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ORIGINAL ARTICLE

Metazoan fish parasites of Segara Anakan Lagoon, Indonesia, and their potential use as biological indicators

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Abstract The present study reports metazoan fish parasites from Segara Anakan, a brackish water lagoon located at the southern Java coast, Indonesia. Seven economically important marine fish species (*Mugil cephalus*, *Siganus javus*, *Scatophagus argus*, *Caranx sexfasciatus*, *Lutjanus johnii*, *Eleutheronema tetradactylum* and *Johnius coitor*) were examined at two different sampling sites within the lagoon for the occurrence of metazoan parasites. A diverse parasite fauna was found, consisting of 43 species/taxa. Ectoparasites (31) were more abundant than endoparasites (12). The fish species *J. coitor*, *M. cephalus* and *S. argus* harboured the most diverse metazoan parasite fauna with 11, 13, and 16 different parasite species, respectively. Prevalence and intensity of infection for each parasite species/taxon is given, including short descriptions for rapid diagnosis. For the first time, we discuss the utilisation of the sampled fish parasites as biological indicator organisms for fish and environmental health within this tropical mangrove ecosystem. Ecto- versus endoparasite ratio and endoparasite diversity are suitable tools to describe the environmental health status at a tropical brackish water locality, and might be applied also for other tropical and possibly non-tropical marine ecosystems.

Keywords Metazoan fish parasites · Biological indicators · Segara Anakan Lagoon · Indonesia

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Introduction

The Segara Anakan Lagoon is a brackish water ecosystem of approximately 4,000 ha. The lagoon is surrounded by about 14,000 ha of mangrove forests and located at the western side of Cilacap (southern coast of Java) (Naamin 1991). It supports a large and productive mangrove system and plays an important role as nursery ground for a variety of fish species (Romimohtarto et al. 1991). Due to high productivity and strategic location, the lagoon is an anthropogenically highly influenced tropical estuarine ecosystem. Located close to the city Cilacap and an oil processing plant on its eastern side, urban and industrial pollutants are released. The pollutants detected in this area are heavy metals, pesticides, hydrocarbons and sediment (Romimohtarto et al. 1991).

To date approximately 3,270 marine and brackish water fish species are known from Indonesian waters (Froese and Pauly 2007). Around 45 fish species have been reported from Segara Anakan Lagoon (White et al. 1989), but the real species number is expected to be much higher (Dudley 2000). According to Naamin (1991) ten of these fish species are of economic importance and regularly caught in the area. Tropical waters are known for a high biodiversity of fish parasites (e.g. Jakob and Palm 2006; Palm 2000, 2004), infecting free living fishes from all trophic levels as well as those under culture conditions (Leong et al. 2006).

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The occurrence of fish parasites is closely related to the distribution of their final and intermediate hosts (Collard 1970; Hine and Kennedy 1974). Their abundance is also influenced by further biotic and abiotic factors, such as the fish feeding ecology (Palm et al. 1998; Walter et al. 2002), water temperature (Rohde et al. 1995), salinity (Roubal 1997), water depth (Collard 1970; Palm 1999) and pollution (Galli et al. 2001).

The close relationship of a highly diverse parasite fauna to its hosts and the environment opens up the opportunity to utilise these organisms as biological indicators. Therefore, fish parasites have already been applied to indicate the ecology of their hosts (e.g. feeding, Palm 1999; migration and recruitment, Williams et al. 1992; Moser 1991) or the conditions of the environment (e.g. water quality, MacKenzie et al. 1995; Galli et al. 2001; pollution, Sures and Reimann 2003; environmental stress, Khan and Thulin 1991; Landsberg et al. 1998). Thus, fish parasites are an important component of the aquatic biodiversity that can be utilised as biological indicators to describe not only the fish health (see below) but also the status of any aquatic environment.

Many fish parasites are known to be causative agents for disease problems and outbreaks within mariculture facilities (Palm 2004; Rückert et al. 2008a, b). Once a fish mariculture is established, parasite and disease outbreaks occur soon thereafter (Moravec 1994). Mortalities in cultured fishes are predominantly caused by monoxenous (single-host) ectoparasites with high reproduction rates (e.g. trichodinid ciliates, monogeneans or crustaceans, Leong 1992; Diamant et al. 1999; Williams and Bunkley-Williams 2000). Normally, heteroxenous (multiple-host) endoparasites do not have any severe effect on the cultivated host; however, different environmental needs of the developmental stages makes them suitable as biological indicators within and around finfish mariculture. The intended extension of the Indonesian finfish mariculture (FIRI 2006) necessitates further information on the available biodiversity of fish parasites and their pathogenic potential, also inside Segara Anakan Lagoon.

The present study will complement our knowledge on fish parasites of free-living economically important fishes in Segara Anakan Lagoon (Yuniar et al. 2007). Special emphasis is given on brief diagnosis and infection levels of the ecto- and endohelminth fauna. We also explore the possibility to utilise the detected parasites as biological indicators for fish and environmental health.

Materials and methods

Samples were taken within the framework of the Science for the Protection of Indonesian Coastal Ecosystems-project (SPICE-project) from August to November 2004 at two different localities in Segara Anakan Lagoon (Central Java). The selected sampling sites Area 2 (Motean and Klaces) and Area 3 (Donan) can be differentiated according to the environmental conditions. Area 2 is located at the centre of the lagoon. It is influenced by freshwater runoff of several large rivers, which causes a lower salinity (19.7–28.0). Area 3 has a higher salinity (29.3–31.2) due to its location close to the outlet into the Indian Ocean.

Fishes were obtained freshly from local fishermen within the lagoon. Seven marine fish species were studied for metazoan parasites: *Mugil cephalus* L., 1758 (35 Area 2/35 Area 3), *Scatophagus argus* (L., 1766) (35/35), *Siganus javus* (L., 1766) (5/−), *Caranx sexfasciatus* Quoy & Gaimard, 1825 (3/5), *Lutjanus johnii* (Bloch, 1792) (4/4), *Eleutheronema tetradactylum* (Shaw, 1804) (2/6), and *Johnius coitor* (Hamilton, 1822) (−/20). The fish samples were kept on ice and then deep-frozen (∼ −20°C) until further examination.

Fish dissection was carried out at the Parasitology and Entomology Laboratory, Biology Faculty, Jenderal Soedirman University, Purwokerto. Total fish length and weight (TL, to the nearest 1.0 cm; TW to the nearest 1.0 g) were measured (Table 1) prior to the parasitological examination. Each fish was examined microscopically for the presence of endoparasitic metazoans following Kabata (1985). The isolated parasites were fixed in 4% formalin and preserved in 70% ethanol. Acanthocephala were transferred to freshwater until the proboscis everted prior to fixation. For identification purposes, Nematoda and Acanthocephala were dehydrated in a graded ethanol series and transferred to 100% glycerine (Riemann 1988). Digenea,

Table 1 Fish species, number (*N*) of dissected specimens in Area 2/Area 3, mean length and mean weight (range in parentheses) of the studied fish species

Fish species	*N*	TL (cm)	TW (g)
Mugil cephalus Area 2	35	16.7 (12–20)	57.7 (20–125)
Mugil cephalus Area 3	35	14.3 (11–24)	40.2 (11–175)
Siganus javus Area 2	5	11.6 (11–13)	27.0 (15–40)
Scatophagus argus Area 2	35	9.5 (7–14)	29.5 (10–100)
Scatophagus argus Area 3	35	11.1 (8–20)	54.1 (11–230)
Caranx sexfasciatus Area 2	3	15.0 (14–16)	73.3 (45–95)
Caranx sexfasciatus Area 3	5	16.6 (15–18)	79.0 (50–100)
Lutjanus johnii Area 2	4	15.8 (12–21)	58.8 (20–150)
Lutjanus johnii Area 3	4	13.8 (13–16)	35.0 (30–40)
Eleutheronema tetradactylum Area 2	2	20.5 (17–24)	65.0 (40–90)
Eleutheronema tetradactylum Area 3	6	22.2 (17–29)	110.0 (55–200)
Johnius coitor Area 3	20	13.9 (11–18)	28.4 (15–50)

N number, *TL* total length, *TW* total weight

Monogenea and Cestoda were stained with acetic carmine, dehydrated, cleared with Eugenol and mounted in Canada balsam. Parasite identification followed standard identification literature and original descriptions.

Pictures were taken by using a digital camera, Canon PC 1015, attached to a microscope (Axioskop 40 Zeiss, Germany) or a stereomicroscope (STEMI SV 11 Zeiss, Germany). Selected specimens were prepared following Robinson et al. (1985) for scanning electron microscopy (SEM). The SEM photomicrographs were made with the help of a Leitz, LEICA MD-2, Canada, reflex camera with AGFA APX 25 professional 135, Ilford FP4 plus 125 and Ilford Panf plus 50 (36 exposures each, black and white film).

The ecological terms in parasitology follow Margolis et al. (1982) and Bush et al. (1997): prevalence (*P*) is the number of infected fish with one or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined (expressed as a percentage). Intensity (of infection, *I*) is the number of individuals of a particular parasite species in a single infected host (expressed as a numerical range); and mean intensity (of infection, m*I*) is the average intensity, or the total number of parasites of a particular species found in a sample divided by the number of infected hosts. The diversity of the parasite fauna was estimated by using the Shannon–Wiener diversity index (H') and the evenness index (E) of Pielou [$H' = -\sum Pi \times lnPi \quad E = H'/lnS$, with H' being the diversity index, Pi the proportion of the individual (ith) species to the total and S the total number of species in the community (species richness), see Magurran 1988]. Additionally, the ratio of ecto- to endoparasites (E/E ratio) was calculated. Species groups (such as Nematoda indet.) that could not be further identified were not included in these calculations.

Results

During the present study, 189 fishes (7 families; 7 species) from the Segara Anakan Lagoon were investigated for the presence of metazoan parasites. All fish species were infected with at least one parasite taxon. Overall, 43 parasite species belonging to the taxa Digenea (4), Monogenea (7), Cestoda (1), Nematoda (6), Acanthocephala (1), Hirudinea (1) and Crustacea (23) were collected (Tables 2, 3). The crustaceans were described by Yuniar et al. (2007).

Metazoan parasite fauna

The prevalence and intensity of infection of the metazoan parasites varied among the sampled fish species and the two different sampling sites (Tables 2, 3). Crustacea were the most common parasites on the examined fishes. *Mugil cephalus*, *Scatophagus argus* and *Johnius coitor* harboured the most diverse metazoan parasite fauna with 13, 16 and 11 different species, respectively. A brief description of the collected helminths with notes on the identification and key literature is given below.

Digenea

Larval stages of didymozoid trematodes were found in the intestine of a single *Johnius coitor*. The larvae can be recognised by the presence of an apical oral sucker and an acetabulum located in the anterior quarter of the body, internal organs and eggs are not developed. A species identification of larval didymozoid trematodes is not possible (Køie and Lester 1985). Two digeneans of the family Haploporidae were isolated from the intestine of *Mugil cephalus*. The first species was identified as *Lecithobotrys* sp. This genus is characterised by a slender body shape (total length 1–1.2 mm), the presence of an oral and ventral sucker (diameter 100–120 µm) and the shape of the caecum (Machida 1996). The second haploporid species was identified as Haploporidae gen. et sp. indet. These specimens showed obvious differences in the morphological characteristics in comparison with *Lecithobotrys* sp., e.g. a bigger oral and ventral suckers (diameter of 175–185 and 380–400 µm, respectively) and a compressed body shape (body length = 800–870 µm). Digenetic trematodes in the intestine of *Scatophagus argus* belonged to the family Waretrematidae and were identified as *Pseudohapladena* cf. *scatophagi* (see Yamaguti 1952). Characters were the slender body, the presence of a big pharynx and the number of testes.

Monogenea

Two dactylogyriid monogeneans were collected. *Metahaliotrema scatophagi* Yamaguti, 1953 (Fig. 2b, c) from the gill filaments of *Scatophagus argus* is characterised by its small size (total length = 300–320 µm), the presence of head organs, two pairs of eye spots, and two pairs of anchors supported by chitinous bars and 14 marginal hooklets in the bilobed opisthaptor (Yamaguti 1953). The monogenetic trematodes on the gill filaments of *Mugil cephalus* were identified as Dactylogyridae gen. et sp. indet. The specimens are characterised by the small size (total length = 60–65 µm), presence of eyespots, and an opisthaptor with two pairs of hooks and several anchors. The monogeneans isolated from the gill cavity of *J. coitor* belong to the family Diclidophoridae. The genus *Choricotyle* (Figs. 1a, 2a, d) is distinguished by having four pairs of equal or subequal, pedunculate clamps with a

Table 2 Prevalence (*P* in %), mean intensity (m*I*) and the range of intensity (*I*) in parentheses for the metazoan parasites of the dissected fish species in Area 2

Parasites (Area 2)	*Mugil cephalus*		*Siganus javus*		*Scatophagus argus*		*Caranx sexfasciatus*		*Lutjanus johnii*		*Eleutheronema tetradactylum*	
	P (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)
Ectoparasites												
Metahaliotrema scatophagi (Mo)					100	37.5 (5–114)						
Dactylogyridae gen. et sp. indet. (Mo)	74	9.6 (1–92)										
Monogenea indet. (Mo)	9	24.6 (1–48)										
Zeylanicobdella arugamensis (H)			20	1.0 (1)	6	2.0 (1–3)			50	3 (3)		
Nothobomolochus sp. (Cr)	9	1.3 (1–2)										
Ergasilus sp. 1 (Cr)	63	6.7 (1–37)										
Ergasilus sp. 2 (Cr)	3	3.0 (3)			6	51.0 (37–65)						
Ergasilus sp. 3 (Cr)					83	16.6 (1–78)						
Ergasilus sp. 4 (Cr)			20	1.0 (1)								
Ergasilidae gen. et sp. indet. (Cr)	20	5.3 (1–15)										
Caligus acanthopagri (Cr)					71	7.0 (1–22)						
Caligus cf. *confusus* (Cr)							67	5.5 (4–7)				
Caligus epidemicus (Cr)			20	1.0 (1)	9	1.3 (1–2)						
Caligus phipsoni (Cr)											50	2 (2)
Caligus cf. *quadratus* (Cr)			100	1.0 (1)								
Caligus rotundigenitalis (Cr)	37	1.5 (1–3)										
Parapetalus hirsutus (Cr)											50	1 (1)
Pseudocaligus sp. (Cr)					11	1.8 (1–4)						
Caligidae gen. et sp. indet. (Cr)	23	1.9 (1–4)			74	8.9 (1–44)						
Thysanote sp. (Cr)					66	1.8 (1–2)						
Lernanthropus polynemi (Cr)											50	5 (5)
Peniculus cf. *scomberi* (Cr)							33	1.0 (1)				
Endoparasites												
Haploporidae gen. et sp. indet. (D)	29	4.1 (1–15)										
Pseudohapladena cf. *scatophagi* (D)					9	10.6 (1–29)						
Procamallanus sp. (N)					14	1.4 (1–2)						
Cucullanus sp. (N)					29	3.2 (1–11)						
Capillaria sp. (N)					14	1.6 (1–3)						
Nematoda indet. (N)	9	2.3 (2–3)										
Filisoma cf. *indicum* (Ac)					29	8.9 (1–21)						

Ac Acanthocephala, *Cr* Crustacea, *D* Digenea, *H* Hirudinea, *Mo* Monogenea, *N* Nematoda

Table 3 Prevalence (*P* in %), mean intensity (m*I*) and the range of intensity (*I*) in parentheses for the metazoan parasites of the dissected fish species in Area 3

Parasites (Area 3)	*Mugil cephalus*		*Scatophagus argus*		*Caranx sexfasciatus*		*Lutjanus johnii*		*Eleutheronema tetradactylum*		*Johnius coitor*	
	P (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)	*P* (%)	m*I* (*I*)
Ectoparasites												
Metahaliotrema scatophagi (Mo)	6	2.0 (2)	89	14.1 (1–44)								
Dactylogyridae gen. et sp. indet. (Mo)	49	6.1 (1–31										
Choricotyle sp. (Mo)											30	1.8 (1–3)
Axinidae gen. et sp. indet. (Mo)					60	1.3 (1–2)						
Microcotyle cf. *polynemi* (Mo)									67	2.3 (1–5)		
Microcotylidae gen. et sp. indet. (Mo)											75	2.6 (1–10)
Monogenea indet. (Mo)	29	7.1 (1–29)										
Zeylanicobdella arugamensis (H)	3	1.0 (1)	11	1.0 (1)			25	1.0 (1)				
Nothobomolochus sp. (Cr)	26	1.8 (1–5)										
Ergasilus sp. 1 (Cr)	40	4.1 (1–30)										
Ergasilus sp. 2 (Cr)			9	2.3 (1–4)								
Ergasilus sp. 3 (Cr)			74	19.0 (1–233)								
Ergasilidae gen. et sp. indet. (Cr)	29	3.2 (1–8)										
Caligus acanthopagri (Cr)			86	3.2 (1–16)								
Caligus cf. *confusus* (Cr)					100	10.6 (4–17)						
Caligus phipsoni (Cr)									83	3.0 (1–6)		
Caligus cf. *quadratus* (Cr)												
Caligus rotundigenitalis (Cr)	11	4.5 (1–15)										
Caligus sp. (Cr)											25	2.0 (1–4)
Parapetalus hirsutus (Cr)									83	1.8 (1–3)		
Caligidae gen. et sp. indet. (Cr)	26	2.6 (1–8)	80	3.5 (1–23)	60	2.0 (1–3)			33	1.0 (1)	30	1.3 (1–2)
Thysanote sp. (Cr)			66	1.7 (1–2)								
Naobranchia cf. polynemi (Cr)									17	1.0 (1)		
Lernanthropus polynemi (Cr)									100	3.5 (2–8)		
Lernanthropus sp. (Cr)											5	1.0 (1)
Peniculus cf. *scomberi* (Cr)											5	1.0 (1)
Cymothoa sp. (Cr)			29	1.4 (1–2)								
Gnathiidae gen. et sp. indet. (Cr)			6	1.0 (1)	20	1.0 (1)						
Endoparasites												
Didymozoidae gen. et sp. indet. (D)											10	1.0 (1)
Lecithobotrys sp. (D)	3	1.0 (1)										
Haploporidae gen. et sp. indet. (D)	26	5.4 (2–11)										
Pseudohapladena cf. *scatophagi* (D)			6	1.0 (1)								
Mixonybelinia southwelli (C)											10	1.0 (1)
Anisakis sp. (N)					20	1.0 (1)					5	1.0 (1)
Procamallanus sp. (N)											45	2.3 (1–6)
Philometra sp. (N)											5	1.0 (1)
Filisoma cf. *indicum* (Ac)			46	3.9 (1–22)								

Ac Acanthocephala, *C* Cestoda, *Cr* Crustacea, *D* Digenea, *H* Hirudinea, *Mo* Monogenea, *N* Nematoda

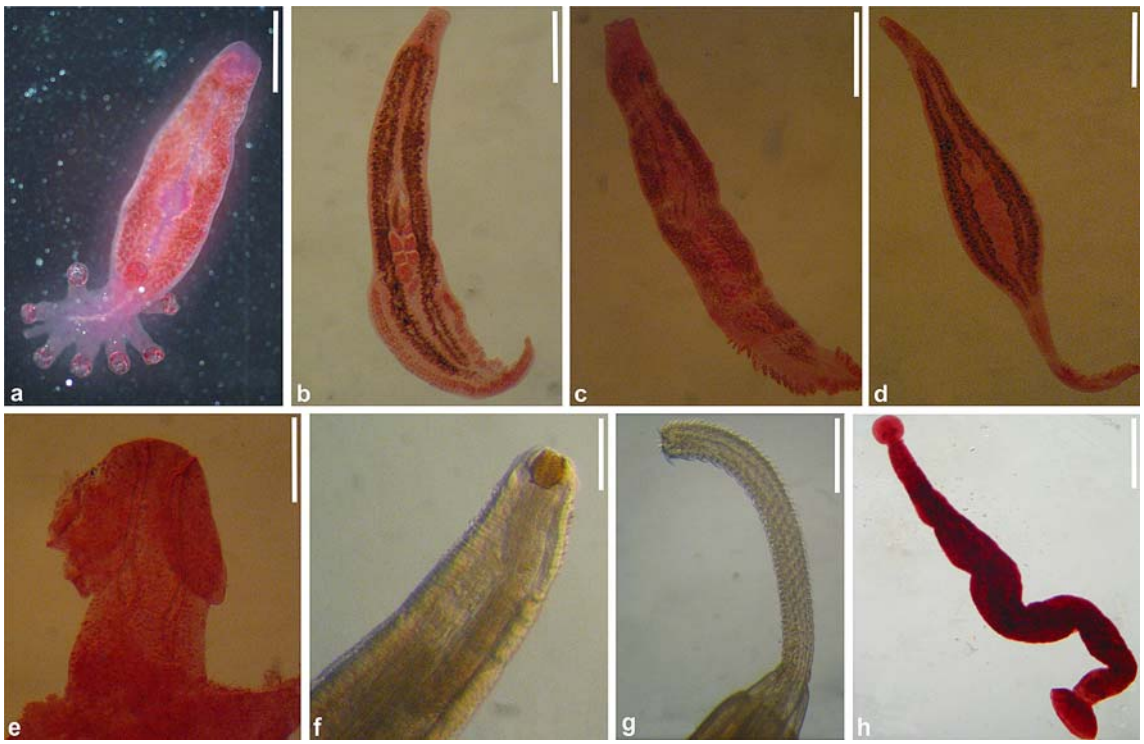

Fig. 1 Light micrographs of metazoan fish parasites from Segara Anakan, Indonesia. **a** *Choricotyle* sp. from *Johnius coitor*, *scale bar* = 510 µm. **b** *Microcotyle* gen. et sp. indet. from *J. coitor*, *scale bar* = 70 µm. **c** Axinidae gen. et sp. indet. from *Caranx sexfasciatus*, *scale bar* = 260 µm. **d** *Metamicrocotyla* sp. from *Mugil cephalus*, *scale bar* = 260 µm. **e** *Mixonybelinia southwelli* from *J. coitor*, *scale bar* = 35 µm. **f** *Procamallanus* sp. (*anterior*) from *Scatophagus argus*, *scale bar* = 30 µm. **g** *Filisoma* cf. *indicum* from *S. argus*, *scale bar* = 50 µm. **h** *Zeylanicobdella arugamensis* from *Siganus javus*, *scale bar* = 60 µm

typical sucker in the expanded inner dorsal quadrant (Schell 1970). A minute, armed or unarmed, terminal lappet is present between the two posterior clamps (Yamaguti 1963). According to the total length of 2–2.5 mm, pharynx length 95–110 µm and sucker diameter 60–70 µm, they match *Choricotyle*. The monogenean found on the gill filaments of *Caranx sexfasciatus* was identified as Axinidae gen. et sp. indet. (Fig. 1c) with a total length of 1–1.2 mm, a slender body and the possession of clamps, which are similar to those in the family Microcotylidae (uniform in structure). In contrast to the Microcotylidae, the clamps in the family Axinidae are considerably reduced on one side of the opisthaptor (Yamaguti 1963). Three monogenean species of the family Microcotylidae were isolated from the studied fishes. *Metamicrocotyla* sp. (Fig. 1d) was found on the gill filaments of *Mugil cephalus*, having a long and slender body shape, a small pharynx, anterior suckers with marginal denticles and the same number of clamps on both sides of the opisthaptor (Yamaguti 1963). *Microcotyle* cf. *polynemi* MacCallum, 1917 was found on the gill filaments of *Eleutheronema tetradactylum*. This species is distinguished by its long and slender body (total length = 1.2–1.6 mm), two oral suckers with rows of minute spines (diameter = 30–40 µm) and a long opisthaptoral region (Yamaguti 1963). Microcotylidae gen. et sp. indet. (Fig. 2e) was found on the gills and inner operculum of *Johnius coitor*. The species is characterised by a total length of 1–1.7 µm and a slender body, the uniform clamps on both sides of the opisthaptor and the absence of terminal anchors (Yamaguti 1963). One monogenean on the gill filaments of *Mugil cephalus* could not be assigned to a definite family. The specimens have an elongated body shape, head organs and two pairs of eyespots. The slender opisthaptor bears two pairs of anchors. Most likely these monogeneans belong to the family Dactylogyridae.

Cestoda

Mixonybelinia southwelli (Palm & Walter, 1999) of the trypanorhynch family Tentaculariidea was found in the stomach wall of *Johnius coitor*, and described in detail by Palm and Walter (1999) and Palm (2004). *Mixonybelinia southwelli* (Fig. 1e) is characterised by a compact scolex, four triangular bothria, elongated bulbs and four long and slender tentacles armed with solid falcate basal and uncinate metabasal hooks of different size.

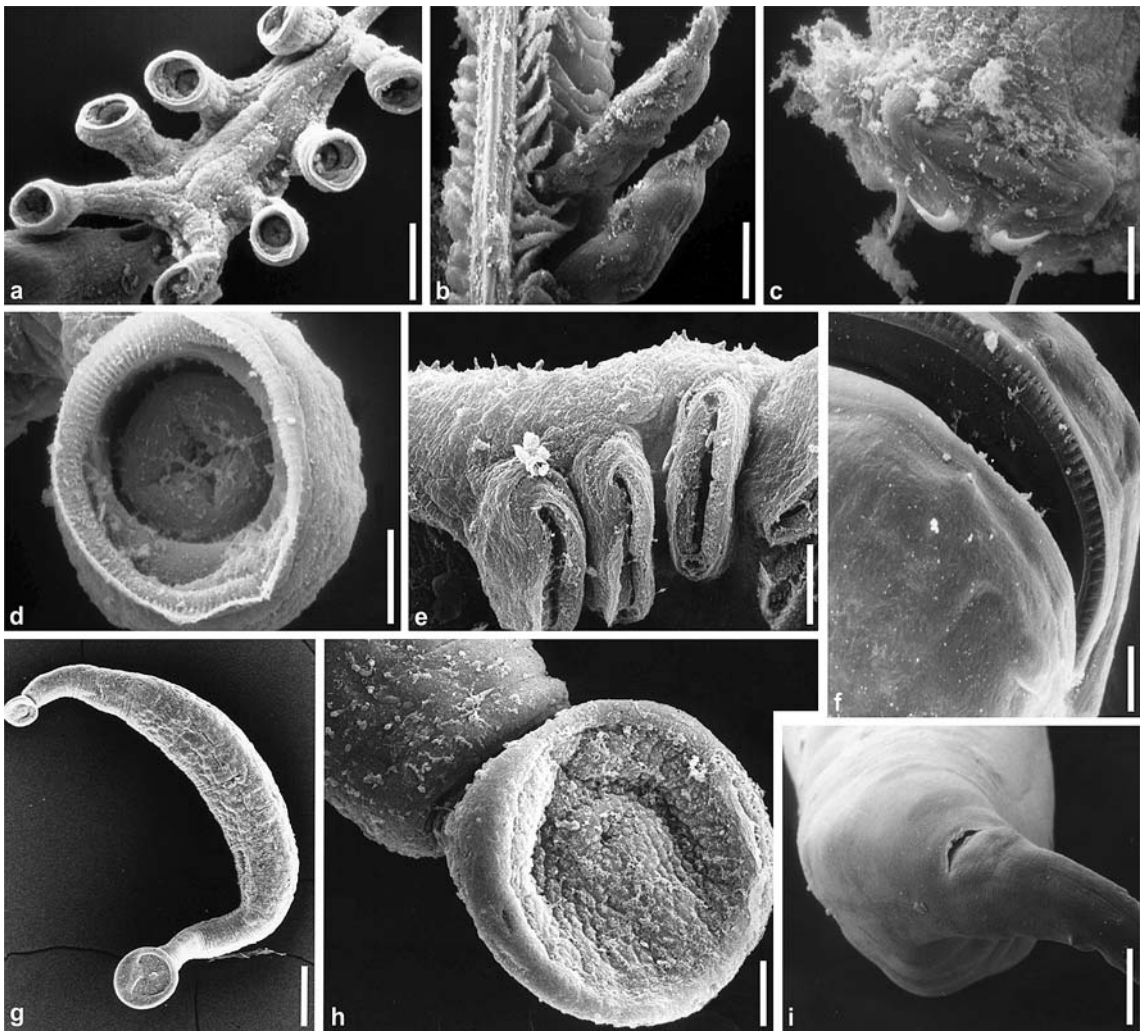

Fig. 2 Scanning electron micrographs of metazoan fish parasites from Segara Anakan, Indonesia. **a** Opisthaptor of *Choricotyle* sp. from *Johnius coitor*, *scale bar* = 55 µm. **b** *Metahaliotrema scatophagi* from *Scatophagus argus*, *scale bar* = 50 µm. **c** Opisthaptor of *M. scatophagi*, *scale bar* = 20 µm. **d** Clamps of *Choricotyle* sp., *scale bar* = 20 µm. **e** Clamps of Microcotylidae gen. et sp. indet. from *J. coitor*, *scale bar* = 25 µm. **f** Anterior end of *Cucullanus* sp. from *S. argus*, *scale bar* = 10 µm. **g** *Zeylanicobdella arugamensis* from *Siganus javus*, *scale bar* = 54 µm. **h** Posterior sucker of *Z. arugamensis*, *scale bar* = 26 µm. **i** Posterior end of *Cucullanus* sp. from *S. argus*, *scale bar* = 40 µm

Nematoda

Larval *Anisakis* sp., family Anisakidae, were isolated from the stomach wall of *Johnius coitor* and *Lutjanus johnii*. The third stage larva of *Anisakis* is characterised by the presence of a boring tooth, the excretory pore in the lip region, a large ventricle and the absence of the ventricular appendage and caecum (Anderson 2000; Moravec 1998). *Cucullanus* sp. (Fig. 2f, i), family Cucullanidae, was found in the intestine of *Scatophagus argus* and resembled the description provided by Anderson (2000). Characteristic features are a long, slender body, a thick cuticle and the dorsal-ventrally elongated oral opening surrounded by a row of numerous minute teeth (Moravec 1998). The nematodes found in the intestinal content of *Johnius coitor*, *Lutjanus johnii* and *Scatophagus argus* belong to the family Camallanidae and were identified as *Procamallanus* sp. (Fig. 1f). This genus is characterised by an orange buccal capsule and a round oral opening (Moravec 1998). In the present study, one species of the family Philometridae was found in *Johnius coitor*. According to Rasheed (1963), the identification of philometrid nematodes is based on the differences in the body shape and size, the cuticle, head and cephalic papillae, oesophagus and the tail. A single female nematode recovered from the gonads of *Johnius coitor* was identified as *Philometra* sp. (see Moravec 1998). This species had a long and slender body (length = 20 mm; width = 140 µm) and a long oesophagus (length = 210 µm). Females of the family

Capillariidae were found in the stomach content of *Scatophagus argus*. *Capillaria* sp. is a thin and long nematode (total length = 46–55 mm; width = 6–10 µm) with a smooth cuticle. The anterior end of the body is narrow and rounded, with indistinct cephalic papillae. Female *Capillaria* sp. can be easily recognised by the presence of characteristically shaped eggs. According to Anderson (2000), egg shape and size are important characters for identification. Some nematodes from the intestine of *Mugil cephalus* could not be identified to the family level. These nematodes had a length of 4–6 mm and a width of 59–70 µm and an anterior and posterior end of peculiar shape.

Acanthocephala

The acanthocephalans from the intestine of *Scatophagus argus* were identified as *Filisoma* cf. *indicum* van Cleave, 1928, family Cavisomidae. This species is distinguished by the unarmed, long and slender trunk and a long and cylindrical proboscis (Fig. 1g). The largest hooks are in the anterior to middle part of the proboscis, gradually decreasing in size anteriorly and posteriorly (Amin and Nahhas 1994).

Annelida

A single hirudinean species (family Piscicolidae) was collected from *Mugil cephalus*, *Scatophagus argus*, *Siganus javus* and *Lutjanus johnii*. The specimens were identified as *Zeylanicobdella arugamensis* De Silva, 1963 (Fig. 1h, 2g, h), with a total length = 20–90 mm and width = 45–60 µm, in the range given by De Silva (1963).

Mugil cephalus and *Scatophagus argus* in Area 2 and Area 3

Prevalence and intensity of infection

The sampling size of 35 specimens for *Mugil cephalus* and *Scatophagus agus* enables comparison of the two sampling sites inside Segara Anakan Lagoon. Some of the detected parasites occurred only in one of the investigated areas. For *M. cephalus*, *Ergasilus* sp. 2 and Nematoda indet. were found in Area 2, whereas *Metahaliotrema scatophagi*, *Zeylanicobdella arugamensis* and *Lecithobotrys* sp. occurred only in Area 3. In Area 2, *S. argus* was infected with *Procamallanus* sp., *Cucullanus* sp., *Capillaria* sp., *Caligus epidemicus* and *Pseudocaligus* sp. In contrast, the isopods *Cymothoa* sp. and Gnathiidae gen. et sp. indet. occurred only in Area 3. The prevalence and intensity of the infestation with monogenean trematodes was significantly higher in Area 2 than in Area 3 for both fish species. The prevalence of *Metahaliotrema scatophagi* on *S. argus* was 89% in Area 3 and 100% in Area 2 with mean intensities of 14.1 and 37.5, respectively. Of the dissected *M. cephalus*, 49% were infested with Dactylogyridae gen. et sp. indet. in Area 3 and 74% in Area 2. The infection with digenetic trematodes was low for both species and habitats. Haploporidae gen. et sp. indet. infected 29% of *M. cephalus* in Area 2 and 26% in Area 3 with mean intensities of 4.1 and 5.4, respectively. The prevalence of *Pseudohapladena* cf. *scatophagi* was 9% in Area 2 and 6% in Area 3 with a higher mean intensity of 10.6 in Area 2 compared to 1.0 in Area 3. Nematodes occurred only in *S. argus* from Area 2, the prevalence ranged from 14% (*Procamallanus* sp. and *Capillaria* sp.) to 29% (*Cucullanus* sp.). The only acanthocephalan isolated in this study was *Filisoma* cf. *indicum* from *S. argus*. The prevalence was higher in Area 3 with 49% compared to 29% in Area 2. The most abundant parasites for both fish species in both areas were crustaceans. Caligidae gen. et sp. indet. consisting mainly of the Chalimus stages was the only parasite taxon, which occurred in both fish species and sampling sites with higher prevalence for *S. argus* in both sampling sites 74% in Area 2 and 80% in Area 3 compared to *M. cephalus* with 23 and 26%, respectively. Most of the crustaceans isolated from *S. argus* showed a higher prevalence at both sampling sites than the species found on *M. cephalus*. The highest prevalence values for *M. cephalus* were calculated for *Ergasilus* sp. 1 in Area 2 (63%) as well as in Area 3 (40). All other crustaceans occurred with a prevalence below 40% in both habitats. With 86% *Caligus acanthopagri* was the most common crustacean infesting *S. argus* in Area 3, and with 83% *Ergasilus* sp. 3 was the most common crustacean in Area 2. *Caligus acanthopagri*, Caligidae gen. et sp. indet. and *Thysanote* sp. had also a high prevalence in Area 2 with 71, 74 and 66%, respectively. In Area 3 *Ergasilus* sp. 3, Caligidae gen. et sp. indet. and *Thysanote* sp. occurred with prevalences between 66 and 80%. The rest of the crustaceans infesting *S. argus* in both habitats showed prevalence values below 30%.

Ratio of ecto- to endoparasites

Both fish species were infected with more ectoparasites than endoparasites. In Area 2 *M. cephalus* was infected with eight ecto- and just two endoparasites. In contrast, *S. argus* harboured nine ecto- but also five endoparasite species. In Area 3, the number of ectoparasites (9) and endoparasites (2) for *M. cephalus* was almost the same as in Area 2. For *S. argus* in Area 3, the number of ectoparasites (9) was the same as in Area 2, but the number of endoparasites (2) was lower. The resulting E/E ratios calculated for *M. cephalus* were 7 and 4 in Area 2 and Area 3, respectively. In contrast the E/E ratio for *S. argus* was lower in Area 2 (1.8) compared with Area 3 (5).

Parasite diversity

With a total of 16 parasite species/taxa *S. argus* was infected with more parasites than *M. cephalus* (13). With 11 (Area 2) and 12 (Area 3) species, there was no significant difference in the number of parasite species/taxa for *M. cephalus* at both sampling sites. There was a more obvious difference for *S. argus* with 14 species/taxa in Area 2 and 11 parasites in Area 3. For *M. cephalus* the Shannon-Wiener diversity was 1.39 in Area 2 and 1.87 in Area 3. The diversity for *S. argus* in Area 2 was slightly higher (1.46) than for *M. cephalus*. With 1.5, the parasite diversity for *S. argus* in Area 3 was almost the same compared with that of Area 2, whereas *M. cephalus* had a diversity of 1.87. As ectoparasite numbers can be underestimated due to the handling procedure before fish examination (Grutter 1995), diversity values were also calculated excluding ectoparasites. The resulting values were significantly lower because the main groups contributing to the parasite fauna of both fish species were ectoparasites. In Area 2, the endoparasite diversity could only be calculated for *S. argus* and was relatively high with 1.25. In contrast, both diversity values in Area 3 were low, with 0.37 and 0.14 for *M. cephalus* and *S. argus,* respectively.

Discussion

The extraordinarily high biodiversity of the marine fauna in the Indonesian Archipelago is a result of its geographical location and geological history (Froese et al. 1996; Tomascik et al. 1997). Although, less than 10% of the Indonesian marine and brackish water fish species have yet been studied for parasites, this group of organisms appears to be highly diverse. Palm et al. (1999) estimated about three different metazoan parasites for each marine fish species, suggesting that over 9,000 marine metazoan fish parasites occur in Indonesia. Within the present study, 18 parasite species or genera are recorded for the first time from Indonesian waters or the southern coast of Java. In addition, 14 new host records could be established. Some of the recorded parasites might represent so far undescribed species.

The present study is the first large scale investigation of metazoan fish parasites in Segara Anakan Lagoon. The fauna consisted of marine, brackish water and probably also freshwater components. The parasite fauna was dominated by ectoparasites, in contrast to few endoparasites. Most ectoparasites were monoxenous species and therefore able to complete the life cycle without intermediate host. All endoparasitic digeneans, cestodes and acanthocephalans found in this study typically inhabit marine environments. In contrast, the isolated nematodes belonged to genera of marine and freshwater origin (Moravec 1987; Moravec 1998; Anderson 2000). Ergasilid copepods occur mostly in fresh- or brackish waters, and only a few species are known from marine environments (Boxshall and Halsey 2004). Copepod families such as Bomolochidae, Caligidae, Lernanthropidae, Lernaeopodidae and Penellidae as well as the isopods *Cymothoa* sp. and Gnathiidae are mainly or exclusively known as parasites of marine fishes (Kabata 1979; Boxshall and Halsey 2004; Möller and Anders 1986).

As stated by Williams et al. (1992) and Arthur (1997), the parasite species composition of distinct fish species reflects differences in the food sources, feeding preferences and habitats. Three of the studied commercially important fish species (*M. cephalus*, *S. argus* and *S. javus*) are mainly herbivorous. During the present study, 13 parasite species were recorded from *M. cephalus*. Ectoparasites especially the monogeneans and copepods occurred with high prevalence and intensities. Only three endoparasites were detected at a prevalence below 30%. *Mugil cephalus* has a wide geographical distribution and is well studied for its parasites, especially for digeneans and copepods (Overstreet 1971; Paperna and Overstreet 1981; El-Rashidy and Boxshall 1999). According to Overstreet (1971), *M. cephalus* hosts a large number of trematodes. Machida (1996) described three digeneans from Ambon, Indonesia. The fact that only two digeneans were found in the present study is very unusual and suggests that Segara Anakan Lagoon seems to be an anthropogenic highly effected habitat.

Scatophagus argus showed the highest number of parasite species (16), dominated by monogenean and crustacean ectoparasites. The parasite fauna of *S. argus* differed almost completely from that of *M. cephalus*, mainly due to different feeding habits. *S. argus* feeds on algae as well as small benthic organisms. *Zeylanicobdella arugamensis*, Ergasilus sp. 2 and Caligidae gen. et sp. indet. occurred on both fishes. In contrast, the monogenean *Metahaliotrema scatophagi* and *Ergasilus* sp. 3 seem to require a specific host. This result is not surprising, because many monogeneans and copepods show a high degree of host specificity (Santos et al. 2001; Boxshall and Halsey 2004). Only one digenean *Pseudohapladena* cf. *scatophagi* was found in *S. argus*. Yamaguti (1952) also described only *P. scatophagi* from Ujung Padang, Sulawesi, Indonesia and Arthur and Lumanlan-Mayo (1997) listed three digenean parasites from *S. argus* from the Philippines. Nematodes were only present in fish from Area 2 and occurred with prevalences below 30%. For *Procamallanus*, the intermediate host is a copepod (Anderson 2000). The development and transmission of marine Cucullanidae are still imperfectly known. Vertebrates are supposed to be

intermediate hosts (Anderson 2000). Nematodes of the genus *Capillaria* are either directly transmitted or via an invertebrate intermediate host (Anderson 2000). The infection of *S. argus* with nematodes takes place through the food chain by feeding on invertebrates or free swimming larval stages. The acanthocephalan *Filisoma* cf. *indicum* could only be isolated from *S. argus*. Four out of eight species within that genus have already been recorded as parasites of *S. argus* (Golvan 1969; Amin and Nahhas 1994). To complete the life cycle, Acanthocephala use amphipods or copepods as first intermediate hosts. However, the intermediate hosts for *Filisoma* spp. are still unknown. The parasitic isopod *Cymothoa* sp. was collected from the mouth cavity of *S. argus*, a suitable host for this isopod. Larval stages of this parasite search actively for their fish host (Bunkley-Williams and Williams 1998).

Siganus javus harboured only four different ectoparasites, a result influenced by the low number of studied fish. A wide range of parasites is known to infect fish belonging to the genus *Siganus* (e.g. Diamant et al. 1999). The carnivorous fishes *Caranx sexfasciatus*, *Lutjanus johnii*, *Eleutheronema tetradactylum* and *Johnius coitor* show an entirely different parasite fauna. However, even though the potential of being infected with heteroxenous parasites is higher for carnivorous compared to herbivorous fishes (Te 1998), ectoparasites were the predominant group. *Caranx sexfasciatus*, *E. tetradactylum* and *L. johnii* had a low level of parasite infection, while *J. coitor* harboured a rich parasite fauna. This might be explained by different food and habitat preferences, as *C. sexfasciatus*, *E. tetradactylum* and *L. johnii* live in the water column and feed near the surface, whereas *J. coitor* lives close to the bottom and feeds on benthic organisms. In general, bottom-dwelling fish have a more diverse parasite fauna (Klimpel et al. 2006). Similar to *S. javus*, the number of studied fish was low.

The ectoparasites of the five carnivorous fish species were Monogenea, Hirudinea and Crustacea. Most of the fish species were infested with Monogenea except for *L. johnii*. According to Santos et al. (2001), monogeneans are most specific, selective and adapted to specific sites, hosts and macro-environments. All recorded monogeneans were specific to their respective hosts and in most cases were found on a single fish species. The hirudinean *Zeylanicobdella arugamensis* was collected from the herbivorous fish and also occurred on *L. johnii*. According to Cruz-Lacierda et al. (2000), *Z. arugamensis* has a wide host range, including marine, brackish and freshwater fishes. All fishes were infested with copepods, and *C. sexfasciatus* harboured the isopod Gnathiidae gen. et sp. indet. (see Yuniar et al. 2007). According to Boxshall and Halsey (2004), many parasitic copepods are highly host specific, such as *Lernanthropus polynemi* and *Parapetalus hirsutus* on polynemid fishes (Pillai 1962; Ho and Lin 2001; Piasecki and Hayward 2002; Yuniar et al. 2007). However, four out of five carnivorous fish species were infested with *Caligus* spp., a genus known for low host specificity (Ho and Lin 2004).

Larval stages of the ascaridoid nematode *Anisakis* sp. were isolated from the stomach wall of *C. sexfasciatus* and *J. coitor* at low prevalence and intensity. According to Möller and Anders (1986), the adults reach maturity in marine mammals while the larval stages are known to infest various carnivorous marine fishes as second intermediate hosts. Palm et al. (2008) recorded *Anisakis typica* from *Auxis rochei rochei* and *Coryphaena hippurus,* and a closely related genotype from the carangid *Decapterus russelli* and the serranid *Epinephelus areolatus* from the southern Java and Balinese coast. Together with the present record, a total of 23 fish species are known to harbour *Anisakis* spp. or *A. typica* in Indonesia, with 36 fish species known to be infected with anisakid nematodes. The occurrence of *Anisakis* sp. larvae in Segara Anakan Lagoon indicates that the final hosts occur in the surrounding waters, dolphins in the case of *A. typica* (Palm et al. 2008). There are plenty of marine mammals known to be abundant along the Java coast (Tomascik et al. 1997). According to Palm (2004), anisakid nematodes and trypanorhynch cestodes have similar life cycle ecologies, and might follow similar pathways through the marine food web. Only few *Anisakis* sp. were found, and although trypanorhynch cestodes are one of the most abundant marine endoparasites along the southern Java coast, only a single species was isolated from within the lagoon. This raises the question why parasites that are able to occur in freshwater influenced habitats cannot enter the Segara Anakan Lagoon. Extreme conditions in terms of salinity changes, eutrophication or pollution might explain these findings. The typical first intermediate hosts for anisakid nematodes and trypanorhynch cestodes might not sustain within the lagoon due to adverse environmental conditions.

Pathogenic potential of ectoparasites in Segara Anakan Lagoon

Ectoparasites are known to be causative agents for disease outbreaks in finfish mariculture. In the present study, they were the predominant group of parasites. Different monogeneans have been reported to infect cultured marine fish in the Asia-Pacific region (compare Leong et al. 2006), among these were capsalid (e.g. *Benedenia* spp.), diplectanid (e.g. *Pseudorhabdosynochus* spp.), dactylogyrid (e.g. *Haliotrema* spp. and *Dactylogyrus* spp.) and microcotylid monogeneans (e.g. *Heterobothrium* sp., *Heteraxine* sp., *Microcotyle* spp. and *Choricotyle* sp.). Except for *Lutjanus johnii*, all dissected fish species within the present study were infected with either dactylogyrid (*Metahaliotrema scatophagi* and Dactylogyridae gen. et sp. indet.) or

microcotylid monogeneans (*Choricotyle* sp., Axinidae gen. et sp. indet., *Microcotyle* cf. *polynemi* and Microcotylidae gen. et sp. indet.) or both. A total of 10 caligid copepod species were collected from seven fish species. Three species (*Caligus acanthopagri*, *C. epidemicus* and *C. rotundigenitalis*) have already been reported to cause severe problems in Asian mariculture (Ho and Lin 2004). In Indonesia, several reported cases of increased fish mortalities are related to *Caligus* spp. infections (compare Yuasa et al. 1998; Zafran et al. 1998; Yuniar et al. 2007). Five species of ergasilid copepods were collected from fish in Segara Anakan Lagoon. Lin and Ho (1998) reported two ergasilid species in a brackish water fish culture in Taiwan. Snapper (*Lutjanus johnii*) cultured in floating net cages in Malaysia was infested with *Lernanthropus* sp. (Leong and Wong 1989), which was also found, infesting *J. coitor* and *Eleutheronema tetradactylum* in Segara Anakan Lagoon. Isopods belong to the common parasites in mariculture facilities. Koesharyani et al. (2001) reported cymothoid isopods in the nasal and gill cavity of groupers cultured in Bali. A single cymothoid species was found in the mouth cavity of *S. argus*.

Segara Anakan Lagoon is species rich in terms of monogenean and crustacean ectoparasites with fish pathogenic potential. The reason for such an accumulation of problematic species cannot be seen at present. However, these parasites should be kept in mind due to their potential as causative agents for increased fish mortalities in the case of future mariculture activities within Segara Anakan Lagoon (compare Yuniar et al. 2007).

Metazoan parasites as biological indicators in Segara Anakan Lagoon

Several metazoan fish parasites have been successfully applied as biological indicators for pollution. Sures et al. (1994) used Acanthocephala as indicators for heavy metal pollution, a taxon represented by *Filisoma* cf. *indicum* in *S. argus*. Paperna (1975) showed an increase of the monogenean *Benedenia* sp. in an oil-polluted area in the Gulf of Suez, and Khan (1990) similarly reported an increased Monogenea infestation to be associated with oil pollution. Contrasting these findings, the monogenean *Metahaliotrema scatophagi* from *S. argus* had a higher prevalence in the 'non-polluted' Area 2. This might be explained with a different susceptibility of parasite species to the toxicity of pollutants, their concentration and exposure time (Lafferty 1997; Marcogliese and Cone 1997). According to Broeg et al. (1999), the use of a single parasite species as a biological indicator is possible, if this species is common at the location to be investigated, easy to identify and reacts sensitive to environmental changes before the majority of less sensitive organisms is affected. However, none of the detected species fulfilled these requirements, possibly due to the lack of data (carnivorous fish) and identification problems to the species level for some parasite species at this tropical locality.

The huge biodiversity and weak study efforts in tropical ecosystem make environment-related research problematic. Consequently, the current potential to utilise single parasite species as fish health and environmental indicators is fairly limited. Within the present study more monoxenous (mostly ectoparasites) than heteroxenous (mostly endoparasites) parasites were found. There was an obvious lack of one typically species rich group of endoparasites, the digeneans. Even though the examined fishes are known to host a variety of digeneans (e.g. Overstreet 1971), they were almost completely absent within the present study. This is in concordance to the nearly absence of trypanorhynch cestodes, indicating the Segara Anakan as an anthropogenic highly influenced and/or polluted habitat. Diamant et al. (1999) stated that endoparasites with complex life cycles favour stable and non-polluted waters, where the full range of their required hosts is present (Diamant et al. 1999). In contrast, monoxenous parasites with simple life cycles can dominate impoverished environments. According to Dzikowski et al. (2003), the occurrence of heteroxenous parasites decreases while the prevalence of monoxenous parasites increases in polluted environments. Our data for *Scatophagus argus* can support this statement. There were more endoparasites in Area 2 compared with Area 3. The latter is supposed to be more polluted, due to an oil processing plant, fertilizer and cement factories in that area and the city of Cilacap. In contrast to *S. argus*, there was almost no difference between the two areas for *Mugil cephalus*. However, this might be related to the migratory behaviour of this species compared with the more stationary *S. argus*.

By using the number of ecto- versus endoparasites, the calculated ratio can be utilised to describe the environmental conditions within Segara Anakan Lagoon. Fishes under natural conditions accumulate the maximum possible parasite load, consisting of more endoparasites than ectoparasites. Therefore, a smaller resulting coefficient indicates more natural environmental conditions. The calculated ecto- versus endoparasite ratio (E/E ratio) for *S. argus* supports this idea, because the resulting value was lower in the non-polluted Area 2 (with 1.8) compared with the more polluted Area 3 (with 5). However, the differences in the E/E ratios for *M. cephalus* were not so obvious, it was higher in Area 2 (7) compared with that in Area 3 (4). More data from other fish species and habitats and a denser sampling is needed to utilise the E/E ratio for the description of tropical marine environmental conditions. To choose non-migratory local species as an adequate fish-parasite system host, however, is a vital process to apply this method.

As parasites with complex life cycles may provide information on the biological properties of a specific habitat within an ecosystem by synthetically recording the presence of intermediate, paratenic and definitive hosts (Cone et al. 1993; Galli et al. 2001), we calculated the parasite diversity in both habitats inside the lagoon. Given that in general heteroxenous parasites decrease in polluted areas while the prevalence of monoxenous parasites increases (Dzikowski et al. 2003), the total parasite diversity including endoparasites (mostly heteroxenous) and ectoparasites (mostly monoxenous) calculates two opposite processes. Consequently, the total diversity values for parasites of *M. cephalus* (1.39 in Area 2/1.87 in Area 3) and *S. argus* (1.46 in Area 2/1.5 in Area 3) were fairly high in both habitats, and could not clearly distinguish between the two habitats. The utilisation of the endoparasite diversity, however, provided a very different picture. The endoparasite diversity for *S. argus* was fairly high (1.25) in Area 2 in contrast to low diversity values for *M. cephalus* (0.37) and *S. argus* (0.14) in Area 3. Again, the occurrence of endoparasites decreases in polluted areas most likely by preventing the completion of the multi-host life cycle (Dzikowski et al. 2003). The omission of ectoparasites from this calculation has another advantage, because ectoparasites underlie a value underestimation that is caused by difficult handling procedures during and after the catch before fish examination (Grutter 1995).

Conclusion

The present study on marine fish parasites from Segara Anakan Lagoon demonstrates the high parasite biodiversity of this tropical brackish water environment. The species rich parasite fauna is dominated by ectoparasites with direct life cycles, indicating a highly influenced marine environment. Differences in the observed parasite fauna of the studied fish species are caused by food sources and habitats. A different metazoan parasite fauna of *Scatophagus argus* from two study areas within the lagoon is caused by the parasite's life cycle ecology and environmental conditions within Segara Anakan. Ecto- versus endoparasite ratio and endoparasite diversity were calculated to better describe and evaluate these differences. Both ecological parameters appear to be useful tools to indicate environmental conditions at a tropical brackish water locality, and might be applied also for other tropical and possibly non-topical marine ecosystems. Further studies from less influenced non-polluted waters are needed to test and further evaluate the range of variability for both parameters at a given tropical ecosystem, and to better explore the possibility to use fish parasites as biological indicators for fish and environmental health.

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