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Parasites of the grouper fish *Epinephelus coioides* (Serranidae) as potential environmental indicators in Indonesian coastal ecosystems

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Abstract

A total of 195 *Epinephelus coioides* (Hamilton, 1822) were studied for fish parasites from Javanese (Segara Anakan lagoon) and Balinese waters. Up to 25 different parasite species belonging to the following taxa: one Ciliata, one Microsporea, five Digenea, one Monogenea, four Cestoda, four Nematoda, one Acanthocephala, one Hirudinea and seven Crustacea were identified with four new host and locality records. The dominant parasites included the monogenean *Pseudorhabdosynochus lantauensis* (53.3–97.1%), the nematode *Spirophilometra endangae* (23.3–42.9%), the digenean *Didymodiclinus* sp. (2.9–40.0%), the nematodes *Philometra* sp. (22.6–34.3%) and *Raphidascaris* sp. (2.9–28.6%), and the isopod *Alcirona* sp. (6.7–31.4%). Regional differences for *E. coioides* were found in terms of endoparasite diversity, total diversity according to Shannon–Wiener, Simpson index and Evenness. A comparison with published data from Sumatera revealed highest endoparasite diversity (Shannon–Wiener: 1.86/1.67–2.04) and lowest ectoparasite/endoparasite ratio (0.73/0.57–0.88) off the Balinese coast, followed by Lampung Bay, Sumatera (1.84; 0.67), off the coast of Segara Anakan lagoon (1.71; 0.71), and in the lagoon (0.30/0.19–0.66; 0.85/0.67–1.00). The presented data demonstrate the natural range of these parameters and parasite prevalences according to habitat and region, allowing adjustment of the scale that has been used in the visual integration of the parasite parameters into a star graph. The parasite fauna of *E. coioides* in Segara Anakan lagoon 'improved' from 2004 until 2008/09, possibly related to earlier oil spill events in 2002 and 2004. The use of grouper fish parasites as an early warning system for environmental change in Indonesian coastal ecosystems is discussed.

Introduction

Coastal marine ecosystems experience a variety of environmental stressors, such as anthropogenic induced pollution, environmental degradation and change (Cooper *et al.*, 2009; Dsikowitzky *et al.*, 2011). Heavy exploitation of the coastal resources leads to overfished fish stocks, altered population sizes and species composition, as well as changed habitats. By 2025, 2.75 billion people worldwide are expected to live close to the coast (Palm *et al.*, 2011), increasing the urgent need for a better

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and sustainable use of the coastal resources. This requires increased understanding and the development of new methodologies to assess and visualize regional environmental conditions and change.

It has been difficult to demonstrate the environmental status of any coastal marine habitat, due to the complexity and natural variability of such systems. According to Kurtz *et al.* (2001), monitoring systems are required, as it is impossible to measure and interpret all the various influencing factors within an ecosystem. So far, many environmental assessment studies have focused on descriptive methodologies with no clear purpose and using uncorrelated methods (Downs *et al.*, 2005), without further potential for practical applications. As summarized by Palm & Rückert (2009) and reviewed by Palm (2011), the status of a marine environment and environmental change can either be studied directly, by using, for example, water quality parameters such as phosphate, nitrate and dissolved organic carbon (DOC), or indirectly by using bioindicators. Such indicator organisms react sensitively to specific environmental conditions. Their occurrence or abundance can be used to describe the current status of the environment, and even environmental change.

Because of the direct linkage and dependence of parasites with multiple-host life cycles to the surrounding animal communities (Hechinger *et al.*, 2007), these organisms have been considered as sensitive bioindicators for aquatic ecosystem health (Overstreet, 1997; Dzikowski *et al.*, 2003). Fish parasites have been used as biological and environmental indicators (for a review see Palm, 2011), especially for environmental change and pollution (Diamant *et al.*, 1999; Dzikowski *et al.*, 2003; Palm & Rückert, 2009) or environmental stress (Landsberg *et al.*, 1998). Sures (2001, 2003) used acanthocephalan parasites to detect heavy metal pollution, because acanthocephalans accumulate 1000 times higher amounts of heavy metals in contrast to their host tissues. Sasal *et al.* (2007) utilised fish parasites to detect anthropogenic influences (urban and industrial pollution) in coral reef lagoons, and Lafferty *et al.* (2008b) suggested that they are a convenient method to assess spatial variation of their final host distribution. Heteroxenous fish parasites (multiple hosts) with complex life cycles can be used to indicate food-web relationships in unaffected marine habitats (e.g. Palm, 1999; Klimpel *et al.*, 2006; Lafferty *et al.*, 2008a). While the occurrence of endoparasites often decreases in polluted waters (Nematoda: Kiceniuk & Khan, 1983), ectoparasitic parasites such as monogeneans can increase (Monogenea: Khan & Kiceniuk, 1988; *Trichodina*: Khan, 1990; Palm & Dobberstein, 1999; Ogut & Palm, 2005). Ectocommensals with direct life cycles, such as trichodinid ciliates,

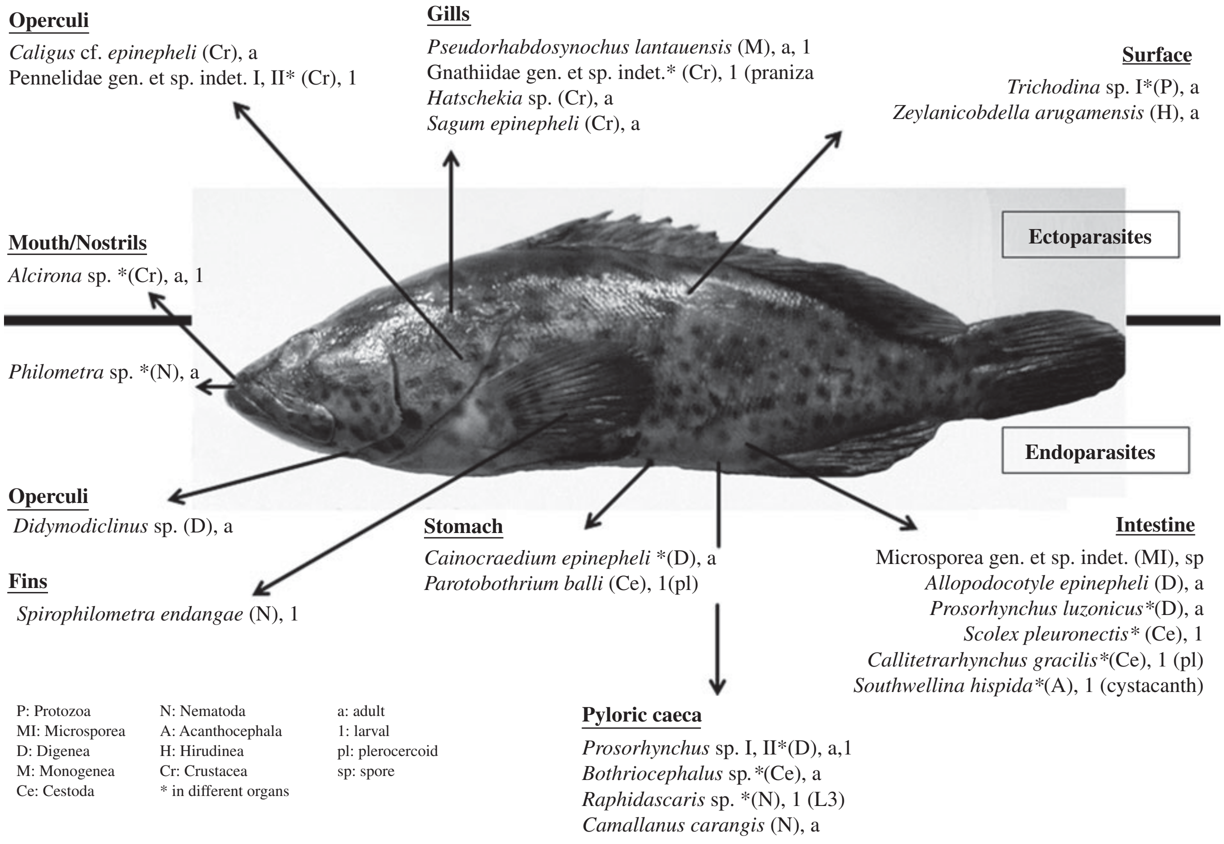

Fig. 1. The occurrence of ectoparasites and endoparasites from the grouper fish *Epinephelus coioides* from Indonesian waters during 2007–2009.

favour polluted waters and can indicate high bacterial load (Palm & Dobberstein, 1999; Ogut & Palm, 2005), in contrast to many endoparasites with complex life cycles that favour stable and non-polluted waters, where the full range of their required hosts is present (Lafferty *et al.*, 2008b).

The Indonesian coastal marine habitat has one of the highest aquatic biodiversities on Earth (Yuniar *et al.*, 2007; Palm, 2011). This includes fish species as well as their parasite fauna, though not more than about 4% of the estimated fish parasite fauna in Indonesia has been explored (Jakob & Palm, 2006). Palm & Rückert (2009) applied a method to visualize environmental differences by using fish parasites. They used the star-graph method according to Bell & Morse (2003). The authors also sampled *Epinephelus coioides*, from the wild and coastal mariculture in Lampung Bay, Sumatera, and from inside the anthropogenic influenced Segara Anakan lagoon in Central Java. As exemplified by Palm *et al.* (2011) from a mariculture facility in the Thousand Islands, six different parasite metrics from *Epinephelus fuscoguttatus* demonstrated a significant change in parasite composition and abundance over six consecutive years. The authors suggested that groupers might also be useful biomarkers to monitor environmental change in the wild. Kleinertz *et al.* (2012) have shown regional differences in the parasite composition of free-living *Epinephelus areolatus* from Indonesian waters using the same methodology.

Fish parasites of groupers (e.g. *Cromileptes altivelis*, *E. areolatus*, *E. fuscoguttatus*) from tropical marine waters have been of special interest in recent years. The groupers are of high commercial value and, consequently, of importance for fisheries as well as finfish mariculture (Rimmer *et al.*, 2004). This steadily growing business is also playing a significant role in the Indonesian economy, ensuring food availability and improving the living standards of the coastal communities (Rückert *et al.*, 2010). Grouper (*Epinephelus* spp.) mariculture production in Indonesia has increased 340% from 2004 to 2009 (DJPB, 2009).

Taxonomical and ecological studies on fish parasites from Indonesia have been intensified in recent years (e.g. Palm *et al.*, 2007, 2008, 2011; Yuniar *et al.*, 2007; Palm, 2008, 2011; Bray & Palm, 2009; Kuchta *et al.*, 2009; Rückert *et al.*, 2009a, b, 2010; Kleinertz, 2010, Kleinertz *et al.*, 2012, Dewi & Palm, 2013; Kuhn *et al.*, 2013), taking into account the high parasite biodiversity at this tropical location. The purpose of the present study is an assessment of the fish parasite fauna of *E. coioides*, a widely distributed and rapidly developing mariculture species in Indonesia, from additional sampling sites. We have correlated the observed parasite fauna with regional differences in the sampled regions. Being aware that limited sample replications of theoretically ‘impacted’ versus ‘healthy’ environments can be tested in Indonesia, we herewith apply ecological and parasitological parameters that were used to monitor regional differences and environmental change by Palm & Rückert (2009) and Palm *et al.* (2011). The use of grouper fish parasites as an early warning system for environmental change in Indonesian coastal ecosystems is discussed.

Materials and methods

Collection and examination of fish

Samples were taken within the framework of the SPICE project (Science for the Protection of Indonesian Coastal Marine Ecosystems) during the rainy season 2007/08 and 2008/09, and dry seasons 2008 and 2009. A total of 195 *E. coioides* (Hamilton, 1822) were studied from Javanese (Segara Anakan lagoon) (108°46′–109°03′E; 08°35′–08°48′S) and Balinese waters (114°25′53″–115°42′400′E; 8°30′40″–08°50′48″S) in Indonesia (fig. 1, table 1). Additional data were calculated based on Yuniar (2005; dry season 2004) and Rückert (2006; dry season 2003) and Rückert *et al.* (2009a) for comparison (also see table 1).

Table 1. The mean body length (cm) and body weight (g) of wild *Epinephelus coioides* sampled from Indonesian waters in the rainy and dry seasons from 2007 to 2009 for comparison with *Yuniar (2005) and **Rückert (2006); measurements of body length and weight shown in brackets.

Locality	Year	No. of fish	Body length	Body weight
Rainy season				
Segara Anakan	2007/08	35	29 (25.1–38.8)	345.4 (242.0–753.0)
Segara Anakan	2008/09	35	29.3 (25.1–36.4)	374.3 (284.0–715.0)
Off the coast of Segara Anakan	2008/09	30	27.7 (20.3–40.3)	344.1 (182.0–902.0)
Dry season				
Off the coast of Segara Anakan	2008	30	27.3 (21.5–34.2)	294.2 (140.0–579.0)
Bali	2008	35	29.7 (23.3–40.2)	355.6 (201.0–746.0)
Bali	2009	30	33.1 (27.3–46.4)	529 (340.0–1400.0)
Segara Anakan*	2004	21	17.3 (10.0–28.0)	83.2 (13.0–250.0)
Ringgung**	2003	35	23.9 (19.5–34.5)	185.1 (100.9–495.0)

Live fish were obtained from local fishermen using fish traps in Segara Anakan lagoon and from Balinese waters. Groupers from the coastal zone off Segara Anakan were bought at the fish market and were separated into plastic bags directly after the catch. Fish were transported immediately to the laboratory, or kept on ice and then frozen (~ −20°C) until subsequently dissected at the Faculty of Biology, Jenderal Soedirman University, Purwokerto (UNSOED) and the Faculty of Veterinary Medicine, Udayana University, Jimbaran, Bali. Total fish length (L_T), weight (W_T) and liver weight (W_L) were measured to the nearest 1.0 cm and 1.0 g (table 1) prior to the parasitological examination (see Rückert *et al.*, 2009a).

Smears were taken from the gills, surface and the inner opercula of the living fish. The skin, fins, eyes, gills, nostrils, mouth- and gill-cavity were examined for ectoparasites. Inner organs such as the digestive tract, liver, gall bladder, spleen, kidneys, gonads, heart and swim bladder were separated and transferred into saline solution for microscopical examination under the stereomicroscope (Zeiss Stemi DV4; Carl Zeiss, Oberkochen, Germany) in order to allow a quantitative parasitological examination of each organ; belly flaps and musculature (fillets) were examined on a candling table. Isolated parasites were fixed in 4% borax-buffered formalin and preserved in 70% ethanol. Smears from the gills, surface and opercula were stained using silver nitrate ($AgNO_3$) impregnation, after Klein (1926, 1958): 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 40–50 min. Smears were dried after exposure. Finally, the musculature was sliced into 0.5- to 1-cm-thick fillets and pressed between two Petri dishes to identify and isolate parasites from the musculature. Nematoda were dehydrated in a graduated ethanol series and transferred to 100% glycerine (Riemann, 1988). Digeneans, monogeneans and cestodes were stained with acetic carmine, dehydrated, cleared with eugenol and mounted in Canada balsam, whereas crustaceans were dehydrated and transferred directly into balsam. The identification of parasites was based on original descriptions given in Palm *et al.* (2011).

Parasitological parameters

A variety of ecological parameters were evaluated to indicate regional differences, such as the different diversity indices (Shannon–Wiener, Evenness and Simpson index), fish ecological indices (such as the hepatosomatic index) and parasitological parameters (such as ectoparasite/endoparasite ratio and different prevalences of infection of metazoan parasites) (see Palm & Rückert, 2009; Palm, 2011; Palm *et al.*, 2011).

Parasitological calculations were made according to Bush *et al.* (1997). The present study applies the method by Palm & Rückert (2009) and Palm *et al.* (2011) to monitor the parasite community of groupers from Indonesia. This is based on the assumption that certain parasite prevalence data and parameters are characteristic for undisturbed environmental conditions with scenarios of high parasite diversity. The Berger–Parker index characterizes the dominance of a respective parasite

Table 2. The prevalence (%), intensity (I), mean intensity (MI) and mean abundance (MA) of ectoparasites from *Epinephelus coioides* in Javanese (in and off the coast of the Segara Anakan) and Balinese waters.

Locality	Segara Anakan				Off the coast of Segara Anakan				Bali			
Year of sampling	2007/08		2008/09		2008		2008/09		2008		2009	
Parasite species/-taxa	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA
Trichodina sp. I	51.4	3.1 (1–9) 1.57	40.0	2.6 (1–11) 1.03		nc		nc	17.1	11.5 (2–32) 2.00		nc
Pseudorhabdosynochus lantauensis	91.4	79.5 (3–433) 72.73	97.1	37.4 (1–150) 36.31	86.7	39.2 (1–259) 34.00	80.0	33.3 (1–225) 26.57	80.0	43.1 (1–246) 34.5	53.3	8.4 (1–35) 4.50
Zeylanicobdella arugamensis	8.6	2.0 (2) 0.17	–	–	16.7	1.2 (1–2) 0.20	40.0	2.3 (1–11) 0.93	–	–	–	–
Alcirona sp.	31.4	19.6 (1–194) 6.17	–	–	23.3	5.7 (1–33) 1.33	13.3	1.3 (1–2) 0.17	8.6	1.0 (1) 0.09	6.7	2.5 (1–4) 0.17
Gnathiidae gen. et sp. indet.	8.6	1.0 (1) 0.08	40.0	1.7 (1–4) 0.69	6.7	11.5 (1–22) 0.77	13.3	1.8 (1–4) 0.23	5.7	1.5 (1–3) 0.09	10.0	2.7 (1–6) 0.27
Caligus cf. *epinepheli*	–	–	–	–	–	–	–	–	5.7	1.0 (1) 0.60	–	–
Caligidae gen. et sp. indet.	–	–	–	–	–	–	–	–	60.0	25.9 (2–89) 15.6	6.7	1.0 (1) 0.07
Hatschekia sp.*	–	–	–	–	–	–	–	–	14.3	8.0 (1–16) 1.14	–	–
Sagum epinepheli	–	–	–	–	–	–	–	–	2.9	1.0 (1) 0.03	–	–
Pennelidae gen. et sp. indet. I	28.6	5.2 (1–28) 1.49	–	–	–	–	–	–	–	–	–	–
Pennelidae gen. et sp. indet. II	20.0	4.3 (1–11) 0.86	2.9	1.0 (1) 0.03	56.7	2.5 (1–8) 1.43	23.3	94.6 (1–636) 22.10	–	–	33.3	7.0 (1–21) 2.33
Ectoparasite species		7		4		5		5		7		4

*New host record. nc, not calculated.

Table 3. The prevalence (%), intensity (I), mean intensity (MI) and mean abundance (MA) of endoparasites from *Epinephelus coioides* in Javanese (in and off the coast of the Segara Anakan) and Balinese waters.

Locality	Segara Anakan lagoon				Off the coast of Segara Anakan lagoon				Bali			
Year of sampling	2007/08		2008/09		2008		2008/09		2008		2009	
Parasite species/-taxa	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA	(%)	MI (I) MA
Microsporea gen. et sp. indet.	–	–	17.1	5.3 (1–15) 0.91	6.7	4.5 (1–8) 0.60	–	–	–	–	–	–
Didymodiclinus sp.	17.1	4.5 (2–9) 0.77	31.4	4.5 (1–32) 1.43	–	–	40.0	2.1 (1–7) 0.83	2.9	5.0 (5) 0.14	13.3	2.8 (2–4) 0.37
*Cainocraedium epinepheli**	–	–	–	–	–	–	–	–	11.4	4.5 (2–8) 0.51	6.7	2.0 (1–3) 0.13
Prosorhynchus luzonicus	–	–	–	–	6.7	31.0 (1–61) 0.60	–	–	–	–	–	–
Prosorhynchus sp. I	–	–	–	–	–	–	16.7	8.8 (1–29) 1.47	11.4	7.3 (1–23) 0.83	26.7	2.6 (1–7) 0.70
Prosorhynchus sp. II	5.7	6.0 (1–11) 0.34	–	–	–	–	–	–	–	–	–	–
Bucephalidae gen. et sp. indet.	–	–	–	–	–	–	–	–	–	–	3.3	2.0 (2) 0.07
Digenea gen. et sp. indet.	–	–	–	–	–	–	–	–	–	–	3.3	1.0 (1) 0.03
Bothriocephalus sp.	8.6	2.0 (1–3) 0.17	–	–	20.0	1.3 (1–2) 0.27	26.7	2.5 (1–6) 0.67	–	–	–	–
Scolex pleuronectis	2.9	2.0 (2) 0.06	–	–	–	–	–	–	42.9	4.9 (1–32) 2.10	–	–
*Callitetrarhynchus gracilis**	–	–	–	–	–	–	–	–	–	–	3.3	1.0 (1) 0.03
Parotobothrium balli	–	–	–	–	–	–	–	–	8.6	1.7 (1–3) 0.14	–	–
Raphidascaris sp.	2.9	1.0 (1) 0.03	8.6	2.3 (2–3) 0.20	–	–	3.3	1.0 (1) 0.03	28.6	2.9 (1–14) 0.83	20.0	1.3 (1–2) 0.27
Camallanus carangis	–	–	–	–	–	–	–	–	2.9	1.0 (1) 0.03	–	–
Philometra sp.	22.6	1.8 (1–3) 0.40	34.3	2.4 (1–7) 0.83	–	–	23.3	1.0 (1) 0.23	34.3	2.8 (1–10) 0.94	33.3	1.3 (1–2) 0.43
Spirophilometra endangae	42.9	3.5 (1–13) 1.51	31.4	3.9 (1–17) 1.23	36.7	2.3 (1–7) 0.83	26.7	3.5 (1–10) 0.93	–	–	23.3	2.9 (1–7) 0.67
Nematoda gen. et sp. indet. I	22.9	2.6 (1–8) 0.60	–	–	3.3	1.0 (1) 0.03	–	–	2.9	2.0 (2) 0.06	3.3	1.0 (1) 0.03
Nematoda gen. et sp. indet. II	2.9	9.0 (9) 0.26	–	–	–	–	–	–	–	–	–	–
*Southwellina hispida**	8.6	1.0 (1) 0.08	2.9	1.0 (1) 0.03	16.7	3.6 (1–7) 0.60	20.0	3.5 (1–13) 0.70	–	–	–	–
Endoparasite species	8		6		5		7		8		7	
Ecto-/endoparasite ratio	0.88		0.67		1.00		0.71		0.88		0.57	

* New host record.

species within the sample BP $= N_{\max}/N$, with $N_{\max}$ being the number of specimens of the most dominant species in relation to the total number of parasites within the sample (N) (Munkittrik *et al.*, 1994). The diversity of the collected metazoan endoparasite fauna of each fish species was determined by using the Shannon–Wiener diversity index (H′) and, according to Kleinertz *et al.* (2012), the Evenness index (E) of Pielou (Magurran, 1988) and other parameters were tested (see below). Microsporean parasites were not considered because it was not possible to calculate their intensity. In the case of trichodinid ciliates, the calculations given in table 2 refer to the density, based on counts from slides with mucous smears obtained from about 1 cm^2 of gill surface area. The ratio of ecto- to endoparasites was calculated (Ec/En ratio (R) = number of ectoparasite species/number of endoparasite species), with trichodinid ciliates treated as present or absent in this calculation. 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 (see Palm *et al.*, 2011). The hepatosomatic index was calculated as a descriptor of a possible pollution impact to the fish host, which may affect increasing liver weights (W_{L}) in relation to the total weight (W_{T}) of the host ($\mathrm{HSI} = W_{\mathrm{L}}/W_{\mathrm{T}} \times 100$) (Munkittrik *et al.*, 1994). The Simpson diversity index was also considered as a bioindicator $\left[\mathrm{D} = 1/\sum_{\mathrm{i}=1}^{s}(n_{\mathrm{i}}/N)^2\right]$, excluding the data for the trichodinids (see the explanation above, only density was recorded), with $s =$ the total number of parasite species collected within the sample (ecto- and endoparasites included), $N =$ the total number of parasite individuals collected within the sample, $n_{\mathrm{i}} =$ number of specimens of a single species i.

Visual integration

The visual integration of the calculated ecological indicators follows Palm & Rückert (2009) for the prevalence of trichodinids, ectoparasite/endoparasite ratio and endoparasite diversity after Shannon–Wiener. The Simpson diversity index, Evenness index and hepatosomatic index were added according to Kleinertz *et al.* (2012). In addition, the prevalences of five different parasite species were used to distinguish among the sampling sites: *Scolex pleuronectis* and *Terranova* sp. (according to Lafferty *et al.*, 2008b; Palm *et al.*, 2011), *Raphidascaris* sp. (Nematoda: Kiceniuk & Khan 1983; Palm *et al.*, 2011), *Zeylanicobdella arugamensis* (Grosser *et al.*, 2001) and *Trichodina* sp. (Khan, 1990; Palm & Dobberstein, 1999; Ogut & Palm, 2005). Values that indicate unnatural environmental conditions are orientated towards the centre of the star graph. Values representing natural and unaffected environmental conditions are arranged towards the frame of the star graph. Based on Palm & Rückert (2009), Palm *et al.* (2011) and Kleinertz *et al.* (2012), we adjusted the parameter ranges to values that represent all available samples of *E. coioides*.

Data analysis

Univariate and multivariate statistical analyses were conducted with the programs STATISTICA (release 6, StatSoft Inc., Tulsa, Oklahoma, USA) and PRIMER (release 6, Primer-E Ltd. 6.1.11, Ivybridge, Devon, UK), respectively. Homogeneously distributed (Levene's test) and normally distributed data (Shapiro test) were tested for significant differences with the *t*-test or with one- or two-factorial analyses of variances (ANOVA), using Tukey's HSD test for post-hoc comparisons. The chi-square test was used to compare each year and sampling site with another for all parameters showing parasite prevalences and ectoparasite/endoparasite ratios (see Palm *et al.*, 2011). All tests were considered statistically significant at $P < 0.05$.

In order to compare the parasite communities, abundance data were square-root transformed. A similarity matrix was constructed using the Bray–Curtis similarity measure. The relation between samples based on the comparison of similarity matrices was displayed using cluster analysis and multi-dimensional scaling (MDS) with stress value estimation: <0.05, excellent; <0.2, reliable; >0.2, start of loss of accuracy. One-way analyses of similarity were applied to identify the differences in parasite species composition between the sampling sites (routine ANOSIM, values close to 1 indicate high differences and close to 0 indicate high similarity between species compositions). Routine SIMPER analysis was applied to test which parasite species contributed most to the shown differences between the sampling sites (Clarke & Warwick, 1994; see also Nordhaus *et al.*, 2009). SIMPER analysis was used to determine which species was most responsible for the differences that have been seen between sites with Bray–Curtis analysis (according to Bell & Barnes, 2003; see also Kleinertz *et al.*, 2012).

Results

In both years, during rainy season 2007/08, 2008/09 and dry seasons 2008 and 2009, fish parasitological studies on *E. coioides* in Segara Anakan lagoon, off the coastal zone of Segara Anakan lagoon and Balinese waters revealed 25 different parasite species, belonging to the following taxa: one Ciliata, one Microsporea, five Digenea, one Monogenea, four Cestoda, four Nematoda, one Acanthocephala, one Hirudinea and seven Crustacea (fig. 1, tables 2 and 3). Four new host and locality records were established for *E. coioides* (tables 2 and 3) mainly in Balinese waters. Information on prevalence, intensity, mean intensity and mean abundance of the collected parasite species is summarized in tables 2 and 3. To analyse the parasite composition and ecological status at the respective sampling sites, the ecological parameters as suggested by Palm & Rückert (2009) and Palm *et al.* (2011) were considered as given below (table 4). Regional differences for *E. coioides* were found in terms of endoparasite diversity, total diversity (Shannon–Wiener), Simpson index and Evenness between Bali and in the Segara Anakan lagoon (table 4, figs 2, 3 and 4; for regional comparison see fig. 5).

Parasite diversity and infection levels

The parasite species richness in Bali (up to 17 taxa, calculated and pooled in the fish samples for both years)

Table 4. Mean values (± SD) of hepatosomatic index and condition factor for the free-living *Epinephelus coioides* together with parasite species diversity in Javanese (in and off the coast of the Segara Anakan) and Balinese waters from 2007 to 2009 for comparison with modified data from *Yuniar (2005), **Rückert (2006) and Rückert *et al.* (2009a).

Locality	Segara Anakan			Off the coast of Segara Anakan		Bali		Ringgung
Year of sampling	2004*	2007/08	2008/09	2008	2008/09	2008	2009	2003**
Host/parasite parameters								
Hepatosomatic index	nc	1.03 (0.04)	1.37 (0.07)	0.77 (0.05)	0.96 (0.07)	1.11 (0.07)	1.02 (0.06)	1.58 (0.12)
Condition factor	1.34 (0.29)	1.38 (0.03)	1.47 (0.03)	1.38 (0.02)	1.57 (0.04)	1.34 (0.03)	1.41 (0.04)	1.29 (0.22)
Shannon–Wiener (endoparasites)	0.66	0.19	0.33	0.43	1.71	1.67	2.04	1.84
Shannon–Wiener (total)	0.92	0.65	0.51	0.81	1.19	0.79	1.67	1.14
Evenness (endoparasites)	0.41	0.09	0.21	0.27	0.88	0.80	1.00	0.71
Evenness (total)	0.42	0.25	0.25	0.37	0.48	0.30	0.70	0.38
Ec/En ratio	1.00	0.88	0.67	1.00	0.71	0.88	0.57	0.67
Simpson index	2.00	1.39	1.31	1.50	2.51	2.26	3.73	2.05
Berger–Parker index	0.67	0.85	0.87	0.81	0.48	0.60	0.45	0.69

Ec/En ratio, ectoparasite/endoparasite ratio; nc, not calculated.

was higher than that in fish off the coast of the Segara Anakan lagoon (14 taxa). For each single sample, the highest species richness of 15 taxa was recorded from both Bali in 2008 and Segara Anakan lagoon in 2007/08 during the rainy season. The lowest species richness of 10 taxa was recorded in fish from both the Segara Anakan lagoon 2008/09 during the rainy season and off the coast of this lagoon in 2008 during the dry season (tables 2 and 3).

The lowest ectoparasite richness (four taxa) was found in the second year (2008/09) of samples from both Segara Anakan lagoon and Bali, and highest (seven taxa) for the same samples in the first year (2007/08) (table 2). The endoparasite richness was highest (eight taxa) in fish from Segara Anakan lagoon and Bali in the first year (2007/08), and lowest (five taxa) in the first sample from off the coast of Segara Anakan lagoon (table 3). Ectoparasite/endoparasite ratios, calculated by using the numbers of ectoparasite species vs. the numbers of endoparasite species, ranged from 0.6 to 1.0 (table 3). Regional differences of the ectoparasite/endoparasite ratio were not significant.

The endoparasite diversity (Shannon–Wiener index) of *E. coioides* of the present study ranged from 0.19 in Segara Anakan lagoon to 2.04 in Bali (table 4). The Simpson diversity index for the whole parasite community was lower for grouper parasites in Segara Anakan lagoon (1.39) compared with Bali (3.73) (table 4). The highest Evenness value (1.00) for endoparasites was recorded for Bali, compared with the lowest value (0.09) in Segara Anakan lagoon. The Berger–Parker index was lowest in Balinese waters (0.45) and highest in Segara Anakan lagoon (0.87) (table 4). The hepatosomatic index ranged from 0.77 off the coast of Segara Anakan to 1.37 in Segara Anakan lagoon (table 4), with a significant difference (ANOVA: $F = 3.74$, $P < 0.001$).

The most predominant parasites, occurring at all sampling sites, were the monogenean *Pseudorhabdosynochus lantauensis* 53.3–97.1%, the nematode *Spirophilometra endangae* 23.3–42.9%, the digenean *Didymodiclinus* sp. 2.9–40.0%, the nematodes *Philometra* sp. 22.6–34.3% and *Raphidascaris* sp. 2.9–28.6%, and the isopod *Alcirona* sp. 6.7–31.4%. The prevalence of infection of the larval tetraphyllidean cestode *Scolex pleuronectis* as well as the larval nematode *Raphidascaris* sp. was different between the different regions during the first year (2007/08). The prevalence for both parasite taxa was significantly higher in Balinese waters compared to Segara Anakan lagoon: 42.9 versus 2.9% and 28.6 versus 2.9%, $P = 0.000$ and 0.003 (table 3). The larval nematode *Terranova* sp. could only be isolated from *E. coioides* from Ringgung (Rückert, 2006; Palm & Rückert, 2009) at a prevalence of 14.3%, resulting in significant regional differences between all sampled groupers of the present study with $n = 30–35$ fish per location and year (see table 1) in contrast to those from Ringgung with $n = 35$ ($P = 0.020–0.025$). The prevalence of infection of the leech *Z. arugamensis* was significantly different during the second year of investigation (2008/09) between Segara Anakan lagoon and off the coast of Segara Anakan, with 0% versus 40.0% in 2008/09, $P = 0.000$. The same trend was observed in the first year (2007/08), with no significant difference (8.6% versus 16.7%, $P > 0.05$) (table 2). The prevalence of

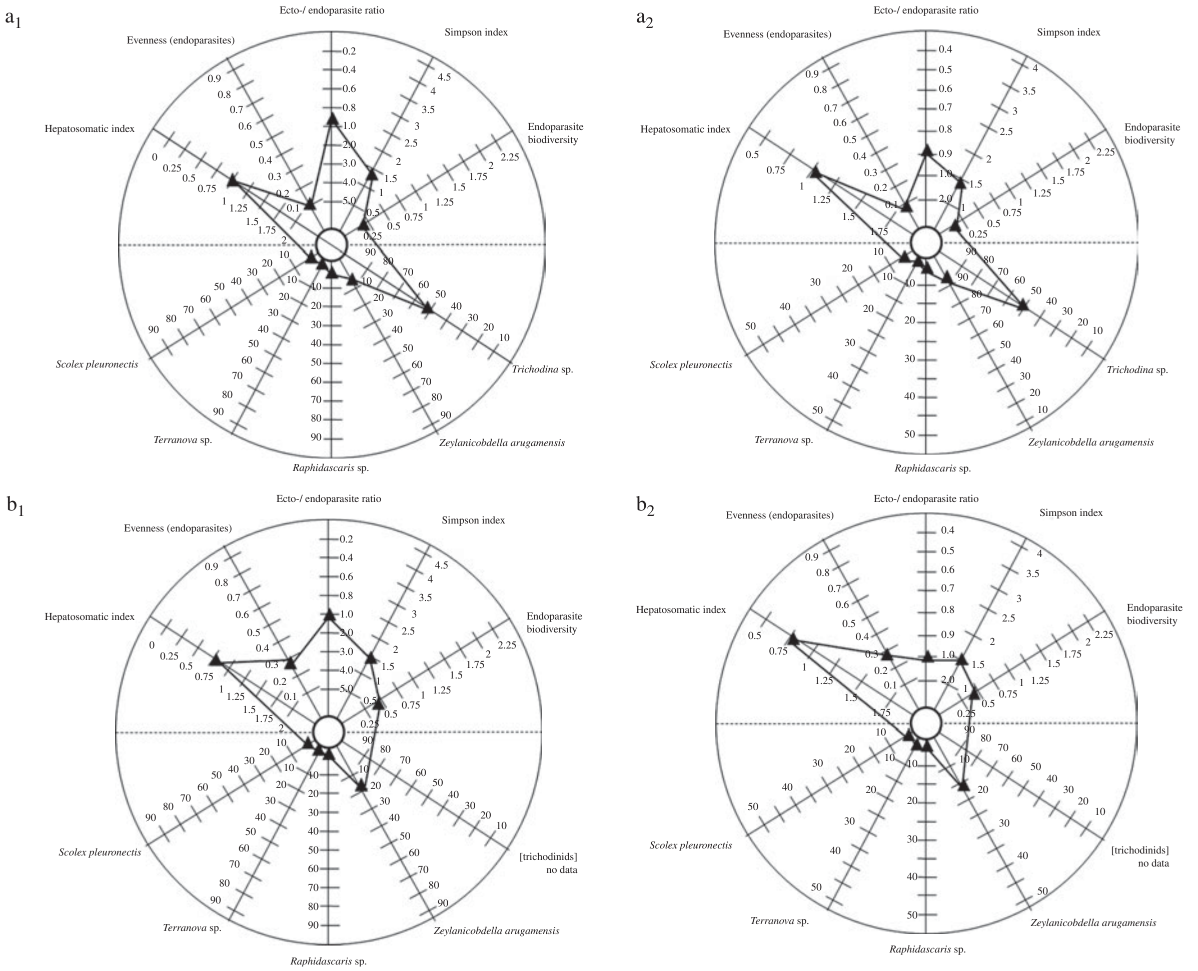

Fig. 2. Visual integration of environmental indicators for free-living *Epinephelus coioides* from Javanese waters: (a_1, a_2) Segara Anakan during the rainy season 2007/08 and (b_1, b_2) off the coast of Segara Anakan lagoon during the dry season 2008, with normal integration $_{(1)}$ and adjusted parameter range $_{(2)}$. Host/parasite parameters are given in the upper half and prevalences (%) of parasite species in the lower half of each star graph.

infection with the ciliate *Trichodina* sp. was significantly higher for fish in Segara Anakan lagoon compared to Balinese coastal waters, with 51.4% and 40.0% versus 17.1%, $P = 0.003$ for 2007/08 and $P = 0.034$ for 2008/09 (table 2).

Regional parasite composition and visual integration

Significant regional differences in species composition were not found between the sampled *E. coioides* from Balinese and Javanese coastal waters in either year. Highest differences regarding to ANOSIM analyses were found between samples from Bali in 2008 and off the coast of Segara Anakan in 2008, with ANOSIM: $R = 0.399$, $P = 0.01$, and between samples from Bali in 2008 and Segara Anakan lagoon in 2007/08, with ANOSIM: $R = 0.342$, $P = 0.01$. There was a distinct separation between the parasite composition of the sampled *E. coioides* from Segara Anakan region (both sites) compared to those from Balinese waters in the first sample with ANOSIM: $R = 0.332$, $P = 0.01$ (fig. 6a). In the following year, the parasite composition was different, without an obvious regional separation, with ANOSIM: $R = 0.217$, $P = 0.01$ (Fig. 6b). With regard to SIMPER analysis, the parasite species contributing most to the regional differences in Segara Anakan lagoon in 2007/08 were *P. lantauensis,* 76.80%; *S. endangae*, 7.71%; *Alcirona* sp., 4.19%; and Pennelidae gen. et sp. indet. I, 3.26%, being also present in the samples from the other sampling sites. Off the coast of Segara Anakan lagoon in 2008, the species contributing most were *P. lantauensis,* 67.68%; Pennelidae gen. et sp. indet. II, 20.70%; and *S. endangae*, 5.55%. Those from Bali in 2008 were *P. lantauensis*, 53.36%; Caligidae gen. et sp. indet., 22.49%; *S. pleuronectis*, 11.91%; and *Philometra* sp., 6.59%.

Ten parasite bioindicators are visualized within a star graph according to Bell & Morse (2003), Palm & Rückert (2009) and Palm *et al.* (2011), to illustrate regional differences between the sampling sites. The presented data demonstrate the natural range of these parameters

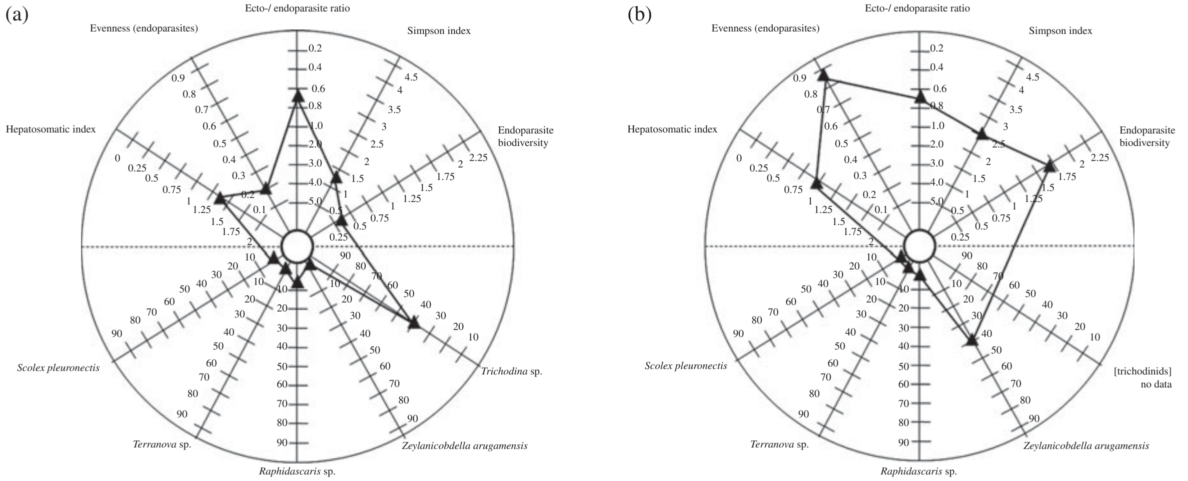

Fig. 3. Visual integration of environmental indicators for free-living *Epinephelus coioides* from Javanese waters: (a) Segara Anakan during the rainy season 2008/09 and (b) off the coast of Segara Anakan during the rainy season 2008/09. Host/parasite parameters are given in the upper half and prevalences (%) of parasite species in the lower half of each star graph.

and parasite prevalences according to habitat and region, allowing an adjustment of the scale to be utilized in the visual integration of the parasite parameters. According to the newly collected and already published data of *E. coioides* parasites from Indonesia, the hepatosomatic index among the sampling sites ranged from 0.77 to 1.58, the Evenness from 0.09 to 1.00, the ectoparasite/endoparasite ratio from 0.57 to 1.00, the Simpson index from 1.31 to 3.73 and the endoparasite diversity according to Shannon–Wiener from 0.19 to 2.04. The prevalence of infection for *S. pleuronectis* was 0–42.9%; for *Terranova* sp., 0–14.3%, *Raphidascaris* sp., 0–31.4%; *Z. arugamensis,* 0–40.0% and for *Trichodina* sp. 14.3–52.4%. Figure 2a$_1$, b$_1$ and fig. 3a, b illustrate the parasite parameters by utilizing a prevalence range from 0 to 100% and the range for the ecological parasite parameters according to Palm & Rückert (2009) and Palm *et al.* (2011), with most of the indicators oriented towards the centre of the star graph in Segara Anakan lagoon and towards the middle in the sample off the coast of Segara Anakan lagoon. According to the parasitological data of *E. coioides* from Indonesia recorded here, the star graphs with adjusted parameter range are given in fig. 2a$_2$, b$_2$. The regional differences in the parasite infection of *E. coioides* between inside Segara Anakan lagoon in 2004 (Yuniar, 2005; Rückert *et al.*, 2009a), Bali (present study) and Ringgung 2003 (Rückert, 2006) are given in fig. 4a$_1$, a$_2$, b$_1$, b$_2$ and fig. 5a$_1$, a$_2$ with and without adjustment of the parameter range, respectively.

Discussion

Grouper parasites

To our knowledge, up to 2009, 28 parasitological studies had been recorded for *E. coioides* worldwide, revealing a total of 57 different parasite species/taxa, belonging to the Ciliata (4), Microsporidia (1), Myxozoa (1), Digenea (7), Monogenea (13), Cestoda (6), Nematoda (13), Acanthocephala (2), Hirudinea (1) and Crustacea (9) (Kleinertz, 2010). Of these records, 77% originate from Indonesian waters; with the present study adding four new host records (see tables 2 and 3). The 25 different parasite species recorded here cover 57% of all previous records from Indonesian waters and 44% of the worldwide records for this host. Kuchta *et al.* (2009) stated that only four bothriocephalideans have been reported so far from Indonesia. Palm & Rückert (2009) added *Botriocephalus* sp. from *E. coioides* from Segara Anakan lagoon; it was also recorded within the present study but so far not identified to the species level. This provides further evidence for the high parasite biodiversity in Indonesian waters (Palm *et al.*, 1999; Palm, 2000; Carpenter & Springer, 2005; Yuniar *et al.*, 2007), encouraging further parasitological studies within the region. Most recently, Justine *et al.* (2010) added one more parasite record, *Argathona rhinoceros,* for *E. coioides* from New Caledonian waters.

Parasite infection according to region and year of sampling

As already stated by Williams *et al.* (1992) and Arthur (1997), the parasite species composition of distinct fish species reflects differences in food sources, feeding preferences and habitats. Consequently, fish parasites are useful for a range of different applications, such as biological-, accumulation-, effect- and ecosystem-indicators (Palm, 2011). According to the selected parasite parameters, the infracommunity of *E. coioides* parasites in Segara Anakan lagoon was significantly different from the other regions studied so far in Indonesia. Segara Anakan can be considered an extreme habitat, with a low stability within the lagoon on the ecosystem and biogeochemical level (Jennerjahn *et al.*, 2009, see below). In addition, it is an area with a high load of organic contaminants (Dsikowitzky *et al.*, 2011). High water mass exchange rates between Segara Anakan lagoon and the coastal region, regularly changing salinities depending on seasons, and possibly natural migration of the sampled

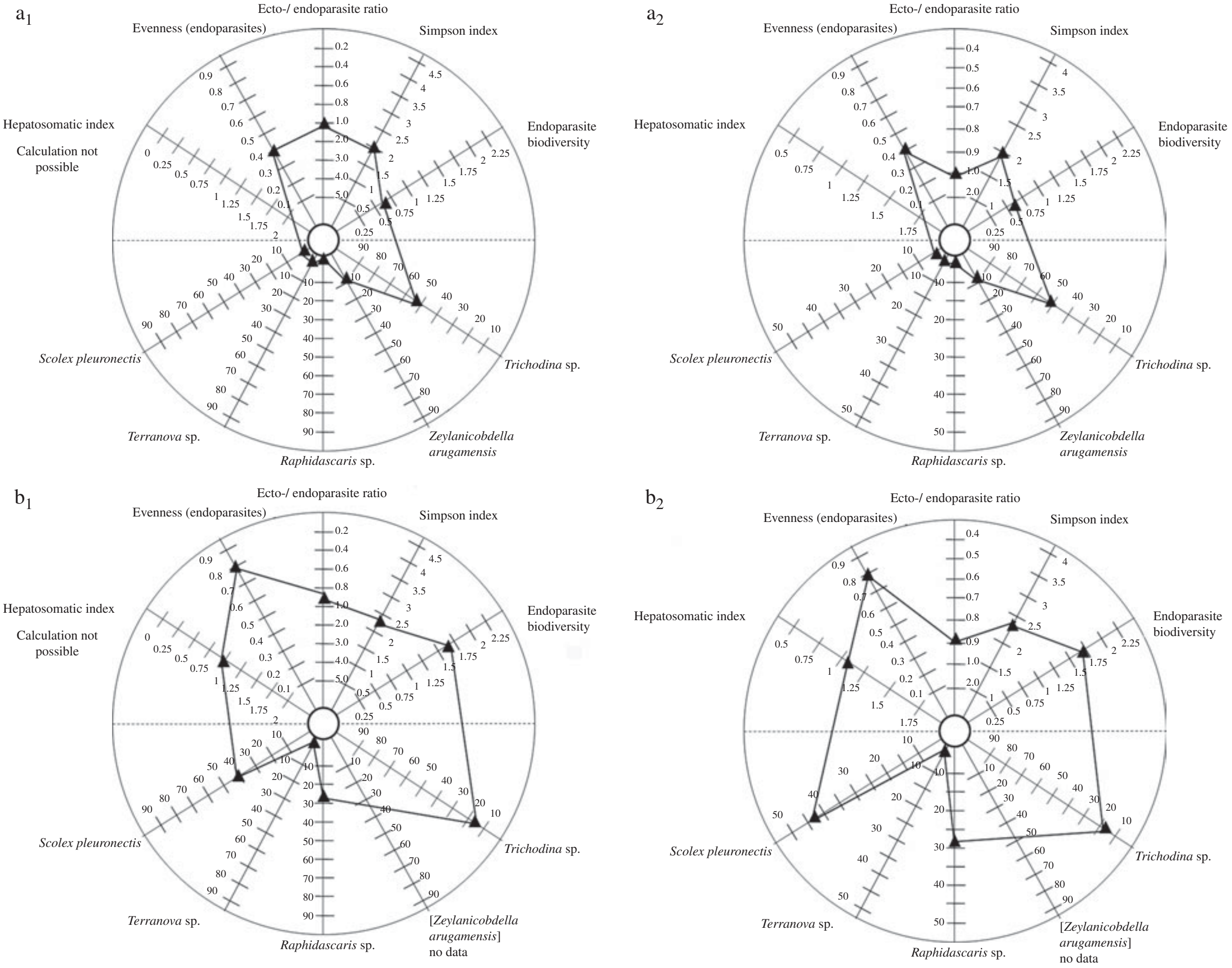

Fig. 4. Visual integration of environmental indicators for free-living *Epinephelus coioides* from Javanese and Balinese waters: (a_1, a_2) Segara Anakan during the dry season 2004 (data modified after Yuniar, 2005) and (b_1, b_2) off the coast of Bali during the dry season 2008, with normal integration $_{(1)}$ and adjusted parameter range $_{(2)}$. Host/parasite parameters are given in the upper half and prevalences (%) of parasite species in the lower half of each star graph.

fish result in a low parasite load, especially of the endohelminths in *E. coioides* (see data for the first year, 2007/08). This is clearly visualized in the resulting star graphs, with most parasite parameters oriented towards the centre (figs 2a_1, a_2, b_1, b_2, 3a, 4a_1, a_2).

A comparison of parasite data revealed the highest richness in 2007/08 with 15 species in the lagoon compared to 12 species during rainy season 2008/09 off the coast. Both values were higher compared to the data from the dry season 2008 off the coast of Segara Anakan and during the rainy season 2008/09 in the lagoon. Groupers off the coast of Segara Anakan were bought on the fish market as dead specimens, and it is possible that the fish from the first sample might have originated as living specimens from inside the lagoon. According to ANOSIM, in the second year, the parasite fauna was different from samples in the lagoon, representing the situation of coastal fish in other regions. A comparison with published data from Ringgung, Lampung Bay, Sumatera in 2003 (Rückert, 2006; Palm & Rückert, 2009) revealed a low endoparasite diversity according to Shannon–Wiener: 0.30/0.19–0.66 and high ectoparasite/endoparasite ratio, with 0.85/0.67–1.00 in the lagoon, followed off the coast of Segara Anakan lagoon at Teluk Bay with 1.71; 0.71 and Lampung Bay with 1.84; 0.67. Highest endoparasite diversity according to Shannon–Wiener: 1.86/1.67–2.04 and a low ectoparasite/endoparasite ratio with 0.73/0.57–0.88 were recorded for *E. coioides* off the Balinese coast, a region that was considered of high environmental quality by Kleinertz *et al.* (2012). The cluster analyses and multi-dimensional scaling plots likewise illustrated these differences; however, they are far less sensitive than the applied star graph method (compare fig. 6 with figs 2, 3, 4 and 5).

During the present study there was only small variability, without any significance, between both years in Segara Anakan lagoon (2007/08 versus 2008/09). However, according to Khrycheva (2009), two fatal oil

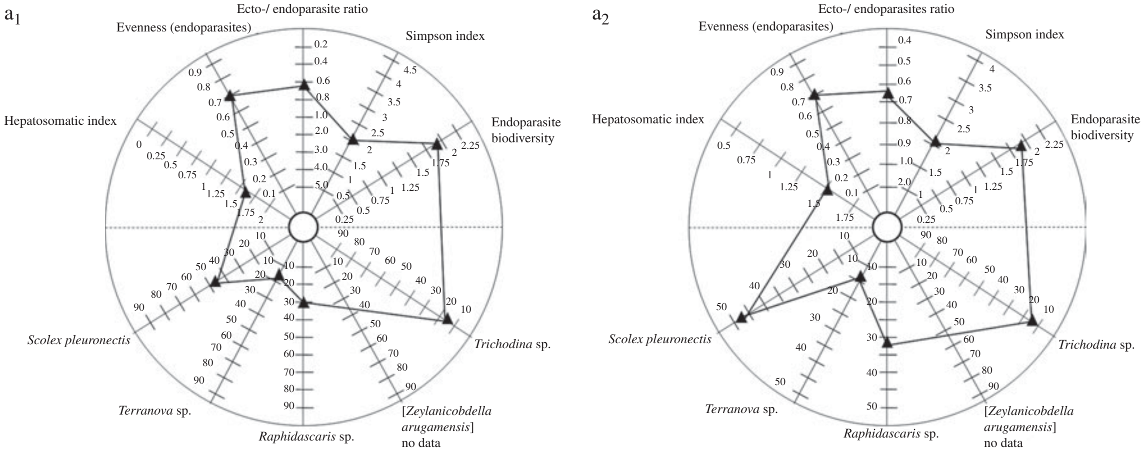

Fig. 5. Visual integration of environmental indicators for free-living *Epinephelus coioides* from Sumatera waters: (a_1, a_2) for cultured *Epinephelus coioides* from Balai Budidaya Laut during the dry season 2003 (data modified after Rückert (2006) and Rückert *et al.* (2009a)), with normal integration $_{(1)}$ and adjusted parameter range $_{(2)}$. Host/parasite parameters are given in the upper half and prevalences (%) of parasite species in the lower half of each star graph.

tanker accidents happened in 2002 and 2004 within the lagoon. Our first data of *E. coioides* from the lagoon originated from the dry season 2004 (fig. 4a_1, a_2), the rainy season 2004/05 and the dry season 2006 (Yuniar, 2005; Palm & Rückert, 2009). The diversity based on the Shannon–Wiener and Simpson indices, as well as the Evenness, were higher during the dry season 2004 compared to 4 years later. However, the ectoparasite/endoparasite ratio changed slightly throughout the different samples, from 1.00 in the dry season 2004 and rainy season 2004/05, to 0.80 in the dry season 2006, 0.88 in the rainy season 2007/08 and 0.67 in the rainy season 2008/09. Having similar parasite species throughout the years might indicate a potential recovery towards the natural parasite fauna of *E. coioides* in the lagoon after both pollution events.

Visual integration

By using the star graph method to integrate different fish parasitological parameters into the same figure, Palm & Rückert (2009) and Kleinertz *et al.* (2012) visualized regional differences within Indonesian waters, and Palm *et al.* (2011) visualized annual changes. Ten different parameters were chosen to describe the parasite communities of *E. coioides* at the sampling sites. The hepatosomatic index describes a possible pollution impact to the fish host (Munkittrik *et al.*, 1994). The Evenness for endoparasites, Simpson index and endoparasite biodiversity according to Shannon–Wiener are used in order to describe natural environmental conditions (Palm & Rückert, 2009; Rückert *et al.*, 2009a; Palm, 2011; Palm *et al.*, 2011; Kleinertz *et al.*, 2012). The prevalence of trichodinid ciliates describes bacteria-enriched waters (Palm & Rückert, 2009). Different leeches have been used as a kind of substandard sensitive marker for definite chemical parameters (Grosser *et al.*, 2001). The authors noted a high sensitivity of these organisms, especially to hypoxic water conditions, high phosphate, heavy metal and organic pollutant concentrations. In the case of our model, we utilized the leach *Z. arugamensis*, a regular parasite in our samples. We are aware that leeches may fall off the fish host during sampling and might not be considered a good bioindicator in all cases. However, *Z. arugamensis* is firmly attached to the groupers, needs to be pulled off with the help of forceps and can be counted,

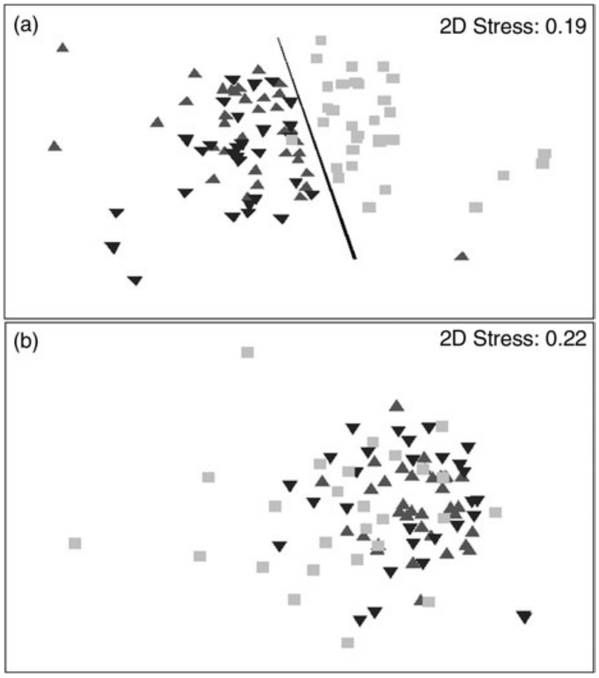

Fig. 6. A multi-dimensional scaling plot of the parasite community of *Epinephelus coioides* from Javanese and Balinese waters in (a) 2007/08 and (b) 2008/09. ▲, Segara Anakan; ▼, off the coast of Segara Anakan; ■, Bali.

especially after collecting the fish in separate plastic bags. The cestode *S. pleuronectis* and the nematode *Raphidascaris* sp. are also common in Indonesian waters, and have been recorded for *E. coioides* in Segara Anakan lagoon (Yuniar, 2005; Rückert *et al.*, 2009b). Groupers represent intermediate hosts in the life cycle of *Raphidascaris* sp., becoming infected via abundant amphipods as first intermediate hosts. Rückert (2006) concluded that epinephelids can also be final hosts for these nematodes. *Terranova* sp., with a possible zoogeographical restriction, has to be considered for the Segara Anakan region (Palm *et al.*, 2011).

Segara Anakan lagoon can be considered an extreme habitat for the fish as well as the parasite fauna. The lagoon has high freshwater influx, mostly from Citanduy River (Holtermann *et al.*, 2009), is governed by tides (Jennerjahn *et al.*, 2009) and can be divided into two major water bodies, mainly connected via a single water-exchange channel. Each of the parts has a direct connection to the ocean (Holtermann *et al.*, 2009). Jennerjahn *et al.* (2009) observed spatio-temporal variations in the distribution of dissolved nutrients in Segara Anakan lagoon, probably the result of seasonally varying interactions of natural (hydrology, geomorphology, soils, vegetation) and anthropogenic (land use, urbanization) factors. The lagoon has been facing a number of environmental problems for decades, because of resource exploitation (Jennerjahn *et al.*, 2009) such as overfishing, logging of mangrove wood, high sediment input by the Citanduy River because of poor upland agricultural practices, agricultural runoff, potential pesticide and oil pollution (White *et al.*, 1989; Jennerjahn *et al.*, 2009; Dsikowitzky *et al.*, 2011). Due to all those facts, we can expect that the high hydrological variability in Segara Anakan lagoon has an important impact on the associated biotics. Consequently, the observed parasite parameters of *E. coioides* in the lagoon, with the characteristic shape of the star graph (figs 2a_1, a_2, b_1, b_2, 3a), represent a heavily disturbed 'natural habitat'. This is in contrast to the coastal zones of Bali, Lampung Bay and even off the coast of Segara Anakan at Teluk Bay, with stable hydrological conditions and less disturbed environments. Thus, our samples represent the greatest possible range of the respective parasite parameters under natural conditions in Indonesia. This leads to the adjustment of the scales that have been used to place the observed parasite parameters into the star graph system for *E. coioides* (see figs 2 and 3). One open question still remains, on how the recorded parasite species react to defined polluted conditions. This will allow final adjustment of the still theoretical range of parameters that we have applied so far for *E. coioides* parasites as environmental indicators in Indonesian coastal ecosystems.

It can be concluded that the presented methodology to visualize fish parasite parameters can distinguish definitive environmental conditions in Indonesian waters under high biodiversity scenarios. So far, regional differences (Palm & Rückert, 2009, Kleinertz *et al.*, 2012; the present study) and long-term annual changes inside a mariculture farm in the Thousand Islands (Palm *et al.*, 2011) and inside the heavily disturbed 'natural habitat' of Segara Anakan have been found. According to these data, free-living *E. coioides* had a high parasite load, similar to those of *E. fuscoguttatus* (Rückert *et al.*, 2010) and *E. areolatus* (Kleinertz *et al.*, 2012). Regular parasitological monitoring of these commercially important fish species will be able to detect environmental conditions and change, possibly serving as an early warning system in Indonesian coastal habitats. We are aware that it is difficult to link directly all observed parasite parameters, without replicates and experiments, to define environmental or anthropogenic factors at all sampling sites and times. However, the star graph system allows direct statements to be made about otherwise highly complex biological scenarios, supporting decision making on the future use of the Indonesian coastal ecosystems.

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Conflict of interest

None.

References

Arthur, J.R. (1997) Recent advances in the use of parasites as biological tags for marine fish. pp. 141–154 *in* Flegel, T.W. & MacRae, I.H. (*Eds*) *Diseases in Asian aquaculture III*. Fish Health Section. Manila, Asian Fisheries Society.

Bell, J.J. & Barnes, K.A. (2003) Effect of disturbance on assemblages: an example using Porifera. *Biological Bulletin* **205**, 144–159.

Bell, S. & Morse, S. (2003) *Measuring sustainability: learning by doing*. London, UK, Earthscan Publications.

Bray, R.A. & Palm, H.W. (2009) Bucephalids (Digenea: Bucephalidae) from marine fishes off the south-western coast of Java, Indonesia, including the description of two new species of *Rhipidocotyle* and comments on the marine fish digenean fauna of Indonesia. *Zootaxa* **2223**, 1–24.

Bush, O., Lafferty, A.D., Lotz, J.M. & Shostak, A.W. (1997) Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *Journal of Parasitology* **83**, 575–583.

Carpenter, K.E. & Springer, V.G. (2005) The center of the center of marine shore fish biodiversity: the Philippine Islands. *Environmental Biology of Fishes* **72**, 467–480.

Clarke, K.R. & Warwick, R.M. (1994) *Change in marine communities: an approach to statistical analysis and interpretation*. 144 pp. Plymouth, Plymouth Marine Laboratory.

Cooper, T.F., Gilmour, J.P. & Fabricius, K.E. (2009) Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes. *Coral Reefs* **28**, 589–606.

Dewi, K. & Palm, H.W. (2013) Two new species of philometrid nematodes (Nematoda: Philometridae) in *Epinephelus coioides* (Hamilton, 1822) from the South Bali Sea, Indonesia. *Zootaxa* **3609**, 49–59.

Diamant, A., Banet, A., Paperna, I., von Westernhagen, H., Broeg, K., Kruener, G., Koerting, W. & Zander, S. (1999) The use of fish metabolic, pathological and parasitological indices in pollution monitoring II. The Red Sea and Mediterranean. *Helgoland Marine Research* **53**, 195–208.

DJPB, (2009) *Kinerja 2008 dan rencana 2009 perikanan budidaya*. Cisaura, Disampaikan oleh direktur produksi-DJPB sebagai bahan diskuksi satgas perikanan budidaya, 5 February.

Downs, C.A., Woodley, C.M., Richmond, R.H., Lanning, L.L. & Owen, R. (2005) Shifting the paradigm of coral-reef 'health' assessment. *Marine Pollution Bulletin* **51**, 486–494.

Dsikowitzky, L., Nordhaus, I., Jennerjahn, T., Khrycheva, P., Sivatharshan, Y., Yuwono, E. & Schwarzbauer, J. (2011) Anthropogenic organic contaminants in water, sediments and benthic organisms of the mangrove-fringed Segara Anakan Lagoon, Java, Indonesia. *Marine Pollution Bulletin* **62**, 851–862.

Dzikowski, R., Paperna, I. & Diamant, A. (2003) Use of fish parasite species richness indices in analyzing anthropogenically impacted coastal marine ecosystems. *Helgoland Marine Research* **57**, 220–227.

Grosser, C., Heidecke, D. & Moritz, G. (2001) Untersuchungen zur Eignung heimischer Hirudineen als Bioindikatoren für Fließgewässer. *Hercynia N.F.* **34**, 101–127.

Hechinger, R.F., Lafferty, K.D., Huspeni, T.C., Andrew, J.B. & Armand, M.K. (2007) Can parasites be indicators of free-living diversity? Relationships between species richness and the abundance of larval trematodes and of local benthos and fishes. *Oecologia* **151**, 82–92.

Holtermann, P., Burchard, H. & Jennerjahn, T. (2009) Hydrodynamics of the Segara Anakan lagoon. *Regional Environmental Change* **9**, 245–258.

Jakob, E. & Palm, H.W. (2006) Parasites of commercially important fish species from the southern Java coast, Indonesia, including the distribution pattern of trypanorhynch cestodes. *Verhandlungen der Gesellschaft für Ichthyologie* **5**, 165–191.

Jennerjahn, T.C., Nasir, B. & Pohlenga, I. (2009) Spatio-temporal variation of dissolved inorganic nutrients related to hydrodynamics and land use in the mangrove-fringed Segara Anakan Lagoon, Java Indonesia. *Regional Environmental Change* **9**, 259–274.

Justine, J.L., Beveridge, J., Boxshall, G.A., Bray, R.A., Moravec, F., Trilles, J.P. & Whittington, I.D. (2010) An annotated list of parasites (Isopoda, Copepoda, Monogenea, Digenea, Cestoda and Nematoda) collected in groupers (Serranidae, Epinephelinae) in New Caledonia emphasizes parasite biodiversity in coral reef fish. *Folia Parasitologica* **57**, 237–262.

Khan, R.A. (1990) Parasitism in marine fish after chronic exposure to petroleum hydrocarbons in the laboratory and to the Exxon Valdez oil spill. *Bulletin of Environmental Contamination and Toxicology* **44**, 759–763.

Khan, R.A. & Kiceniuk, J.W. (1988) Effects of petroleum aromatic hydrocarbons on monogeneids parasitizing Atlantic cod, *Gadus morhua* L. *Bulletin of Environmental Contamination and Toxicology* **41**, 94–100.

Khrycheva, P. (2009) Anthropogenic contaminants in water and sediments from the Segara Anakan lagoon, Indonesia. Master of Science thesis in Marine Biology, University of Bremen.

Kiceniuk, J.W. (1983) Toxicology of chronic crude oil exposure: sublethal effects on aquatic organisms. pp. 425–536 *in* Nraigu, J.O. (*Ed.*) *Aquatic toxicology.* New York, John Wiley.

Klein, B. (1926) Ergebnisse mit einer Silbermethode bei Ciliaten. *Archiv für Protistenkunde* **56**, 243–279.

Klein, B. (1958) The 'dry' silver method and its proper use. *Journal of Protozoology* **5**, 99–103.

Kleinertz, S. (2010) Fish parasites as bioindicators: Environmental status of coastal marine ecosystems and a grouper mariculture farm in Indonesia. PhD thesis of natural sciences, Faculty 2 (Biology/Chemistry), University of Bremen.

Kleinertz, S., Damriyasa, M., Hagen, W., Theisen, S. & Palm, H.W. (2012) An environmental assessment of the parasite fauna of the reef-associated grouper *Epinephelus areolatus* from Indonesian waters. *Journal of Helminthology* doi:10.1017/S0022149X12000715.

Klimpel, S., Rückert, S., Piatkowski, U., Palm, H.W. & Hanel, R. (2006) Diet and metazoan parasites of silver scabbard fish *Lepidopus caudatus* from the Great Meteor Seamount (North Atlantic). *Marine Ecology Progress Series* **315**, 249–257.

Kuchta, R., Scholz, T., Vlcková, R., Ríha, M., Walter, T., Yuniar, A.T. & Palm, H.W. (2009) Revision of tapeworms (Cestoda: Bothriocephalidea) from lizardfish (Saurida: Synodontidae) from the Indo-Pacific region. *Zootaxa* **1977**, 55–67.

Kuhn, T., Hailer, F., Palm, H.W. & Klimpel, S. (2013) Global assessment of molecular identified *Anisakis* spp. (Nematoda: Anisakidae) in the teleost intermediate host. *Folia Parasitologica* **4826**, 1–29.

Kurtz, J.C., Jackson, L.E. & Fisher, W.S. (2001) Strategies for evaluating indicators based on guidelines from the Environmental Protection Agency's Office of Research and Development. *Ecological Indicators* **1**, 49–60.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J.,

Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R. & Thieltges, D.W. (2008a) Parasites in food webs: the ultimate missing links. *Ecology Letters* **11**, 533–546.

Lafferty, K.D., Shaw, J.C. & Kuris, A.M. (2008b) Reef fishes have higher parasite richness at unfished Palmyra Atoll compared to fished Kiritimati Island. *Ecohealth* **5**, 338–345.

Landsberg, J.H., Blakesley, B.A., Reese, R.O., McRae, G. & Forstchen, P.R. (1998) Parasites of fish as indicators of environmental stress. *Environmental Monitoring and Assessment* **51**, 211–232.

Magurran, A.E. (1988) *Ecological diversity and its measurement*. London, Croom Helm.

Munkittrik, K.R., Van der Kraak, G.J., McMaster, M.E., Portt, D.C.B., Van den Heuval, M.R. & Servos, M.R. (1994) Survey of receiving-water environmental impacts associated with discharges from pulp mills. II. Gonad size, liver size, hepatic EROD activity and plasma sex steroid levels in white sucker. *Environmental Toxicology and Chemistry* **13**, 1089–1101.

Nordhaus, I., Hadipudjana, F.A., Janssen, R. & Pamungkas, J. (2009) Spatio-temporal variation of macrobenthic communities in the mangrove-fringed Seagara Anakan lagoon. Indonesia, affected by anthropogenic activities. *Regional Environmental Change* **9**, 291–313.

Ogut, H. & Palm, H.W. (2005) Seasonal dynamics of *Trichodina* spp. on whiting (*Merlangius merlangus*) in relation to organic pollution on the Eastern Black Sea coast of Turkey. *Parasitology Research* **96**, 149–153.

Overstreet, R.M. (1997) Parasitological data as monitors of environmental health. *Parassitologia* **39**, 169–175.

Palm, H.W. (1999) Ecology of *Pseudoterranova decipiens* (Krabbe, 1878) (Nematoda: Anisakidae) from Antarctic waters. *Parasitology Research* **85**, 638–646.

Palm, H.W. (2000) Trypanorhynch cestodes from Indonesian coastal waters (East Indian Ocean). *Folia Parasitologica* **47**, 123–134.

Palm, H.W. (2008) Surface ultrastructure of the elasmobranchia parasitizing *Grillotiella exilis* and *Pseudonybelinia odontacantha* (Trypanorhyncha, Cestoda). *Zoomorphology* **127**, 249–258.

Palm, H.W. (2011) Fish parasites as biological indicators in a changing world: Can we monitor environmental impact and climate change? pp. 223–250 *in* Mehlhorn, H. (*Ed.*) *Progress in parasitology. Parasitology Research Monographs* **2**, 223–250. Berlin, Springer-Verlag.

Palm, H.W. & Dobberstein, R.C. (1999) Occurrence of trichodinid ciliates (Peritricha: Urceolariidae) in the Kiel Fjord, Baltic Sea, and its possible use as a biological indicator. *Parasitology Research* **85**, 726–732.

Palm, H.W. & Rückert, S. (2009) A new approach to visualize fish and ecosystem health by using parasites. *Parasitology Research* **105**, 539–553.

Palm, H.W., Waeschenbach, A. & Littlewood, D.T.J. (2007) Genetic diversity in the trypanorhynch cestode *Tentacularia coryphaenae* Bosc, 1797: evidence for a cosmopolitan distribution and low host specificity in the teleost intermediate host. *Parasitology Research* **101**, 153–159.

Palm, H.W., Damriyasa, I.M., Linda & Oka, I.B.M. (2008) Molecular genotyping of *Anisakis* Dujardin, 1845 (Nematoda: Ascaridoidea: Anisakidae) larvae from marine fish of Balinese and Javanese waters, Indonesia. *Helminthologia* **45**, 3–12.

Palm, H.W., Kleinertz, S. & Rückert, S. (2011) Parasite diversity as an indicator of environmental change – an example from tropical grouper (*Epinephelus fuscoguttatus*) mariculture in Indonesia. *Parasitology* **138**, 1–11.

Riemann, F. (1988) Nematoda. pp. 293–301 *in* Higgins, R.P. & Thiel, H. (*Eds*) *Introduction to the study of meiofauna*. Washington, DC, Smithsonian Institution Press.

Rimmer, M.A., McBride, S. & Williams, K.C. (2004) *Advances in grouper aquaculture*. Australian Centre for International Agricultural Research Monograph, Canberra, ACIAR.

Rückert, S. (2006) Marine fish parasites in Indonesia: state of infestation and importance for grouper mariculture. PhD thesis, Heinrich-Heine University of Düsseldorf, Germany.

Rückert, S., Hagen, W., Yuniar, A.T. & Palm, H.W. (2009a) Metazoan parasites of Segara Anakan Lagoon, Indonesia, and their potential use as biological indicators. *Regional Environmental Change* **9**, 315–328.

Rückert, S., Klimpel, S., Mehlhorn, H. & Palm, H.W. (2009b) Transmission of fish parasites into grouper mariculture (Serranidae: *Epinephelus coioides* (Hamilton, 1822)) in Lampung Bay, Indonesia. *Parasitology Research* **104**, 523–532.

Rückert, S., Klimpel, S. & Palm, H.W. (2010) Parasites of cultured and wild brown-marbled grouper *Epinephelus fuscoguttatus* (Forsskål, 1775) in Lampung Bay, Indonesia. *Aquaculture Research* **41**, 1158–1169.

Sasal, P., Mouillot, D., Fichez, R., Chifflet, S. & Kulbicki, M. (2007) The use of fish parasites as biological indicators of anthropogenic influences in coral-reef lagoons: a case study of Apogonidae parasites in New Caledonia. *Marine Pollution Bulletin* **54**, 1697–1706.

Sures, B. (2001) The use of fish parasites as bioindicators of heavy metals in aquatic ecosystems: a review. *Aquatic Ecology* **35**, 245–255.

Sures, B. (2003) Accumulation of heavy metals by intestinal helminths in fish: an overview and perspective. *Parasitology* **126**, 53–60.

White, A.T., Marosubroto, P. & Sadorra, M.S.M. (1989) *The coastal environment profile of Segara Anakan Cilacap, South Java, Indonesia*. ICLARM, Association of South-east Asian Nations, United States coastal resources management project. Technical report 25. 82 pp. Manila, Philippines, International Center for Living Aquatic Resources Management.

Williams, H.H., MacKenzie, K. & McCarthy, A.M. (1992) Parasites as biological indicators of the population biology, migrations, diet and phylogenetics of fish. *Reviews in Fish Biology and Fisheries* **2**, 144–176.

Yuniar, A. (2005) Parasites of marine fish from Segara Anakan, Java, Indonesia and their potential use as biological indicators. Master of Science thesis in International Studies in Aquatic Tropical Ecology (ISATEC), University of Bremen.

Yuniar, A.T., Palm, H.W. & Walter, T. (2007) Crustacean fish parasites from Segara Anakan Lagoon, Java, Indonesia. *Parasitology Research* **100**, 1193–1204.