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The use of fish parasites as biological indicators of anthropogenic influences in coral-reef lagoons: A case study of Apogonidae parasites in New-Caledonia

Pierre Sasal^{a,*}, David Mouillot^b, Renaud Fichez^c, Sandrine Chifflet^c, Michel Kulbicki^d

^a *Laboratoire de Biologie et d'Ecologie Tropicale et Méditerranéenne, U.M.R. 5244, C.N.R.S. – EPHE – Université de Perpignan, Avenue Paul Alduy, 66860 Perpignan Cedex, France*

^b *UMR CNRS-UMII 5119 ECOLAG, Université Montpellier II CC 093, 34095 Montpellier Cedex 5, France*

^c *IRD/Unité de Recherche Camélia, Universidad Autónoma Metropolitana, Departamento de Hidrobiología, Av. San Rafael Atlixco N° 186, Colonia Vicentina, Mexico DF, Mexico*

^d *UR 128 CoReUS IRD-EPHE, Université de Perpignan, Avenue Paul Alduy, 66860 Perpignan Cedex, France*

Abstract

Parasite species have been widely used as fish host migration tag or as indicators of local pollution. In this paper our approach is to consider the entire parasite community as a biological indicator of the fish environmental conditions. Seven fish species belonging to the Apogonidae, *Apogon bandanensis*, *A. cookii*, *A. doderleini*, *A. norfolkensis*, *A. trimaculatus*, *Cheilodipterus quinquelineatus* and *Fowleria variegata*, were sampled on six stations in two bays (Grand-Rade and Sainte-Marie) around Nouméa (New-Caledonia). The two bays are submitted to urban wastewater inputs alone or combined with additional industrial inputs which influences decrease from the inner part to the entrance of each bay. A total of 592 fish were dissected for macro parasite examination. Parasites were grouped according to their taxonomical rank and development stage for the analysis. We found an inconsistent effect of the confinement between the two bays, revealing that the parasite community is not the same in the two bays. Moreover, the encysted metacercariae found in the pericardic cavity were found to be significant indicators of the specific anthropogenically impacted environmental conditions prevailing in the inner parts of the two bays. Other parasite taxa were found to be significant indicators of specific environmental conditions in one or two stations among the six sampled. Results on parasite specificity and biological life cycle of the parasite taxa found in sampled Apogonid were further compared with environmental parameters.

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1. Introduction

Because of their great diversity in terms of number of species but also because of their number of life history strategies, there is an increasing interest in using parasites as biological or ecological indicators of their fish host life conditions (see reviews in Sindermann, 1979; Thomas, 1990; Williams et al., 1992; Faliex, 1997; Marcogliese and Cone, 1997; Marcogliese, 2005 for examples). The parasite

specificity to their host (i.e. their ability to colonize host species) or their strict environmental requirements (water temperature or salinity variations) have been used to separate stock and to tag populations of fishes or species of commercial value (Frimeth, 1987; Moser, 1991; Overstreet, 1997). Globally, results show that parasite populations can either increase or decrease when facing environmental changes depending on both their life cycle and the nature of the change. Environmental stressors, such as wastewater or industrial pollutants can result in an increase in fish parasites due to a decrease in immunological defences and a lesser resistance to infections (Wedemeyer, 1970; Snieszko,

* Corresponding author. Tel.: +33 4 68 66 20 50; fax: +33 4 68 66 22 81.
E-mail address: sasal@univ-perp.fr (P. Sasal).

1974; Esch et al., 1975; Sindermann, 1979; Møller, 1985; Khan and Thulin, 1991; Steedman, 1991; MacKenzie et al., 1995). On the other hand, some works have reported that pollution may result in a decrease of abundance and prevalence of parasites. This has been mainly the case for complex life-cycle parasites and a local absence or a very low abundance of the intermediate host is usually presented as the most likely explanation (Overstreet and Howse, 1977; Khan and Thulin, 1991; MacKenzie et al., 1995). Because they not only reveal their own abundance but also the local presence of their intermediate and final hosts, complex life cycle parasites may also reflect threats at the ecosystem level. In his meta analysis Lafferty (1997) showed that parasites can be used to detect both heavy metals pollution and eutrophication. Results presented in our paper are more than just another example of the use of parasites as tags of their hosts as they further investigate parasites as potential bioindicators of lagoon environmental conditions, much like insect larvae have been used for years as biological indicators in freshwater systems (Hynes, 1957; Dale and Beyeler, 2001; Compin and Céréghino, 2003). One of the main criteria for using biological indicator is that the indicator should be straightforward, easily measured and sensitive to stress (Williams et al., 1992; MacKenzie et al., 1995; Dale and Beyeler, 2001; Williams and MacKenzie, 2003). Thereafter, we choose to group parasites according to higher taxon level, their life cycles or their development stages as they will reveal the same ecological difference of the studied area and this will pass up pitfall linked with the systematic of parasites.

This study is part of a large survey of the fish communities in two bays around the city of Nouméa (New-Caledonia). In terms of anthropogenic influences, Sainte-Marie's bay (SM) is essentially subject to domestic wastewater inputs whereas Grand-Rade (GR) is subject to combined domestic wastewater and industrial inputs, the latter mainly issuing from a nickel processing industrial unit situated in the inner part of the bay. In parallel to the fish community study, an intensive study was conducted on water chemistry in order to precisely define environmental conditions in the study area. The aim of the present study is to use a family of sedentary reef fishes, the Apogonidae, to test the range of responses of parasite communities to perturbed environmental conditions at a small geographical scale. In particular we wished to investigate if increasing adverse conditions would induce a monotonous response or a discontinuous distribution of the parasites within their hosts.

2. Materials and methods

2.1. Data collection

Sampling stations were located on transects stretching from the entrance (i.e. significantly influenced by lagoon water movements) to the inner part (i.e. longest water

renewal time and strongest anthropogenic influence) of each of the two studied bays (Fig. 1). The sampling zone was surrounded with a 5 m. high net enclosing a water volume of approximately 1500 m³ which was then poisoned with 0.2 ppm rotenone. All dead fishes were collected, identified, counted and weighted (individual total weight for the big fishes and estimation of the population total weight for the small ones). Each area was sampled twice (three times for the middle of the Grand-Rade) and samples were pooled in order to evaluate the fish species richness. We used the Apogonidae to test our hypothesis because of genus diversity (third richest family with 31 species behind the Gobidae and the Pomacentridae 44 and 40 species respectively), abundance (second most numerous with 9016 fish collected after the Pomacentridae 29820 fishes), relative sedentarity and short life-span allowing spatial comparisons at small scales. For the parasite study, seven apogonid species (sampled from one sample of each station), *Apogon bandanensis*, *A. cookii*, *A. doderleini*, *A. norfolkensis*, *A. trimaculatus*, *Cheilodipterus quinquelineatus* and *Fowleria variegata*, were immediately dissected, or frozen for further examination. The digestive tract and the gills were examined. Because of the complex taxonomy of the apogonids parasites, the study focused on parasite taxa and development stages. For the digeneans, we considered the encysted metacercariae (and their position in the host), the juveniles and the adults. All parasite specimens were identified to the family level.

A set of environmental variables was collected for each station. These variables related to: (i) benthic substrate and biota; (ii) water chemistry, (iii) water residence time (Table 1). At each station, substrate and living organisms were sampled along three 50 m long transects with 150 sampling points and according to the line intercept method (English et al., 1994). Water sampled at 3 m depth were immediately analysed for NH₄, conditioned in separate vials and stored in the deep freeze before analysing inorganic and organic dissolved nutrients or filtered in the lab on Whatman GF/F glass-fibre filters and stored in the deep freeze before analysis of particulate organic elements (C, N, P) and chlorophylls. Various parameters generally relating to the concept of water residence times were derived from a 3D hydrodynamic modelling approach (Jouon et al., 2006) and specifically calculated for the three sampling stations of each bay.

2.2. Data analysis

The aim of the analysis was to detect parasite taxa that could be used as biological indicators of the local conditions according to their epidemiological values within the studied host species.

Before extracting indicator species in our assemblage, we had to test the variables (bay and confinement) and their interaction. These variables are fixed factors with two treatments (Grand-Rade and Sainte-Maire) for the first one (bay) and three levels (inner part, middle,

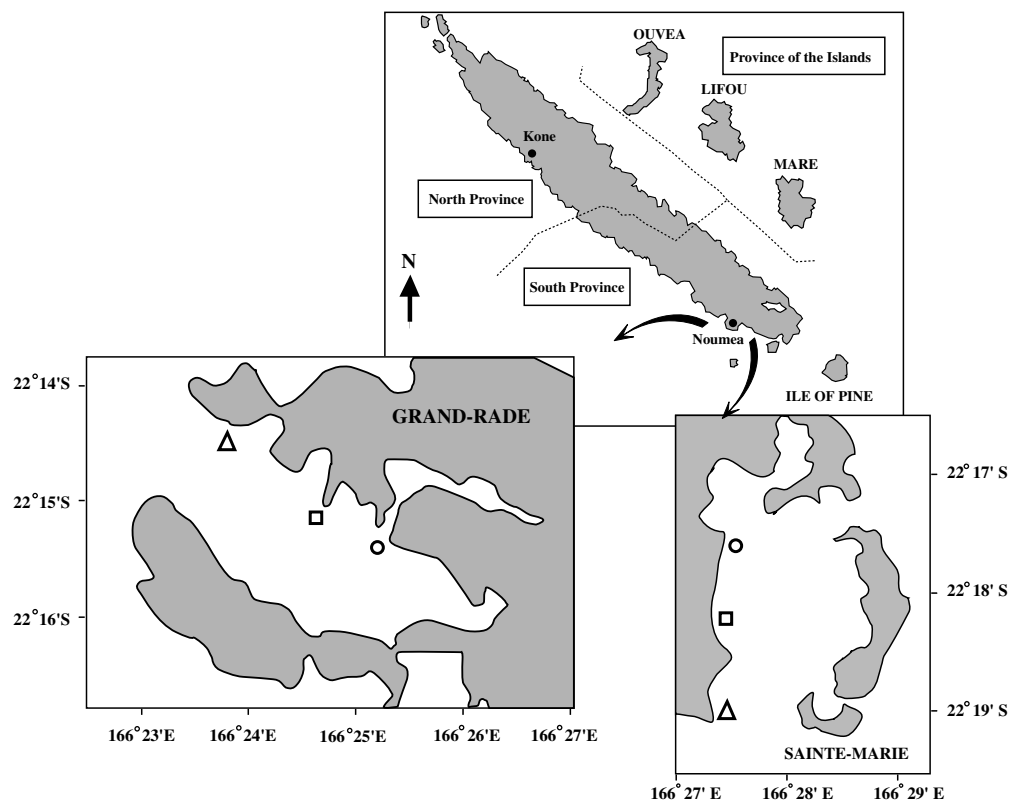


Fig. 1. Localisation of the sampled area. Δ = entrance of the bay; \square = middle of the bay and \circ = inner part of the bay.

entrance) for second one (confinement). This approach requires a multivariate analysis of variance (MANOVA) to test bay and confinement effects on parasite community in all apogonid species. In its traditional parametric form, this analysis needs assumptions like multivariate normality for species abundances and homogeneity of the covariance matrix. Although parametric MANOVA is relatively robust to violations of these assumptions, in ecological studies a non-parametric approach may be preferred (McArdle and Anderson, 2001).

To test the same kind of null hypothesis as in parametric MANOVA, i.e., assemblages from different treatments (factors) are no more different than what could be expected due to randomness, several non-parametric MANOVA methods have been proposed during the last years (Legendre and Legendre, 1998). Recently, Legendre and Gallagher (2001) introduced a new method to test multispecies responses in multifactorial ecological experiments. In this method, the species data are transformed to test the relationship with explanatory variables by using the redundancy analysis (Legendre and Gallagher, 2001).

In our study, we choose the chi-square metric transformation to test the effects of bays and confinement on the parasite assemblage. Legendre and Gallagher (2001) recommended such a transformation when increase the weight of rare species potentially indicating exceptional environmental conditions. As suggested in Legendre and Anderson (1999), two matrices of explanatory variables were used to test the effects of “bay” and “confinement” factors on the

matrix of transformed abundances (named Y). The first matrix contained dummy variables for the interaction in our two-factor orthogonal experiment (named X) and the second one contains dummy variables for the main effects (named X_c). Coding variables for experimental designs is explained in details in Legendre and Legendre (1998) or in Legendre and Anderson (1999).

The effect of bay and confinement on the parasite assemblages was first tested by redundancy analysis and permutation method under the full model implemented in the software CANOCO (1999 permutations), Y being the explained matrix, X the variable matrix and X_c the covariables matrix. When no bay or confinement effect was revealed a second test was conducted to assess the effect of environmental conditions.

2.3. Indicator species

In order to determine which species is an indicator of environmental conditions Dufrene and Legendre (1997) introduced a new and flexible asymmetrical approach based on an indicator value index (IndVal). Ecological advantages of this method have been highlighted by Legendre and Legendre (1998), McGeoch and Chown (1998) or Moullot et al. (2002). For instance this statistical approach allows the identification of an indicator species among parasite species even when those species are rather scarce, exhibit similar abundances but marked area fidelity or exhibit a homogeneous fidelity between sites but a very marked

Table 1
Values of the environmental variables associated to the sampled stations

	Sainte-Marie			Grand-Rade			Effect		
	Inner	Middle	Entrance	Inner	Middle	Entrance	Confinement	Bay	Bay × confinement
Substrate									
Fine sand	0.00	6.00	0.00	1.67	0.89	0.00	**	NS	NS
Coarse sand	0.33	44.00	1.00	15.67	10.00	24.67	***	NS	***
Gravel	0.33	6.00	3.00	9.00	5.78	10.00	NS	***	*
Debris	5.33	8.00	14.00	12.67	8.44	2.00	NS	NS	*
Small blocks	9.33	11.33	8.67	11.67	15.78	11.33	NS	***	NS
Large blocks	8.67	11.33	3.67	5.00	22.67	14.67	**	***	*
Rock	35.00	12.00	29.67	30.67	28.44	34.00	NS	NS	NS
Beach rock	5.33	0.67	2.33	0.00	7.11	3.33	NS	NS	NS
Dead coral	35.67	0.67	37.67	13.67	0.89	0.00	***	***	***
Living organism									
Seagrass	0.00	16.00	0.67	0.00	0.00	0.00	***	**	***
Brown algae	0.33	8.00	13.00	1.33	0.00	12.67	***	*	*
Soft coral	1.67	0.67	4.33	0.00	0.22	0.00	*	***	NS
Branched coral	16.00	0.67	25.00	3.67	0.00	0.00	***	***	***
Digitate Coral	24.33	2.00	17.67	6.00	0.89	10.67	***	***	***
Massive coral	15.00	2.67	2.33	0.67	9.33	14.67	NS	NS	***
Millepora coral	8.33	0.00	0.00	7.00	6.22	0.00	***	**	NS
Sponges	0.33	0.67	1.00	0.33	3.33	2.67	*	NS	NS
Diadema urchins	0.00	0.01	0.00	0.35	0.78	0.07	NS	***	NS
Holothurians	0.03	0.04	0.09	0.04	0.02	0.00	NS	*	*
Chemical and physical values									
NH ₄	0.843	0.315	0.284	0.150	0.220	0.166	**	***	**
NO ₃	0.451	0.054	0.029	0.012	0.060	0.016	NS	NS	NS
PO ₄	0.282	0.104	0.051	0.041	0.050	0.035	***	***	***
Si	3.416	2.685	2.459	4.740	6.272	3.804	*	***	**
Dissolved organical nitrogen	8.290	6.924	6.438	6.452	6.687	6.597	*	NS	***
Dissolved organical phosphorus	0.347	0.284	0.246	0.222	0.254	0.238	NS	*	*
Particular organical nitrogen	2.551	1.809	1.439	1.291	1.540	1.272	**	***	***
Particular organical phosphorus	0.320	0.278	0.247	0.200	0.225	0.177	NS	NS	NS
Particular organical carbon	18.377	12.030	7.885	7.639	8.726	6.797	**	**	*
Chlorophyll-A	1.560	0.791	0.418	0.367	0.524	0.343	**	***	**
Turbidity	2.582	1.566	1.053	0.915	1.382	0.956	NS	*	*
Water masses									
Flushing lag	21.3	20.7	19.9	43	28.4	23.4			
Local flushing time	16.2	13.1	12.5	45.4	38.5	22.2			
Export time	10.4	8.5	7.3	15.7	11.4	11.3			
Transit time	31.7	29.2	27.2	58.7	39.8	34.7			

The effect of confinement or bay or both was tested. NS = effect not significant.

- * $p < 0.05$
- ** $p < 0.01$.
- *** $p < 0.001$.

specificity or abundance. Moreover, this method can be used with data which contain a high proportion of tied zero scores, present non-normal distributions and exhibit a wide variability.

In this approach, a randomisation procedure is used to test the statistical significance of the species indicator value (Dufrene and Legendre, 1997). Based on abundance, the indicator values were calculated for each parasite taxon j and for each station k as:

$$\text{IndVal}_{kj} = 100 \times A_{kj} \times B_{kj}$$

where A_{kj} is a measure of specificity ($A_{kj} = N\text{individuals}_{kj} / N\text{individuals}_{+k}$) and B_{kj} is a measure of fidelity ($B_{kj} = N\text{fish}_{kj} / N\text{fish}_{k+}$).

In our case, $N\text{individuals}_{kj}$ is the mean abundance of parasite taxon j for the fishes examined in area k . $N\text{individuals}_{+k}$ is the sum of the mean abundance of taxon j within the various fishes of area k . $N\text{fish}_{kj}$ is the number of fishes in area k where parasite taxon j is present and $N\text{fish}_{k+}$ is the total number of fishes in that area.

The indicator value of parasite taxon j (IndVal _{j}) for an area k is the largest value of IndVal _{kj} observed over all the areas. This index is maximum (100%) when all the individuals of a parasite taxon j are observed in the fishes belonging to a single area. These values were tested using the randomisation test proposed by Dufrene and Legendre (1997). The calculations of IndVal and the associated tests were performed using the PC-ORD 4.0 software (McCune

and Mefford, 1999); a total of 10,000 iterations were performed.

This procedure was conducted first for all species pooled then separately for the host species which were present in all sampled stations.

2.4. Environmental variables

In order to identify changes in the environmental variables between stations, factorial ANOVAs were conducted on the substrate, living organisms and water column variables, the factors tested being bay and confinement.

3. Results

3.1. Environmental variables

Substrates variables (Table 1) displayed significant differences according to bay, confinement and the interaction between these two factors. In particular, dead coral and large blocks, two variables linked to the detrital status of the substrate, showed marked differences in their spatial distribution within and between bays. Variables related to biota (Table 1) were significantly influenced by bay and confinement even though a clear pattern did not appear. The sole patterns were for: (1) *Millepora* which were mainly present in the inner part of each bay plus in the middle of Grand-Rade; (2) digitate corals which were present at the entrance and inner part of each bay; (3) brown algae which were typically present at the entrances of each bay; (4) sponges which were significantly less abundant in the inner parts of the bays. Chemical and physical variables (Table 1) displayed marked inner to entrance gradients either in Sainte-Marie bay or in Grand-Rade. More specifically, turbidity and all variables related to trophic status (particulate and dissolved nutrients, chlorophyll a) significantly decreased from the inner part to the entrance of the bay. Except for dissolved Si, an indicator of terrigenous input, all nutrient values were higher in Sainte-Marie than in Grand-Rade hence revealing a higher eutrophication level in Sainte-Marie than in Grand-Rade. As resulting from model calculation, water residence time variables could not be tested statistically. However, water residence time logically increased as a function of confinement hence from the entrance to the inner part of each bay. Average residence time in Grand-Rade was consistently higher than in Sainte-Marie bay, thus indicating renewal of water masses to be slower in Grand-Rade than in Sainte-Marie bay. The combination of the high eutrophication indices together with a shorter average residence time in Sainte-Marie bay suggested a strong influence of domestic waste inputs.

3.2. Fish community

A total of 47,759 fishes, belonging to 59 families and representing 351 species, were sampled in the two bays. Species

richness was 244 (109, 146 and 155 from the inner part to the entrance of the bay) for Sainte-Marie (SM) and 290 (141, 194 and 210 from the inner part to the entrance of the bay) for Grand-Rade (GR). Only 37 fish species were found in all the sampled areas. Among these fishes, 592 apogonids belonging to seven species were dissected (Table 2).

We compared fish sizes between stations because host size may have an effect on parasite abundance or community structure. A non-parametric Kruskal–Wallis tests revealed that none of the studied species showed differences in SM but *A. cookii* ($H = 13.5$; $p = 0.001$), *A. norfolkensis* ($H = 33.78$; $p < 0.001$), *A. trimaculatus* ($H = 21.77$; $p < 0.001$) and *F. variegata* ($H = 11.83$; $p = 0.003$) were significantly larger at the entrance or in the middle than in the inner part of Grand-Rade.

F. variegata was the only species reaching highest densities in the inner parts of the two bays. For other species, lowest densities were measured in the inner part of GR (except for *A. cookii* for which densities were low in all the GR sampled sites). In Sainte-Marie bay, two species, *A. doderleini* and *C. quinquelineatus*, were totally absent in the inner part of the bay while *A. trimaculatus* was present in low densities all through the bay and that the density of *A. norfolkensis* was very low in the inner part of the bay.

3.3. Comparison of the epidemiological data between stations

The parasites found, were grouped according to higher taxon level, their development stage or their position in the fish body. Crustaceans were ergasilids, cestodes were larvae of tetraphylids, digeneans larvae (non mature worms) and adults were opoecelids and hemiurids and nematodes were unidentified larvae. Encysted metacercariae were not identified. Epidemiological values for each parasite taxon are given in Table 3.

In the first step the 592 abundance values of the seven parasite taxa were transformed by chi-square metric. The redundancy analysis (RDA) detected a significant multivariate interaction between bay and confinement effects for the parasites community of all Apogonidae species ($p < 0.01$). From an ecological point of view, it means that the effect of confinement was inconsistent between the two bays, i.e. there is a differential effect of the type of pollution on the parasite community.

We searched for indicator parasite taxa for the different levels of confinement in each bay in two analyses. We only retained parasite taxa presenting a significant potential as indicator of confinement of each bay (Figs. 2 and 3).

When considering all host species pooled (Fig. 2), the results showed that only the presence of encysted metacercariae in the pericardic cavity was significantly higher in the inner parts of the two bays. Two accompanying variables were also strongly linked to position in each bay, *Millepora* corals being more abundant and sponges less abundant in the inner parts of the bays. Nematodes were significantly more abundant in the middle of Sainte-Marie bay and the inner part of Grand-Rade. The only accompanying

Table 2
Fish density (in 10^{-3} fish/m³) and mean standard length (in mm \pm se) of the dissected fish in each station

Fish species	Sainte-Marie			Grand-Rade			Total
	Inner	Middle	Entrance	Inner	Middle	Entrance	
<i>Apogon bandanensis</i>	82.67 52 \pm 1 (15)	136.00 58 \pm 2 (15)	24.00 58 \pm 3 (20)	77.00 51 \pm 1 (21)	79.78 47 \pm 2 (19)	198.33 46 \pm 2 (15)	98.10 52 \pm 1 (105)
<i>A. cookii</i>	26.67 58 \pm 1 (15)	22.33 56 \pm 2 (15)	66.67 57 \pm 1 (17)	14.00 40 \pm 2 (12)	11.56 53 \pm 3 (15)	7.33 51 \pm 2 (15)	23.74 53 \pm 1 (89)
<i>A. doderleinii</i>	– – –	71.33 54 \pm 2 (15)	11.33 54 \pm 2 (15)	5.00 54 \pm 2 (15)	29.56 53 \pm 2 (19)	44.67 57 \pm 2 (15)	27.18 55 \pm 1 (79)
<i>A. norfolcensis</i>	9.00 73 \pm 2 (15)	105.00 73 \pm 3 (14)	40.33 77 \pm 2 (15)	7.67 66 \pm 2 (16)	52.89 46 \pm 1 (11)	12.67 82 \pm 2 (14)	39.08 70 \pm 1 (85)
<i>A. trimaculatus</i>	4.67 82 \pm 14 (6)	3.67 82 \pm 6 (11)	0.67 – –	9.00 58 \pm 5 (17)	39.30 88 \pm 3 (15)	21.00 97 \pm 4 (15)	15.08 81 \pm 3 (64)
<i>Cheilodipterus quinquelineatus</i>	– – –	65.67 58 \pm 2 (15)	2.67 63 \pm 2 (8)	42.33 62 \pm 2 (20)	67.11 65 \pm 2 (25)	69.33 63 \pm 3 (15)	43.03 63 \pm 1 (83)
<i>Fowleria variegata</i>	112.00 48 \pm 3 (15)	41.67 47 \pm 3 (15)	49.67 47 \pm 3 (16)	118.00 44 \pm 2 (11)	42.44 49 \pm 2 (15)	35.67 56 \pm 2 (15)	64.72 49 \pm 1 (87)

The number between parentheses indicates the number of fish dissected.

Table 3
Abundance (\pm SE) and range of number of parasites for each parasite taxon for the entire host community on the different station

	Sainte-Marie				Grand-Rade			
	Inner	Middle	Entrance	Total	Inner	Middle	Entrance	Total
Crustaceans	0.23 \pm 0.13 0–8	0.56 \pm 0.20 0–13	0.87 \pm 0.20 0–10	0.58 \pm 0.11	0.25 \pm 0.07 0–5	0.68 \pm 0.20 0–12	0.34 \pm 0.09 0–5	0.43 \pm 0.80
Digeneans CP	0.79 \pm 0.20 0–7	0.49 \pm 0.09 0–3	0.24 \pm 0.08 0–4	0.48 \pm 0.07	0.41 \pm 0.11 0–8	0.49 \pm 0.09 0–2	0.28 \pm 0.09 0–6	0.26 \pm 0.05
Encysted metacercariae	0.80 \pm 0.31 0–19	0.53 \pm 0.25 0–22	2.33 \pm 1.54 0–100	1.24 \pm 0.56	0.04 \pm 0.02 0–2	0.37 \pm 0.09 0–6	0.77 \pm 0.14 0–6	0.38 \pm 0.06
Digeneans larvae	0.89 \pm 0.42 0–18	0.60 \pm 0.28 0–24	0.11 \pm 0.06 0–5	0.50 \pm 0.16	0.35 \pm 0.18 0–19	0.43 \pm 0.17 0–15	0.04 \pm 0.03 0–3	0.28 \pm 0.09
Digeneans adults	0.53 \pm 0.13 0–4	0.28 \pm 0.07 0–4	0.10 \pm 0.04 0–3	0.28 \pm 0.05	0.30 \pm 0.12 0–11	0.19 \pm 0.04 0–2	0.10 \pm 0.06 0–5	0.19 \pm 0.05
Nematodes	0.12 \pm 0.06 0–2	0.43 \pm 0.09 0–4	0.06 \pm 0.03 0–2	0.22 \pm 0.04	1.12 \pm 0.28 0–16	0.69 \pm 0.26 0–20	0.14 \pm 0.05 0–3	0.66 \pm 0.14
Cestodes	0.08 \pm 0.03 0–1	0.30 \pm 0.12 0–11	0.12 \pm 0.07 0–6	0.18 \pm 0.06	0.23 \pm 0.09 0–7	0.69 \pm 0.43 0–50	0.02 \pm 0.01 0–1	0.33 \pm 0.16

variables displaying comparable spatial patterns were fine sand and beach rock, two variables associated to low habitat availability and diversity. Adult digeneans were significantly more important in the inner part of Sainte-Marie bay where trophic status variables, reached the highest levels. Millepora and digitate corals (potential intermediate hosts of digeneans) were also most abundant at this

station, whereas coarse sand and gravel were poorly represented. Crustaceans and the other encysted metacercariae were significantly more abundant at the entrance of the two bays. The entrance of Sainte-Marie bay was specifically characterized by the lowest value of local flushing time associated to the lowest concentrations in all chemicals and high abundance of brown algae, soft corals and

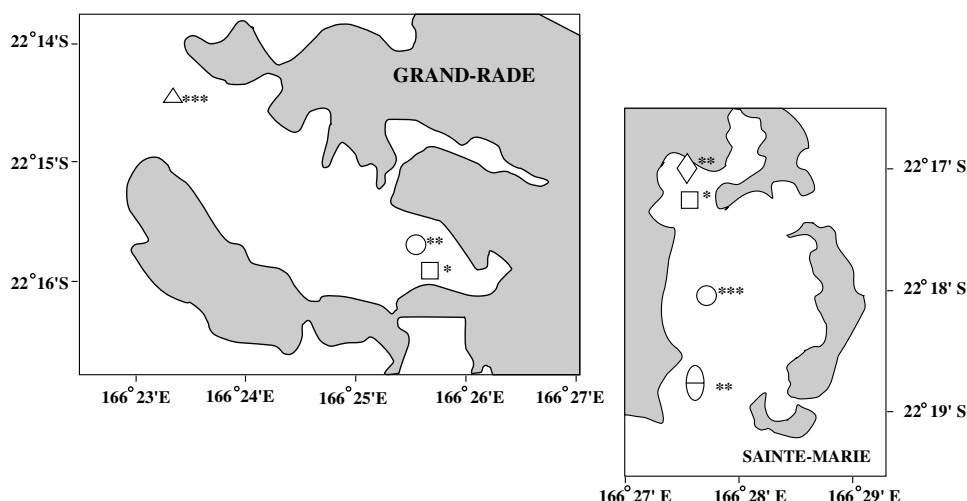


Fig. 2. Localisation of the significant parasite taxon as indicators of the considered station when all fish species were considered. Δ = encysted metacercariae; \square = metacercariae in the pericardic cavity, \circ = nematodes, \ominus = crustaceans and \diamond = adults digeneans. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

branched corals. Local flushing time was much higher at the entrance of Grand-Rade but concentrations in all chemicals were still close to those measured at the entrance of Sainte-Marie bay and brown algae, digitate and massive corals were abundant.

In order to investigate the potential use of single host species as carriers of indicator parasite species, the four host species found in all stations were analysed separately (Fig. 3). Results are consistent with those of the previous paragraph (all host species pooled). In particular, for three out of these four host species, encysted metacercariae in the pericardic cavity and adults of digeneans were also significant indicators of the inner part of Sainte-Marie bay. Similarly, for two out of the four host species, nematodes were indicators of the inner part of Grand-Rade bay and, encysted metacercariae were indicators of the entrance of that bay. However, some differences may be noticed, such as cestodes as indicators of the middle part of Sainte-Marie bay for two host species and the presence of three parasite groups (crustaceans, cestodes and adults of digeneans) as indicators of the middle part of Grand-Rade bay in *A. cookii*.

4. Discussion

The aim of this work was to establish if parasites could be used as bioindicators of the environmental conditions to which their hosts are exposed. The specificity of this work is that it relied on a high taxonomic level approach to test the efficiency of parasites as biological tags of environmental conditions whereas most previous studies focused on one parasite group or/and one host species (see Faliex, 1997; Lafferty, 1997 or Marcogliese and Cone, 1997 for reviews). That choice was justified by the need to simplify the use of parasites as biological indicators hence to bypass limitations imposed by the complex systematic of parasites only mastered by a few specialists worldwide.

The spatial distribution of biogeochemical parameters showed that there was a strong decreasing gradient from the inner part to the entrance of the bays studied especially for turbidity or trophic status variables such as nitrates, ammonia or Chlorophyll a concentrations. Our results were consistent with previous work describing Sainte-Marie's bay as essentially impacted by anthropogenic eutrophication due to waste water inputs and Grand-Rade's bay as impacted with a combination of anthropogenic eutrophication and industrial inputs (Breau, 2003).

Apogonids were selected for their high sedentarity, their abundance and their species diversity in the studied area. When considering the fish community alone, results showed that the species richness was globally 30% higher in Grand-Rade than in Sainte-Marie bay. Within each bay, host species richness increased from the inner part to the entrance. This result may be related to the joint influence of pollution and confinement. Comparison was logically conducted on the parasites community, in fish species common to both bays. However, two host fish species were absent from the inner parts of the bays (*A. doderleini* and *C. quinquelineatus*).

All the parasite groups were found in all the stations. This pattern revealed the parasites to be host-unselective as parasites strongly linked to specific host species would be much more affected by the spatial distribution of host species. This may be linked to a low specificity of the encountered parasites to their hosts as more specific parasites could be more sensitive to a local absence of their host. However, there were significant differences depending on parasite taxon both within and between stations. This is one of the prerequisite for using parasites as biological indicators (MacKenzie et al., 1995; Williams and MacKenzie, 2003).

The only ectoparasites detected on the apogonids were ergasilid crustaceans on the gills. Our results suggest that they were significant indicators for the entrance of the

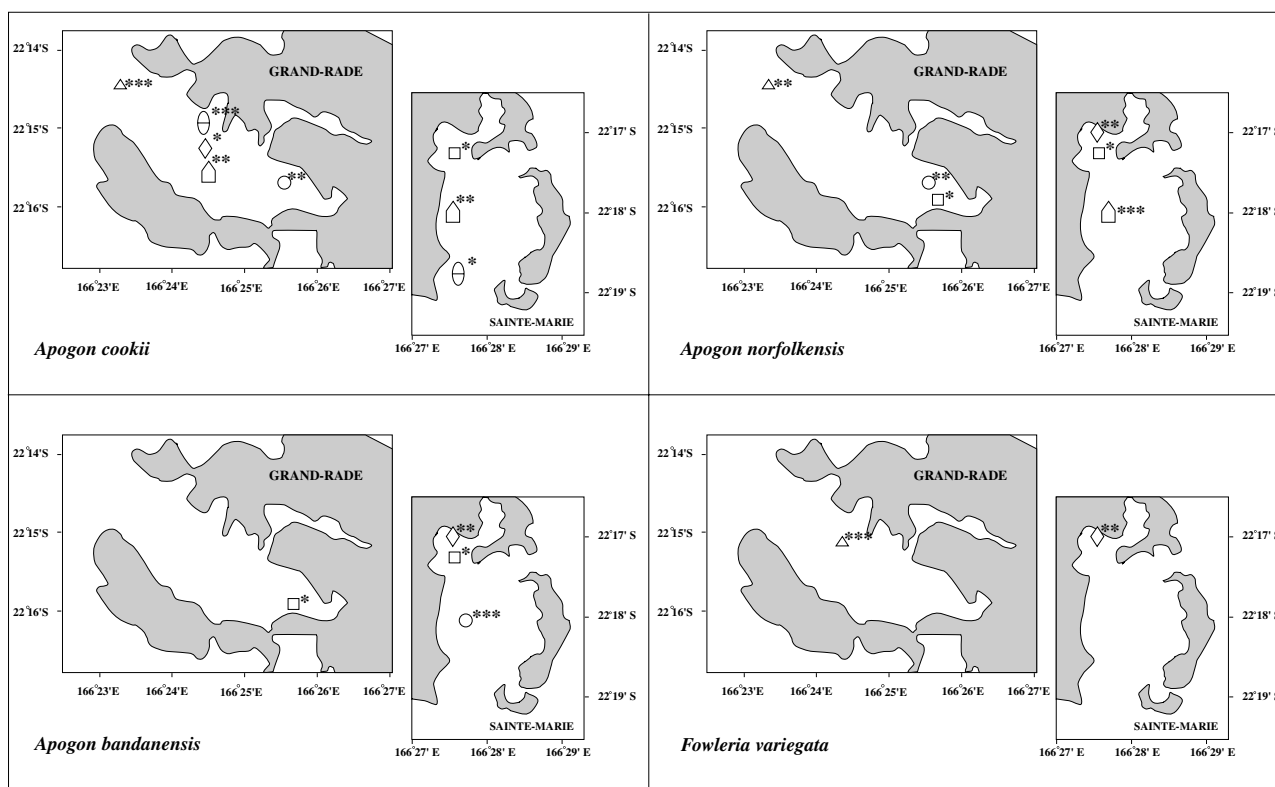


Fig. 3. Localisation of the significant parasite taxon as indicators of the considered station when fish species were considered separately. Δ = encysted metacercariae; \square = metacercariae in the pericardic cavity, \circ = nematodes, \ominus = crustaceans, \diamond = adults digeneans and \triangleleft = cestodes. * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

SM bay. Moreover, when considering host species independently, crustaceans appeared to be more abundant in the middle or at the entrance of the bays. The better water conditions in these stations, both in term of water quality and water renewal (lower residence time) may have favoured the presence of these crustaceans. On the opposite, eutrophic and polluted conditions could prevent their high abundance in the other stations. It is interesting to note that the crustaceans appeared to be significant indicators only for the entrance of SM despite global host diversity higher in GR (ergasilid crustaceans are known to have low host specificity). This may indicate a higher sensitivity to eutrophication due to waste water inputs than to industrial inputs. Because ectoparasites are in direct contact with the environment they might be more affected by differences in local conditions and as a consequence be better biological indicators. There is little information on the use of crustacean parasites as indicators of water quality, as most of the bibliography referring to ectoparasites deals with monogeneans which usually appear to be more abundant in eutrophic waters (Valtonen et al., 1987) or other polluted areas (Skinner, 1982). However, Dzikovski and collaborators (Dzikovski et al., 2003) found that crustaceans were by far more abundant in their polluted station. They explain this result by possible immune suppression of the host. It seems highly probable that the level of pollution in our sampled station is lower than the one observed in the “extreme polluted harbour” studied by these authors and that fish in our study were not immuno suppressed.

Digeneans are complex life cycle parasites and apogonids appeared to be both second intermediate hosts (harbouring encysted metacercariae) and final hosts (harbouring digenean mature or/and non mature worms). It is important to consider each development stage separately as environmental conditions may have different effects on larval or adult stages of the parasite (Poulin, 1992; Lafferty, 1997). Because of their strict specificity to the first intermediate host (generally a mollusc), digeneans are generally considered as good biological tags (Køie, 1984; MacKenzie et al., 1995). The specificity is generally less strict for second intermediate hosts and could be even weaker for the final host. Consequently the local presence of these parasites could be linked to the considered stage of the cycle. In the case of apogonids as intermediate hosts, encysted metacercariae were significantly more abundant in the middle or in the inner part of the bays. This may be linked with the presence of the mollusc species acting as the first intermediate host only in the more polluted sampled stations, but also the most sheltered in term of water hydro dynamism. This was especially true for the cysts found in the pericardial cavity. Despite no information in term of parasite identification at the specific level is yet available, a cardiac pathology is usually associated with infection of the pericardial cavity and the pumping performance of heart of infected fish may be reduced (Tort et al., 1987; Watson et al., 1992). As a consequence, we may hypothesize that at the entrance of the bays where fish

species and consequently fish predators present a higher diversity, infected fish could be preferentially predated. As for the other encysted metacercariae, their abundance varied greatly depending on stations and host species. However, they appeared to be good biological indicators of the entrance of GR. In this case, the local presence of the first intermediate host (higher abundances of brown algae could support higher densities of first intermediate hosts molluscs) seems to be the most plausible reason to explain the parasite distribution, but data on mollusc densities would be required to confirm this hypothesis. When considering apogonids as final hosts, only *C. quinquelineatus* in GR seem to harbour differences in digenean abundances. When taking into consideration the apogonid community, adults digeneans were indicators of the inner part of SM. This is consistent with an increase of digenean abundance with eutrophication as generally observed (Lafferty, 1997). The same distribution pattern was observed for the nematodes.

Finally our results showed that it may be important to take into account the distance from the source of disturbance when considering parasite communities and when using them as indicator of the environmental conditions of the hosts. The fact that the effect of the confinement was inconsistent between the two bays may be explained by at least two variables: the type of pollution and the hydrodynamics of the bays (SM has a much shorter residence time than GR). Our results also revealed that it could be important to consider more than one host species when using parasites as biological tags of the environmental conditions. Lafferty (1997) already pointed out that a community-level monitoring would be more sensitive than a single host species or single parasite species approach. To our knowledge, this study is the first to use a panel of fish hosts and their parasites to evidence differences in environmental conditions. However, when host species were considered separately, there was evidence that some species were more efficient in revealing indicator parasite species (e.g. *A. cookii*) than others (e.g. *F. variegata*). This could be interesting in long term survey or when working in protected areas in order to avoid sacrificing hosts.

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