

Use of parasites as indicators of estuarine health and the presence of important host species

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Abstract: *Trematode parasites in snail hosts have been proposed as potential bioindicators of estuarine ecosystem health. Both the definitive and snail host must be present in the system for successful transmission and the diversity and abundance of larval trematodes in snail first intermediate host populations directly reflects the diversity and abundance of definitive hosts in the ecosystem. In addition, trematodes in snail hosts are negatively influenced by environmental impacts and abundance and diversity are lower in impacted habitats. Estuarine snails proposed as suitable bioindicators include species from the Potamididae, Cerithiidae, Nassariidae, Batillariidae and Hydrobiidae. The northern hemisphere snail Nassarius obsoletus hosts nine species of parasite from eight families that infect fish, birds or reptiles. Very little is known about parasites of snails in Australia estuaries. A study of three estuarine locations in Capricornia compared the parasites of three species of Nassarius: N. dorsatus, N. olivaceus and N. pullus. Trematodes from six families that infect fish and birds were collected, including eight species from a single family of fish parasites. Fish parasites were present across all locations, but bird parasites were collected from a single location. This location is listed as an important bird habitat, both for endangered and migratory species. No reptile parasites were collected from these locations, although a reptile parasite was recently reported from N. dorsatus in Townsville. In addition to indicating ecosystem health, parasites in snail hosts may indicate the relative importance of selected locations for particular types of definitive host species; especially birds, reptiles or mammals. Using parasite information as an additional bioindicator for estuarine health may help to inform the presence of important definitive hosts.*

Introduction

The integration of 'ecoassays' (bioindicators based on ecological processes) into monitoring analyses may provide better information about the ecology of an environment than measures of ecosystem components, as such bioindicators relate to the functioning of the ecosystem (Fairweather, 1999). Parasites with complex life cycles are potential bioindicators of environmental quality and complexity because they reflect the relationship between the parasite, the relevant hosts and the environmental conditions necessary for successful transmission (Kuris and Lafferty, 1994).

Trematode parasites in snail hosts have been proposed as potential bioindicators of estuarine ecosystem health (Hudson, Dobson, and Lafferty, 2006; Huspeni, Hechinger, and Lafferty, 2005). Hudson, Dobson and Lafferty (2006) argued that a healthy ecosystem would be rich in parasite species because the diversity of parasites reflects the diversity of the free-living organisms in the system. Correlation has been shown between the abundance and diversity of definitive hosts and the abundance and diversity of snail parasites (Hechinger and Lafferty, 2005; Hoff, 1941; Smith, 2001).

A typical digenean trematode life cycle includes a definitive host and two intermediate hosts (see Figure 1), although there are life cycles with fewer or more hosts (Combes, Bartoli, and Theron, 2002; Esch, Barger, and Fellis, 2002; Sukhdeo and Sukhdeo, 2004). The first intermediate host is almost always a mollusc, usually a snail. Transmission from the definitive host to the first intermediate host is

frequently via a free-living larval miracidium; from the first to second intermediate host via a free-living cercaria; and to the definitive host by ingestion of the second intermediate host (Sukhdeo and Sukhdeo, 2004).

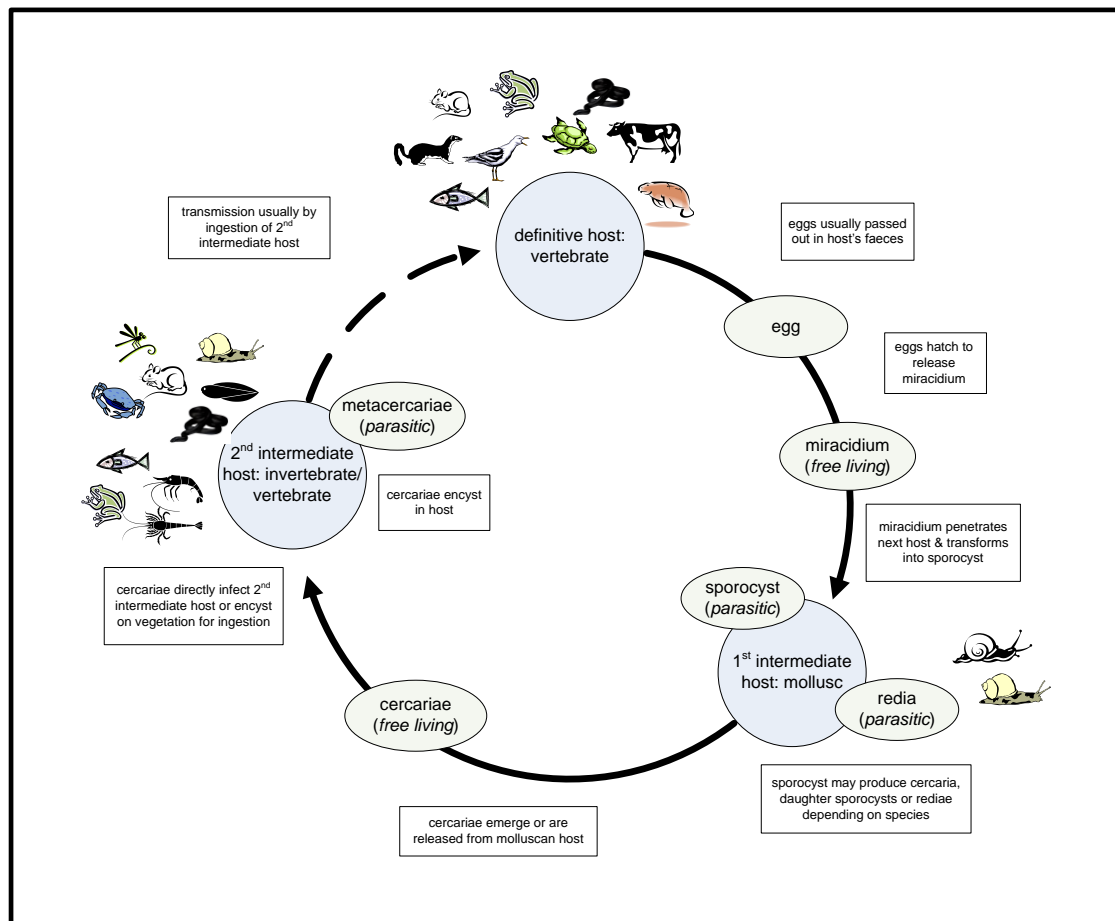


Figure 1: Stylised typical life cycle of a digenean trematode (modified from Sukhdeo and Sukhdeo, 2004, page 296).

To have successful transmission from the definitive to the snail host, both hosts must be present in the system. The infection of snails is dependent on the presence of infective stages (eggs, miracidia) from the definitive host and these infective stages must survive for transmission to occur.

The abundance of trematodes in snail hosts also reflects water quality and environmental conditions essential for survival of the free-living stages of the parasite. As the free-living infective stages (miracidia, cercariae) have direct contact with the environment, they are sensitive to toxins and are adversely affected by poor water quality. Numerous studies have demonstrated that cercariae and miracidia are negatively affected by extreme temperature, extreme pH, salinity shifts and pollutants (particularly organics and heavy metals) (Pietroock and Marcogliese, 2003; Shostak, 1993; Shostak and Esch, 1990; Thieltges and Rick, 2006).

Huspeni, Hechinger and Lafferty (2005) proposed a protocol for using snail parasites as bioindicators of ecosystem structure and trophic links in estuarine communities. They listed snails that would be suitable bioindicators and these included snails from the Potamididae, Cerithiidae, Nassariidae, Batillariidae and Hydrobiidae. They noted that nassariid snails were likely to have rich parasite fauna, despite few species having been studied apart from the northern hemisphere species *Nassarius*

obsoletus, which hosts nine species of trematodes from eight families that infect either birds, fish or reptiles (Curtis, 2002).

Huspeni, Hechinger and Lafferty (2005) also suggested that ecoassays involving snail parasites would allow inferences about the final host communities present, as most families of digenean trematodes are restricted to a specific type of definitive host (such as fish, birds, reptiles or mammals).

The nassariid snails *N. olivaceus*, *N. dorsatus* and *N. pullus* are common scavengers in the intertidal mangroves and adjacent mudflats of Capricornia. *Nassarius olivaceus* is found in the mid intertidal zone, amongst mangroves and ranging out into adjacent inundated areas, while *N. dorsatus* and *N. pullus* are predominantly found in the low intertidal zone.

A recent study in Townsville, Queensland, reported digenean parasites from the families Acanthocolpidae and Pronocephalidae in *N. dorsatus* (Terwijn, 2007). Acanthocolpidae most commonly infect fish as definitive hosts while Pronocephalidae infect reptiles. Acanthocolpidae have also been reported from *N. dorsatus* and *N. olivaceus* in Capricornia (Barnett, Smales, and Cribb, 2008).

Large areas of estuarine mangroves are situated on the central Capricornia coastal strip. Corio Bay is registered as a wetland of international importance under the Ramsar Convention on Wetlands, and is one of a few large estuarine systems retaining a relatively undisturbed catchment (WI, 1995). Corio Bay is a declared Fish Habitat Area (management level A), and has a high diversity of native fauna (DEWHA, 2008a; DPIF, 2001). The mangroves, tidal mudflats and salt-flats are important habitats for local and migratory waterbirds, and a variety of migratory bird species are listed for Corio Bay (DEH, 2004). The adjacent Iwasaki Wetlands, an extensive area of saline and freshwater wetlands linked to Corio Bay, is a significant breeding habitat for large numbers of waterbirds including magpie geese, black swans, brolgas and cotton pygmy geese, and also supports significant transitory populations of migratory birds (DEWHA, 2008b).

Ross Creek is a small tidal creek draining into Keppel Bay. The catchment for the creek includes a wide area of urban development, and water quality is reduced by pollution from stormwater runoff (DEWHA, 2008c).

The estuary at the mouth of Cawarral Creek is also a declared Fish Habitat Area level A (DPIF, 2001), and is also listed as relatively undisturbed (DEWHA, 2008c). Cawarral Creek is a popular recreational fishing area.

This study compared the digenean parasites of *N. dorsatus*, *N. olivaceus* and *N. pullus* at three estuarine locations in Capricornia: Sandy Point at Corio Bay, Ross Creek and the mouth of Cawarral Creek.

Methodology

Snails were collected by hand from mudflats at Sandy Point, Corio Bay (22°58' S, 150°46' E), Ross Creek, Yeppoon (23°8' S, 150°45' E) and the mouth of Cawarral Creek, Keppel Sands (23°19' S, 150°47' E) in Central Queensland, Australia. A total of 1766 *N. dorsatus*, 1908 *N. olivaceus* and 1614 *N. pullus* were collected between August 2004 and May 2006. Up to approximately 100 snails of each species were collected quarterly from each location. Infections were diagnosed by cercarial emergence. Snails were held in filtered seawater at room temperature (20–28°C) and examined for naturally emerged cercariae every 1–3 days for up to one month. Freshly emerged cercariae were transferred to a cavity block in a small volume of seawater and heat-killed by pouring several volumes of near boiling seawater into the dish. Cercariae were fixed in 5% formalin and 70% ethanol and examined as temporary wet mounts without flattening. Live specimens were also observed as temporary wet mounts. Cercariae were identified to family level using the key of Schell (1970) and Acanthocolpidae were further characterised to species level.

A subset of putatively uninfected *N. dorsatus* and *N. olivaceus* was dissected to confirm the absence of infection. Of 556 *N. dorsatus* (31.5% of 1766) and 399 *N. olivaceus* (20.9% of 1908) dissected, there were few undiagnosed infections of Acanthocolpidae, Echinostomatidae, Lepocreadiidae,

Microphallidae or Zoogonidae. This result suggests that the infection prevalence from emergence closely approximates the actual prevalence, except for Opecoelidae, which were under-diagnosed. Findings here are based on emergence data only.

Findings

There were 1908 *N. olivaceus*, 1766 *N. dorsatus* and 1614 *N. pullus* collected from the three locations (Table 1). Snails from a wide size and age range were collected from Sandy Point and Cawarral Creek. Fewer snails were collected from Ross Creek as this location had a small community of snails and the location was sampled for only seven collections. Most *N. olivaceus* collected from Ross Creek were large adult snails, with very few young snails seen at the location. Collection of *N. pullus* was reduced at both Ross Creek and Cawarral Creek after the first year, as no infected *N. pullus* were collected from those locations.

Table 1: Number of snails collected from each location.

Location	<i>N. olivaceus</i>	<i>N. dorsatus</i>	<i>N. pullus</i>	Total
Sandy Point	820	710	964	2494
Ross Creek	185	241	198	624
Cawarral Creek	903	815	452	2170
Totals	1908	1766	1614	5288

There were 115 infections from six digenean families (Table 2), with both the highest prevalence of infection and number of infections in *N. olivaceus* (prevalence 4.8%; n=91; 79% of infections), followed by *N. dorsatus* (1.1%; 20; 17%) and *N. pullus* (0.2%; 4; 4%). *Nassarius olivaceus* also displayed the highest diversity of infections at family level, being infected by six families compared to *N. dorsatus* (4) and *N. pullus* (1) (Table 2). Because of the higher diversity and prevalence of infection, *Nassarius olivaceus* would have more utility as a bioindicator than *N. dorsatus* or *N. pullus*. *Nassarius pullus* would be the least effective species to use as a bioindicator as it was infected with only one parasite family at a single location, Sandy Point (see also Table 3). Of the families collected, Acanthocolpidae, Lepocreadiidae, Opecoelidae and Zoogonidae usually infect fish as the definitive host, while Echinostomatidae and Microphallidae most frequently infect birds. There were no Pronocephalidae collected from these locations.

Table 2: Infections by trematode family for each snail species

Family	Definitive host	<i>N. olivaceus</i>	<i>N. dorsatus</i>	<i>N. pullus</i>	Total
Acanthocolpidae	Fish	40	14	0	54
Echinostomatidae	Birds	20	3	4	27
Lepocreadiidae	Fish	13	2	0	15
Microphallidae	Birds	8	1	0	9
Opecoelidae	Fish	9	0	0	9
Zoogonidae	Fish	1	0	0	1
Total		91	20	4	115

The highest number of infections occurred at Sandy Point (n=65; 57% of infections) (Table 3,

Figure 2), followed by Cawarral Creek (30; 26%) and then Ross Creek (20; 17%). The number of infections at Ross Creek was disproportionate as there were only 624 snails collected, compared to 2494 at Sandy Point and 2170 at Cawarral Creek. However, the majority of *N. olivaceus* collected at Ross Creek were large adult snails, while a broad range of sizes was collected at the other locations. Large snails are expected to have an increased probability of infection compared to smaller and younger snails as they have had a longer period of exposure.

Echinostomatidae and Microphallidae were collected only from Sandy Point, while Opecoelidae and a single Zoogonidae were collected only from Cawarral Creek. Acanthocolpidae had the highest number of infections (54; 47%), while there was only a single Zoogonidae infection at Cawarral Creek.

Table 3: Infections by trematode family for each location

Family	Definitive host	Sandy Point	Ross Creek	Cawarral Creek	Total
Acanthocolpidae	Fish	22	16	16	54
Echinostomatidae	Birds	27	0	0	27
Lepocreadiidae	Fish	7	4	4	15
Microphallidae	Birds	9	0	0	9
Opecoelidae	Fish	0	0	9	9
Zoogonidae	Fish	0	0	1	1
Total		65	20	30	115

The infection data for *N. olivaceus* displays the same patterns of distribution as for the total infections from all snail species (Table 4,

Figure 2). The same infecting families are reported from each location as in Table 3, and the highest number of infections was again seen at Sandy Point, with 54% of the total infections. The number of infections with Acanthocolpidae and Lepocreadiidae was slightly higher at Sandy Point than at the other locations.

Table 4: Infections by trematode family from *Nassarius olivaceus* for each location

Family	Definitive host	Sandy Point	Ross Creek	Cawarral Creek	Total
Acanthocolpidae	Fish	15	13	12	40
Echinostomatidae	Birds	20	0	0	20
Lepocreadiidae	Fish	6	3	4	13
Microphallidae	Birds	8	0	0	8
Opecoelidae	Fish	0	0	9	9
Zoogonidae	Fish	0	0	1	1
Total		49	16	26	91

Figure 2 shows the similarity in patterns of distribution of infection for both the total infections and for the *N. olivaceus* infections. The relative infection rates for each family remain similar when considering only the infections from *N. olivaceus*. This result indicates that for this study, collection of only *N. olivaceus* was sufficient to gain a comparison of the infections at the locations and that collection of *N. dorsatus* and *N. pullus* was not essential.

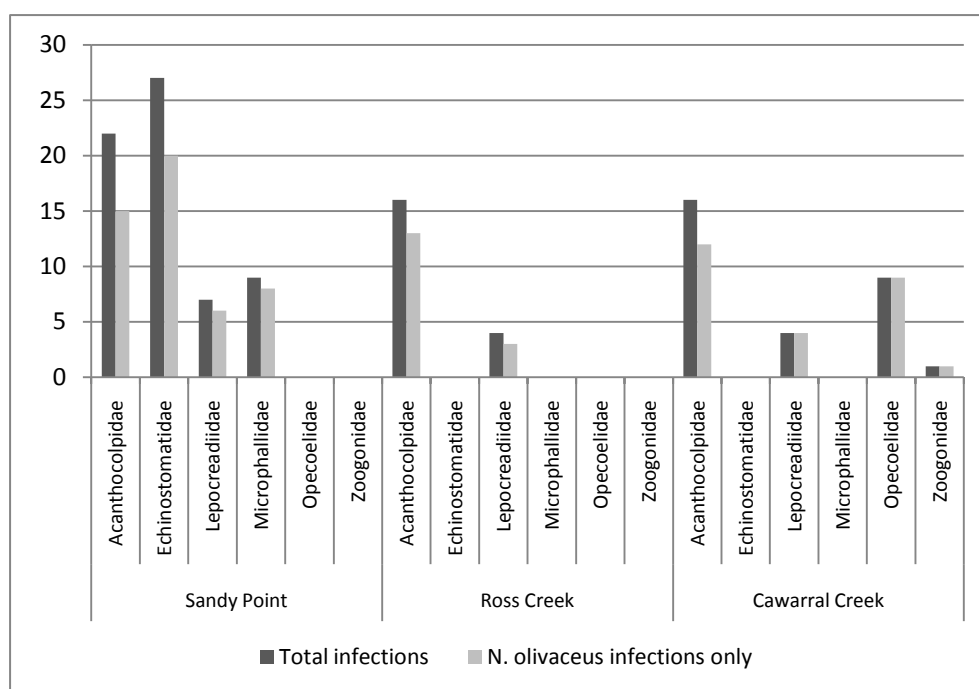


Figure 2: Families of trematodes at each location (total infections and infections from *Nassarius olivaceus*)

Acanthocolpidae were characterised to species level. There were eight species of Acanthocolpidae: four species infecting *N. olivaceus* only, three infecting *N. dorsatus* only and one species which infected both *N. olivaceus* and *N. dorsatus* (*Cercaria capricornia* VII). All five species infecting *N. olivaceus* were recorded at Ross Creek, four at Cawarral Creek and only three at Sandy Point (Table 5). Many of these infections were at very low prevalence (less than 0.1%). The presence of low prevalence infections may indicate that parasite identification to species level was not helpful in this study, as the likelihood of missing infections present in the system was increased.

Table 5: Acanthocolpidae infections from *Nassarius olivaceus* by parasite species at each location.

Species	Sandy Point	Ross Creek	Cawarral Creek	Total
<i>Cercaria capricornia</i> I	0	1	2	3
<i>Cercaria capricornia</i> II	9	7	8	24
<i>Cercaria capricornia</i> III	0	2	0	2
<i>Cercaria capricornia</i> IV	3	2	1	6
<i>Cercaria capricornia</i> VII	3	1	1	5
Total	15	13	12	40

Comparison of infections diagnosed in *N. olivaceus* at the quarterly collections shows different trends for the three locations (Figure 3). At Sandy Point, infections reduced over subsequent collections, while at Cawarral Creek infections maintained a reasonably constant number of infections after the initial collection. Infections at Ross Creek increased until the final collection when no infections were diagnosed (from only 10 snails). Although some infections were diagnosed at each collection for the majority of collections, the number of infections at each collection is low.

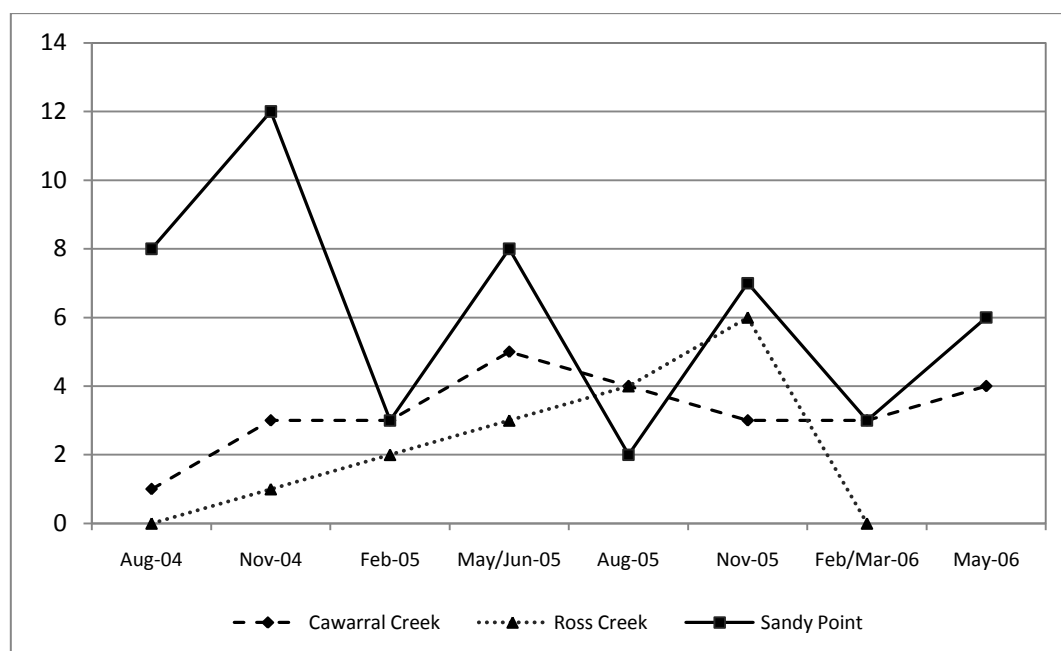


Figure 3: Total *Nassarius olivaceus* infections at each location for each collection trip.

Discussion

These results indicate that *N. olivaceus* would be more suitable than *N. dorsatus* or *N. pullus* as a bioindicator in Capricornia. *Nassarius olivaceus* was infected with up to six families (comprising at least ten species, although this number is expected to increase once all specimens have been characterised to species level). The prevalence of infection was sufficient at family level to record infections over most collections at all locations, although not all families were recorded at each collection. There were two collections at Ross Creek with no infections recorded, but the number of snails sampled at those times was extremely low (4 and 10 individuals).

Both Sandy Point and Cawarral Creek are described as relatively undisturbed estuaries on the estuarine database and both are listed as Fish Habitat areas. Both locations are considered to be more 'healthy' than Ross Creek and presented a wider variety of infecting families with four fish parasite families at Cawarral Creek and two fish and two bird parasite families at Sandy Point. Only two fish parasite families were recorded at Ross Creek, and these two families were recorded across all locations. This result supports the suggestion that more healthy locations display higher diversity of parasites at the family level.

There were also more infections at Sandy Point and Cawarral Creek than at Ross Creek, although the number of infections from Ross Creek was difficult to compare directly using the data in this study. The greatest number of infections was recorded at Sandy Point, a location which is listed as having a high diversity of native fauna.

Interestingly, if taken to species level, the number of Acanthocolpidae species collected was higher at Ross Creek than at the other two locations (all five species compared to four at Cawarral Creek and three at Sandy Point). Since many of these infections were at very low prevalence (0.05%) and collection may fail to find infections that are present, working to species level may not be helpful except for species that occur at higher prevalence. Also, this considered only one family, and once all families have been characterised this trend may vary.

Fish parasites from Acanthocolpidae and Lepocreadiidae were recorded at all three locations. Opacoelidae and a single Zoogonidae were recorded only at Cawarral Creek. This may indicate the presence of fish species at Cawarral Creek that are not found at Sandy Point or Ross Creek. The 1997

RFISH diary program, ranked recreational catch at Cawarral Creek and Causeway Lake listed 58 fish species (561,641 fish, 1.1% Queensland total) compared to Corio Bay with 18 species (58,054 fish, 0.1% Queensland total) (NLWRA, 2001a, 2001b). It is possible that Cawarral Creek has a greater diversity of fish species. For fish parasites, comparison of parasite diversity may be suitable for showing relative importance of a location for fish diversity, although lower biodiversity may also be a function of poorer ecosystem health.

Parasites which use birds as a definitive host (Echinostomatidae and Microphallidae) were recorded only from Sandy Point. Sandy Point and the adjacent Iwasaki Wetlands are noted as important habitats for numerous domestic and migratory bird species. Neither Ross Creek nor Cawarral Creek are listed as important bird habitat, and no bird parasites were collected from either location. This suggests that the likelihood of collecting bird parasites is higher at sites where birds are important members of the ecosystem.

No Pronocephalidae were collected in this study, but were reported recently from *N. dorsatus* in Townsville (Terwijn 2007). Pronocephalidae use reptiles (turtles) as the definitive host, and this may reflect the lesser importance of the Capricorn Coast as important turtle habitat. Although some turtle nests have been reported, the Capricornia Coast region is not registered as an important turtle rookery.

Conclusions

Although this study was not designed to test the suitability of snail parasites as bioindicators, the results indicate that they may provide valuable data for assessing ecosystem health. Using family level results for the locations sampled here, a general comparison of system health was obtained.

In addition to indicating ecosystem health, parasites in snail hosts may indicate the relative importance of selected locations for particular types of definitive host types; especially birds, reptiles or mammals.

Snail parasites may be used to show this in a number of ways. They can be used as a snapshot to show the relative importance of host types in a system. By studying a parasite community over time, comparisons may also indicate recovery or stress in important host types by the increase or loss of diversity within the system.

Recommendations

The incorporation of snail parasites as bioindicators of ecosystem health in monitoring programs should be further explored. If further evidence of the correlation between the presence of important host species and their parasites can be demonstrated, snail parasites may prove to be a suitable bioindicator for the presence of key host types such as mammals, reptiles and birds.

Future research

Very little is known about the parasites of marine snails. Research on parasites of other snail species would help to determine the ideal species to use as bioindicators. *Nassarius olivaceus* has been shown to have a variety of infecting parasites, although many of these infections are at low prevalence. Research of snail parasites from the Potamididae and Cerithiidae in the Capricornia and nearby regions would allow an educated choice of snail species. Any research on the parasites of marine snails will also add valuable data for both parasite taxonomy and ecology.

Comparison of the findings of this research with parasite communities of *N. olivaceus* and *N. dorsatus* in other regions would also be informative, especially if conducted in ecosystems where reptile, bird or mammal hosts were important.

In addition, a trial of parasites as bioindicators in an area where regular environmental monitoring was already in place would provide an opportunity to compare the use of parasite bioindicators to already established monitoring protocols.

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