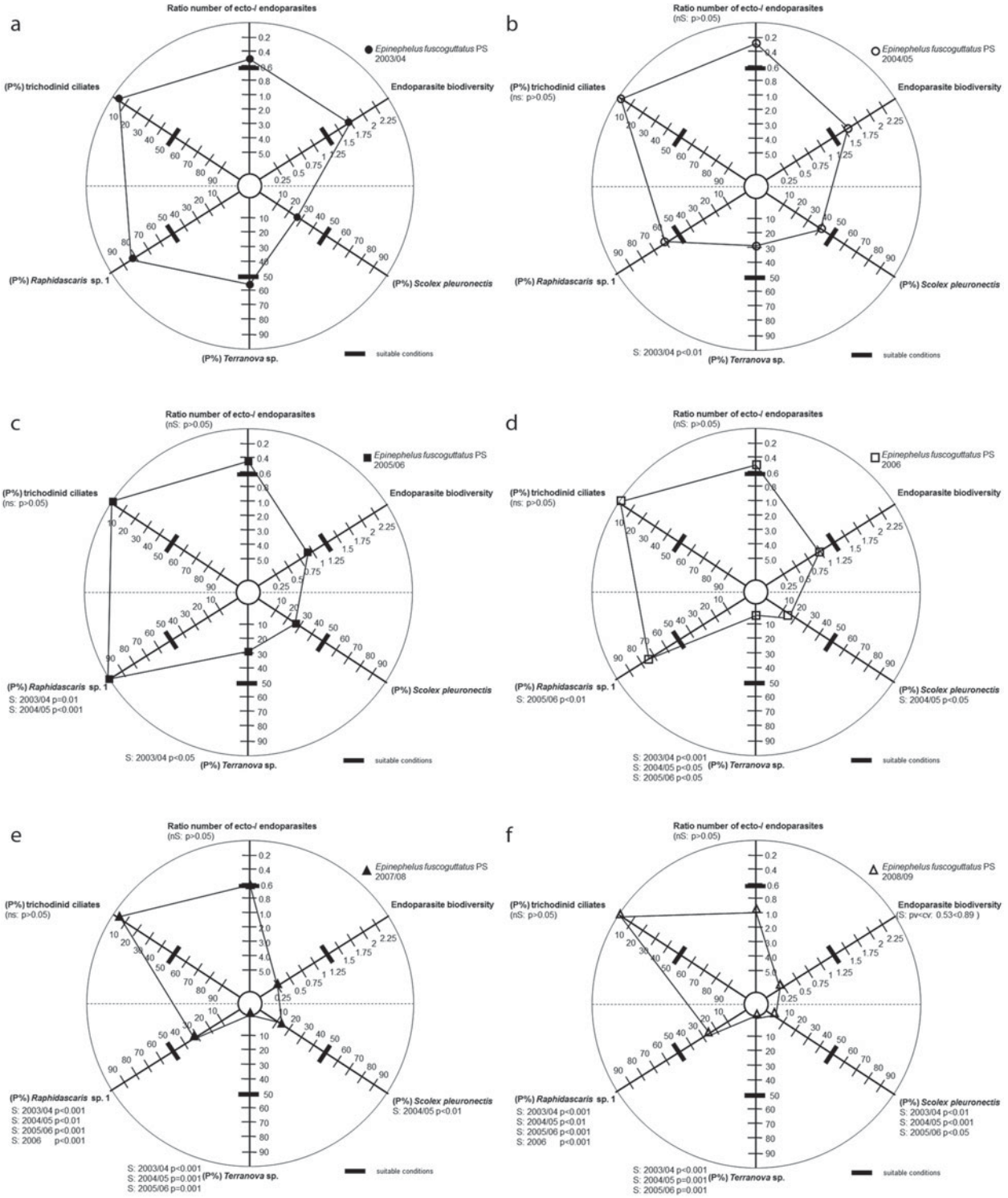

Fig. 3. Visual indicator integration for *Epinephelus fuscoguttatus* in six consecutive years, during rainy seasons 2003/04, 2004/05, 2005/06, dry season 2006, and rainy seasons 2007/08 and 2008/09. The black bars show theoretical thresholds values to distinguish natural and non-natural holding conditions. Positive values (natural conditions) are oriented towards the outer tip of the axes within the star graph. Thousand Islands (PS), significant (s), non-significant (ns).

freshwater bath or sorting did not change since the testing phase of the farm in 2001–2002 until today and no major disease outbreak occurred, other factors must have impacted the parasite infracommunity of the sampled fish. According to Rückert *et al*. (2009*b*, 2009*c*), trash fishes are responsible for the transmission of endohelminths into *E. coioides* and *E. fuscoguttatus* mariculture in Lampung Bay, Indonesia, depending on the trash fish species and the use of different body parts. The infestation of pellet-fed fish with endohelminths demonstrated that infestation could also occur via organisms that naturally live in, on, and in the surroundings of the net cages (Rückert *et al*. 2009*b*). Five of the

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bioindicators utilized here describe the occurrence of endohelminths that are transmitted to the groupers through the intermediate hosts. The *E. fuscoguttatus* studied were fed with trash fishes that originated mainly from local fishermen between 2001 and 2006 and in part also from Jakarta fish market since 2006 until 2009. The trash fishes, especially those bought in larger quantities in Jakarta, were kept deep frozen and given in daily portions directly into the grouper cages. Consequently, some of the parasites recorded might have been introduced through the regular feed, though the use of frozen trash fish in larger quantities since 2006 could have minimized this effect.

Another explanation of the observed parasite transmission is mainly through the intermediate hosts that live on the net cages and in their surroundings (Rückert *et al*. 2009*b*). The infestation of the sampled groupers may therefore reflect the parasite infestation of natural host organisms surrounding the mariculture farm. As mesh sizes and treatments did not change over the years of our samplings, a significant decrease in the grouper parasitisation might have been caused by an overall reduced number of fish parasites within the natural intermediate hosts. Palm and Rückert (2009) explained the high diversity of fish parasites in the sampled groupers in 2004/05 by the novelty of the mariculture site (founded in 2001) and the use of a low number of open-water floating net cages with low stocking densities. High parasite biodiversity in the surroundings of the net cages, combined with the use of local (possibly fresh?) trash fishes must have been responsible for the observed high biodiversity of endohelminth parasites in the beginning of our sampling, between 2003/04 and 2004/05. The Thousand Islands, one of the marine national parks, can be seen as a relatively clean environment at the sampled site (Fig. 1). Under such a scenario, the fish parasites studied could be used as biological indicators for a significant ecosystem change. The low trichodinid prevalence in 2003/04 and 2007/08 and 2008/09 still indicates low bacterial load and presumably little eutrophication in the system (see Palm and Rückert, 2009, Rückert *et al*. 2009*a*). The disappearance of the elasmobranch-parasitic *Scolex pleuronectis* and *Terranova* sp., however, might be connected with a transition of a relatively undisturbed marine environment at the beginning of the mariculture activity into a more affected tropical habitat, lacking important biota such as elasmobranchs as the most common top predators (Lafferty *et al*. 2008*b*). This can be either caused by the mariculture activity itself or through a possible increased fishing pressure from the surrounding islands.

Under exclusion of a direct parasite transfer through the net cage, our data would indicate a significant change in management methods. A change in fish treatment would affect the presence of ectoparasites, thus having a greater effect on the Ec/En ratio and the presence of trichodinid ciliates. During our sampling, the ectoparasite numbers were relatively stable, and also the presence of some ectohelminths within all samples and the low number of trichodinids indicates no major shift. This can be either related to a similar and possibly 'optimal' treatment of the fish or to a relatively stable water quality (see above). Consequently, a major change in fish treatment during the 6 years of sampling is unlikely and cannot explain the observed result. The only other possibility is a change in feed use, from fresh local trash fishes at the beginning of the sampling in 2004 to the addition of deep frozen trash fishes from Jakarta fish markets that might have killed the endohelminth larvae before feeding them to the groupers. In summer 2009, it was also observed that cultured milkfish was used as additional grouper feed, practically preventing parasite transmission (only a single adult digenean, *Isorchis parvus*, was found in 35 milkfish from the netcages, with a prevalence of 83·3% in the intestine and pyloric caecae). However, some endohelminth species such as *Lecithochirium* sp., *Prosorhynchus* sp., *Raphidascaris* sp. and *Scolex pleuronectis* occurred in the groupers throughout the sampling period, suggesting that at least a portion of the feed use must not have changed, originated as fresh trash fishes from local fishermen, or directly from the surroundings of the net cage (see above).

According to Vidal-Martínez *et al*. (2010), the circumstances under which parasites can be used as biological indicators of environmental impact have not been demonstrated conclusively. This is on the one hand caused by different physiological and ecological adaptations of the different parasite species, leading to conflicting evidence if different indicator species are used. Changes in parasite abundance can be biased by sampling size, host collection and migration, or by spatial or seasonal variation. On the other hand, significant interactions have been demonstrated between different parasite taxa and environmental stressors, where it remains difficult to attribute a numerical change in parasite prevalence and intensities to a single stressor in the field. The present study demonstrates that regular systematic, parasitological studies of fish (methodology see Palm and Rückert, 2009) can supervise and monitor feed use and management practice within a commercially run mariculture farm. By routinely sampling a separate batch of cultured fish that is exclusively fed with trash fishes from the surroundings of the farm, groupers can be used as a model organism to monitor the mariculture environment. As such they can be introduced as a methodological standard to regulate and monitor grouper finfish mariculture, providing useful information to the fish farmers and decision makers. To protect the cultured fishes and private investment, better parasite and disease control while monitoring the health status of

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the environment is the prerequisite for a sustainable and environmentally friendly mariculture in Indonesia.

We have applied a method that can visualize long-term change by using parasite metrics together on a single star graph (Palm and Rückert, 2009). By using this approach we could demonstrate that the chosen fish parasite metrics successfully indicate a significant change in maricultured fish under holding conditions, either caused by a different culture management (especially feeding) or by environmental change. A high diversity of recorded grouper parasites, and the possibility to use the groupers and their parasites to monitor long-term changes, also suggests that this model might be useful to monitor environmental change in the wild. Though the present study is based on a series of taxonomic and systematic studies in Indonesian waters (Palm, 2000, 2004, 2008; Palm and Overstreet, 2000; Jakob and Palm, 2006; Yuniar *et al*. 2007; Palm *et al*. 2008; Rückert *et al*. 2008; Bray and Palm, 2009; Kuchta *et al*. 2009), several recorded parasites could not be identified to species level, and the taxonomic status of many of them is far from being resolved. This, however, is important for a broader application of fish parasites as bioindicators for environmental impact and change. Because such applications are especially useful under high biodiversity conditions – as in tropical and subtropical regions that underlie anthropogenic stressors caused by pollution, over-exploitation as well as shrimp or finfish mariculture – further taxonomic research on marine fish parasites in these regions is urgently needed.

The use of parasite diversity as an indicator of environmental change is very promising, considering the high species number of fish, their parasites and parasitic life cycle stages. Depending on the host and parasite systematics and ecology, specific model organisms are more useful than others. This necessitates taxonomic expertise, especially in tropical countries where high species diversity is common. This, however, often does not reflect reality, where most emphasis is given to short-term applied research, combined with a severe lack of taxonomic expertise in many public research institutions. We propose that fish parasites and the presented methodology should be included within regular environmental impact monitoring programmes (e.g. in Indonesia), providing further data on the parasite biodiversity in tropical countries. While gathering important information on regional environmental health aspects in tropical waters, this would also promote further interest in fish parasite systematics and taxonomy in so far underdeveloped regions.

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