Invasion of a South African *Anguilla mossambica* (Anguillidae) Population by the Alien Gill Worm *Pseudodactylogyrus anguillae* (Monogenea)

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Invasion of a South African *Anguilla mossambica* (Anguillidae) population by the alien gill worm *Pseudodactylogyrus anguillae* (Monogenea)

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The parasitic gill monogenean *Pseudodactylogyrus anguillae* is alien to Africa. In an investigation of 227 longfin eel, *Anguilla mossambica,* and 26 mottled eel, *Anguilla marmorata,* sampled from four river systems in the Eastern Cape, South Africa, this parasite was only present on the gills of *A. mossambica* sampled from the Great Fish River system. In the Great Fish River, it infected 73.2% of the sampled population at a mean intensity of 63.8 ± 34.3 parasites per fish. High prevalence and intensity are indicative of a well-established alien invasive parasitic species. Results showing the absence of *P. anguillae* from the co-occurring but less abundant *A. marmorata* indicate that this species may be less susceptible to *P. anguillae* infection.

Key words: invasive, monogeneans, longfin eel, mottled eel.

INTRODUCTION

South Africa has a long history of intentional fish introductions (Van Rensburg *et al.* 2011). As is commonly the case, these intentional introductions acted as pathways and the alien fish as vectors for the introduction of a variety of alien fish parasites into South Africa. A good example is the Asian tapeworm *Bothriocephalus acheilognathi* Yamaguthi, 1934 that was introduced with grass carp from Germany and has since infected a variety of native cyprinid fishes (De Moor & Bruton 1988; Schulz & Schoonbee 1999; Retief *et al.* 2007). The recent discovery of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) Gussev, 1965 on captive *Anguilla mossambica* Peters, 1952 (glass eels) that had been harvested from Eastern Cape rivers and transported to a university aquaculture facility prior to inspection, raised concerns that this parasite had invaded South African eel populations (Christison & Baker 2007). This is of concern because eels are important components of aquatic ecosystems. In the Eastern Cape for example, *Anguilla marmorata* Quoy & Gaimard, 1824 and *A. mossambica* are the only large native predatory fish species (Jubb 1964).

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Pseudodactylogyrus anguillae is a monogenean naturally inhabiting the gills of *Anguilla japonica* Temminck & Schlegel, 1846, one of the two indigenous species of river eels in East Asia (Buchmann *et al.* 1987; Hayward *et al.* 2001). This parasite is expanding its range and was first reported in Europe in 1977, when infected European eels (*Anguilla anguilla* Linnaeus, 1758) were discovered in an eel production plant in the western Soviet Union (Buchmann *et al.* 1987). The parasitic monogenean was thought to have been introduced to Europe through the importation of infected *A. japonica* from Japan and by 1990 had spread throughout Europe (Buchmann *et al.* 1987; Køie 1991; Kennedy 2007). It has also been reported in North America (Cone & Marcogliese 1995) and more recently from the small Indian Ocean Island of Réunion (Sasal *et al.* 2008).

The biology and pathology of *P. anguillae* were reviewed by Kennedy (2007). In summary, this anguillid host-specific monogenean feeds on gill epithelia and blood which, at high infection levels, can result in hyperplasia of gill tissues, fusion of secondary lamellae, and in some cases leads to hyperanaemia (Buchmann *et al.* 1987).

While infections on wild populations of their natural host *A. japonica* seldom result in infection intensities that lead to mortality (Kennedy 2007), parasite infection under aquaculture conditions is often so severe that it results in reduced aquaculture production and high mortality (Kennedy 2007).

A range of dynamic interactions between monogeneans and fish hosts are responsible for host finding, host specificity and host immunity (Buchmann & Lindenstrøm 2002). Following identification and attachment on their natural hosts, the parasites must cope with various immunological defense systems developed by the host. As a result, abundances on natural hosts are often lower than on naïve hosts that lack the ability to cope with the parasite because they exhibit weaker immunological responses to the alien parasite (Buchmann & Lindenstrøm 2002; Taraschewski 2006). For example, high prevalence and intensities of *P. anguillae* have been reported for *A. mossambica* on Réunion Island subsequent to its introduction via Europe (Sasal *et al.* 2008). The recent discovery of *P. anguillae* in elvers from the Eastern Cape (Christison & Baker 2007) therefore required further investigation because this alien parasite may impact wild populations and may result in potential losses if South Africa attempts to develop eel aquaculture.

Christison & Baker (2007) obtained their samples of *P. anguillae* from elvers that had been captured from various rivers and mixed before being held in a culture facility in Stellenbosch. The authors of this study pointed out that 'The paucity of data regarding the distribution, intensity and prevalence of *P. anguillae* infections in wild *A. mossambica* and other anguillid populations in South African combined with the general lack of other parasite data renders it difficult to make firm conclusions as to the origins and status of *P. anguillae* locally' (Christison & Baker 2007: 284), but acknowledged that the presence of this parasite was likely to be as a result of an inadvertent introduction. To address this shortfall, the current study investigated the distribution, prevalence, intensity and mean abundance of *P. anguillae* in adult *A. mossambica* and *A. marmorata* from eight sites in four river systems in close proximity to the source of the infected glass eels investigated by Christison & Baker (2007). The aim of the study was to provide a first assessment of the establishment of this alien parasite in selected rivers in the Eastern Cape, South Africa.

MATERIALS & METHODS

Eels were collected from sites in four river systems in the Eastern Cape (Fig. 1). Sites F1, F2, and F3 were on the Great Fish River system. Sample site F1 was on the lower stretches of the Great Fish River system, which is now a fast flowing, turbid, perennial system as a result of water augmentation from the Orange River inter-basin transfer scheme while the F2 sample site was located in the upper reaches of the river. Sample site F3 was at the Koonap River tributary of the Great Fish River. In the Sundays River system, which is also part of the Orange River inter-basin transfer system, sampling was conducted in the lower reaches of the mainstream (S1) and in Slagboom Dam (S2) which is a 10 ha clear-water impoundment located on the lower section of the Wit River tributary. The Nahoon River was sampled in the area below the Nahoon Dam wall (N1), where a weir creates an impoundment with a rocky substrate. Finally, two reservoirs in the Kowie River system (K1 and K2) were also investigated.

Eels were sampled during the southern hemisphere summer and autumn (February–June) of 2008, 2009 and 2010. They were collected using non-baited fyke nets that were set parallel to the river flow at suitable depths and habitats along the length of sampling areas. A maximum of 12 fyke nets were set in a single sampling event. For descriptive purposes, temperature, conductivity, oxygen (%) and pH were recorded at each sampling site using hand-held instruments. After capture, live eels were transported back to the laboratory and euthanased by decapitation and pithing. Each eel was then identified to species level, measured to the nearest mm total length (TL) and weighed to the nearest gram.

The gills were subsequently removed and inspected for monogenean parasites. All monogenean parasites were preliminarily identified as *P. anguillae* based on morphology and this identification was later confirmed by a specialist (K. Buchmann, Department of Veterinary Pathobiology, Section of Fish Diseases, University of Copenhagen, Frederiksberg, Denmark). An attempt was made to count all parasites on all gill arches. When large numbers of parasites were encountered, the total number of parasites was estimated by subsampling a random gill arch and counting all parasites on it and its associated gill filaments. Under the assumption that parasites would be similarly distributed on all gill arches, this count was then

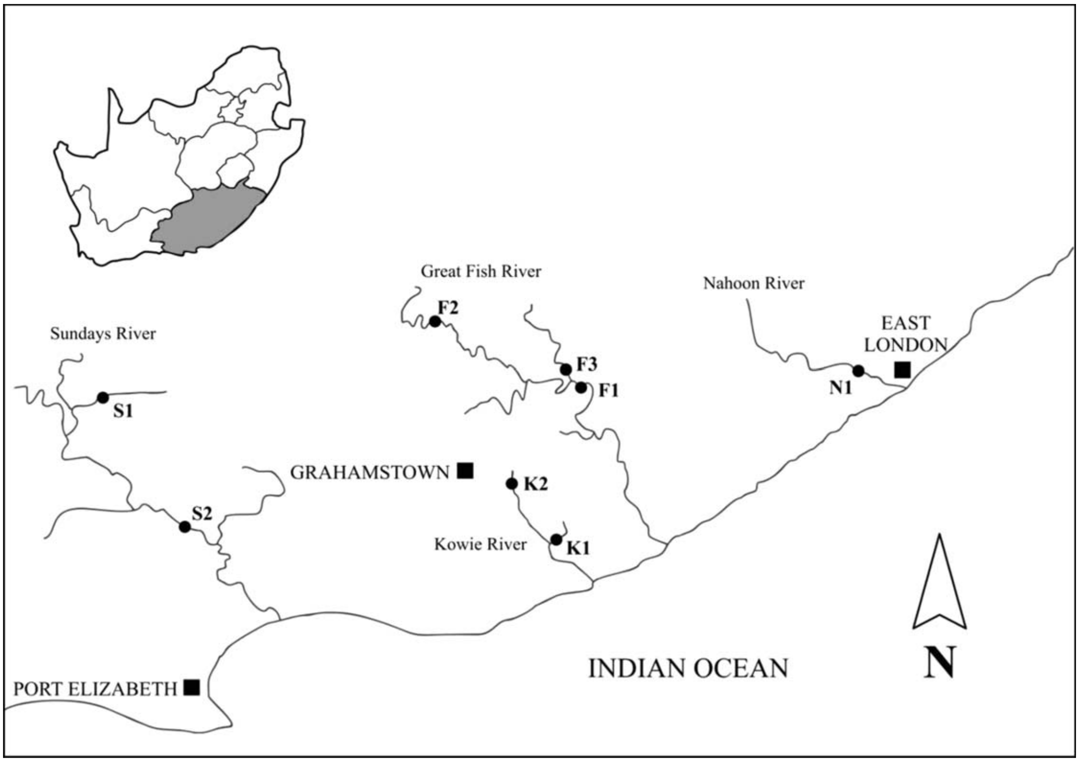

Fig. 1. Sampling area, including sampled sites (●), major cities/towns (■) and sampled river systems in the Eastern Cape, South Africa.

multiplied by the total number of gill arches to give an estimate of total parasite number. The underlying assumption of random distribution of parasites on gills was considered reasonable because Matejusova *et al.* (2003) demonstrated no preference by *P. anguillae* for specific gill arches in *A. anguilla.*

Parasite loads were expressed using the terminology recommended by Bush *et al.* (1997) in terms of prevalence (the number of hosts infected with one or more individuals of a particular parasite species divided by the number of eels examined), intensity (the number of individuals of a particular parasite species in a single infected host), mean intensity (the mean number of individuals of a particular parasite species among the infected members of a particular host species) and mean abundance (the total number of individuals of a particular parasite species in a sample of a particular host species divided by the total number of hosts of that species examined, including both infected and uninfected hosts). To test for differences between sites a chi-square test for homogeneity was performed on the parasite intensity data.

RESULTS

A summary of sampling sites, measured water quality, eels inspected and the presence or absence of *P. anguillae* is presented in Table 1. A total of 253 eels were sampled in this study; 227 were *A. mossambica* and 26 were *A. marmorata. Anguilla marmorata* was only present in three of the eight sample sites; Sundays River, Nahoon River and the Potgieter Farm (Table 1). *Anguilla mossambica* were found in all eight sample sites, with lengths ranging from 275 mm TL to 1110 mm TL. Mean ± standard deviation TL of *A. mossambica* differed between sample sites with Koonap River (F3) eels being smallest (363 ± 50 mm TL) and those from the Fairyvale Dam (K1) largest (698 ± 219 mm TL).

All eels found to be hosting *P. anguillae* were of the species *A. mossambica,* with no record of this monogenean infecting *A. marmorata* in this study. *Anguilla mossambica* individuals hosting *P. anguillae* were only sampled from the three Great Fish River sites (F1, F2 and F3). There was no evidence of infection of *A. mossambica* from sites at the Nahoon River, Sundays River or the Kowie River systems. The prevalence of *P. anguillae* in *A. mossambica* was

Table 1. *Pseudodactylogyrus anguillae* presence/absence observational data for *Anguilla mossambica* and *Anguilla marmorata* in the samples sites, supplemented with mean fish length (TL) mm and basic water quality parameters of each sample site.

Sample site	Habitat	Coordinates	Eel species	*P. anguillae*	Eel length (TL) mm	*n*	pH	Conductivity (µS)	Oxygen (%)
Potgieter Farm (F1)	River	33°02′73.7″S, 26°39′70.7″E	*A. mossambica*	Present	379 ± 64	34	7.70–8.35	868–1013	91.4–97.9
			A. marmorata	Absent	787 ± 76	6			
Middleton (F2)	River	32°57′10.9″S, 25°48′55.0″E	*A. mossambica*	Present	392 ± 25	9	9.77*	>3999*	107.6*
Koonap River (F3)	River	33°02′81.4″S, 26°38′09.2″E	*A. mossambica*	Present	363 ± 50	39	7.6–7.76	117–278	84.5–95.2
Nahoon River (N1)	River	32°54′36.4″S, 27°48′55.2″E	*A. mossambica*	Absent	403 ± 73	35	7.5–7.7	356–349	65.4–70.3
			A. marmorata	Absent	563 ± 144	2			
Slagboom Dam (S1)	Dam	33°22′42.2″S, 25°40′45.4″E	*A. mossambica*	Absent	506 ± 104	44	6.1–7.0	262–650	44.1–141.2
Sundays River (S2)	River	33°36′90.8″S, 25°40′00.8″E	*A. mossambica*	Absent	502 ± 95	40	6.52–7.9	1135–1798	80.4–190.0
			A. marmorata	Absent	821 ± 171	18			
Southwell Dam (K1)	Dam	33°30′37.4″S, 26°43′28.5″E	*A. mossambica*	Absent	654 ± 143	18	8.6–8.7	1238–1283	Not recorded
Fairyvale Dam (K2)	Dam	33°19′22.4″S, 26°35′25.9″E	*A. marmorata*	Absent	698 ± 219	8	Not recorded	532*	Not recorded

*Values without ranges were only sampled once.

very similar for all the sites where this parasite was present (68–78%). In contrast, intensity was highly variable, ranging from one parasite per fish to 1020 parasites per fish (Table 2). An intensity frequency analysis demonstrated the large between- and within-site variation of infection (Fig. 2). As a result of high within-site variation, intensity distributions did not differ significantly between sites (3 sites × 6 category contingency table; chi-square: $T_{(10)} = 7.52$, $P = 0.68$).

DISCUSSION

The current study indicates that *P. anguillae* is not widespread in the Eastern Cape, being restricted to Great Fish River sites. This preliminary data suggests that the invasion of the *P. anguillae* may be restricted to this system and its connecting tributaries. Taraschewski *et al.* (2005) noted the absence of *P. anguillae* in eels collected from the Nahoon River in 1996 and our data indicate that *P. anguillae* has not yet invaded the Nahoon River system nor the Sundays and Kowie River systems. The absence of *P. anguillae* from *A. mossambica* from the Sundays River system was surprising as this river is connected to the Great Fish River through an interbasin water transfer scheme which links the Orange River, first to the Great Fish and then, through a series of tunnels and canals, to the Sundays River (Cambray & Jubb 1977). Further investigation involving rivers not surveyed in this particular study is, however, required to be able to confidently state the invasion boundary of this non-native monogenean.

The high prevalence rates exhibited by *P. anguillae* in this study (68–78%) are indicative of well-established alien invasive species inhabiting an unnatural host. In a comparative susceptibility study done on eels using two *Pseudodactylogyrus* species, it was found that the unnatural host *A. anguilla* was substantially more susceptible to *Pseudodactylogyrus* infections than its natural host, *A. japonica* (Fang *et al.* 2008). The reasons behind increased susceptibility of fish to the alien parasite are a result of complex interactions that involve evolutionary mechanisms that include innate and acquired host immunity, physiological factors such as chemoattractive substances and parasite biology (Fang *et al.* 2008). Fang *et al.* (2008) documented a mean intensity of 4.2 *P. anguillae* per fish on the natural host, *A. japonica*, while in the non-native host *A. mossambica*, Sasal *et al.* (2009) documented intensities of between 1 and 30 *P. anguillae* and *P. bini* Kiknuchi, 1929. On the novel

Table 2. Sample size (*n*), parasite prevalence (%), mean abundance, intensity range and mean parasite intensity observed on *Anguilla mossambica* from the three sample sites where *Pseudodactylogyrus anguillae* was present.

Sample site	*n*	Prevalence (%)	Intensity			Abundance
			Min	Max	Mean	
Potgieter Farm (F1)	34	67.6	1	1020	90.6	60.3
Middleton (F2)	9	77.8	4	125	25.1	19.6
Koonap River (F3)	39	76.9	1	510	75.6	58.1

host, *A. mossambica,* from the Great Fish River, mean intensities were considerably higher, ranging between 25 and 91 parasites per infected fish between the sites (Table 2). The high intensity of pseudodactylogyrids in the wild *A. mossambica* population supports Christison & Baker's (2007) hypothesis that *P. anguillae* is alien to South Africa.

The present study sampled 26 *A. marmorata,* revealing that none of these individuals hosted *P. anguillae.* However, only six *A. marmorata* were taken from the Great Fish River system, none of which were found to be infected by *P. anguillae.* Sasal *et al.* (2008) found that *Pseudodactylogyrus* species commonly occurred on *A. mossambica* (61.0%), but were rarely found on *A. marmorata* (2.5%), and were absent on *Anguilla bicolor* McClelland, 1844, from eels of Réunion Island. Furthermore, a parasite–host list compiled by Nagasawa *et al.* (2007) revealed that *A. marmorata* generally exhibit low parasite richness. The study lists the number of parasites recorded from eel species occurring in Japan. The native *A. japonica* hosted 43 parasite species, the introduced *A. anguilla* hosted 10 parasite species and the native *A. marmorata* only hosted a single parasite species (Nagasawa *et al.* 2007). This substantiates the finding that *A. mossambica* is more susceptible to infection by *P. anguillae* than *A. marmorata.* Further investigation into the prevalence of *P. anguillae* in the *A. marmorata* populations of the Eastern Cape is, however, necessary.

Physiological tolerances are a defining factor when demarcating suitable habitats of a species, and therefore an important tool used to predict the spread of an alien species. *Pseudodactylogyrus anguillae* is primarily a freshwater parasite, but it is argued to be halotolerant and is often found in brackish environments, and able to reproduce at salinities of up to 20 g/kg (Køie 1991). A recent study describing the salinity dependence of parasite infections in *A. anguilla* found that *P. anguillae* had a higher prevalence in freshwater and a

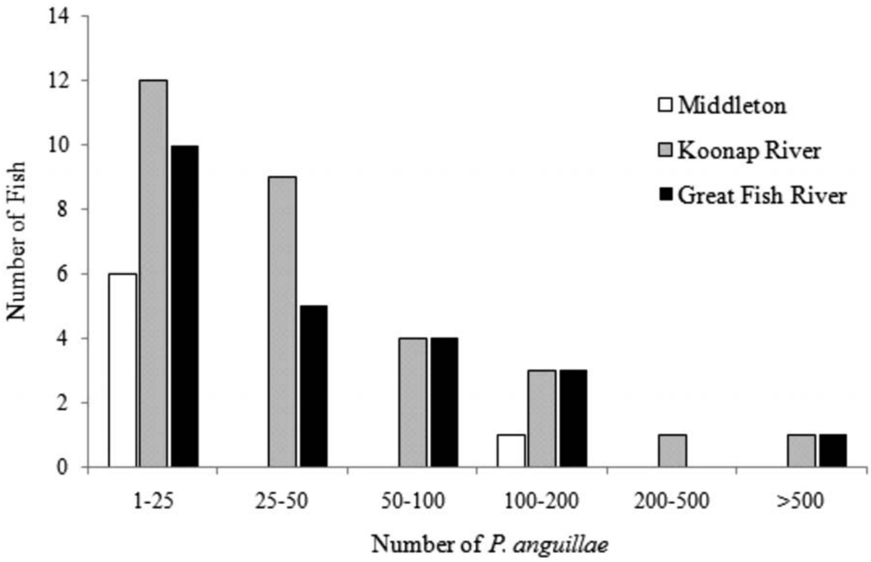

Fig. 2. *Pseudodactylogyrus anguillae* intensity frequency analysis on the host species *Anguilla mossambica* from the three sample sites on the Great Fish River system.

moderate to high prevalence in brackish water, but was not found to infect eels caught at sea (Jakob *et al.* 2009), suggesting that the species is intolerant of a marine habitat. It is therefore unlikely that the parasite was introduced into South Africa via the marine pathways proposed by Christison & Baker (2007).

As the range of *P. anguillae* appears to be restricted, the most plausible pathway for introduction is a result of the freshwater introduction of infected eels. Unfortunately there is a lack of official documentation of eel introductions but the possibility of undocumented eel importations for experimental culture purposes cannot be ruled out.

While the introduction of *P. anguillae* appears to have been restricted to the Great Fish River system and/or its tributaries, infected eels have already been transported through the country resulting in Christison & Baker's (2007) discovery of the parasite in an experimental facility in Stellenbosch.

The understanding of parasites and their interactions with fish populations is often hindered by the lack of long-term data. The effect that the introduced *P. anguillae* will have on the intricate and fragile life history of the indigenous *A. mossambica* is not yet fully understood. Evidence suggests that the impacts of parasites on host organisms will be particularly severe when the parasite is alien and infects a novel host that has not co-evolved with it (Taraschewski 2006).

Although it seems unlikely that the parasite will spread to other river systems through natural means, the threat of anthropogenic-facilitated translocation is still high. This is particularly relevant if there are future attempts to develop an eel aquaculture industry in South Africa. As the current study has demonstrated that *A. mossambica* is vulnerable to *P. anguillae* infections, it is likely that the presence of *P. anguillae* in South Africa will lead to increased risk of mortality and associated production losses under aquaculture conditions. Therefore a precautionary approach needs to be applied and steps must be taken to mitigate the translocation of eels from the Great Fish River system to other water bodies for aquaculture or any other purposes. The introduction of non-indigenous eel species to South African water systems should also be prohibited to prevent additional alien parasite introductions such as *P. bini*; a natural parasite of *A. japonica* which has also exhibited a high pathogenic potential in novel hosts (Fang *et al.* 2008).

Finally, the infection of a novel host in South African rivers presents opportunities for future research. Such research should include not only research on the health impact of infections on the host organism but also on the biology, seasonal abundance, population growth and microhabitat preferences of the parasite.

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