Working towards optimising oral praziquantel for treating monogenean ectoparasites of captive fishes

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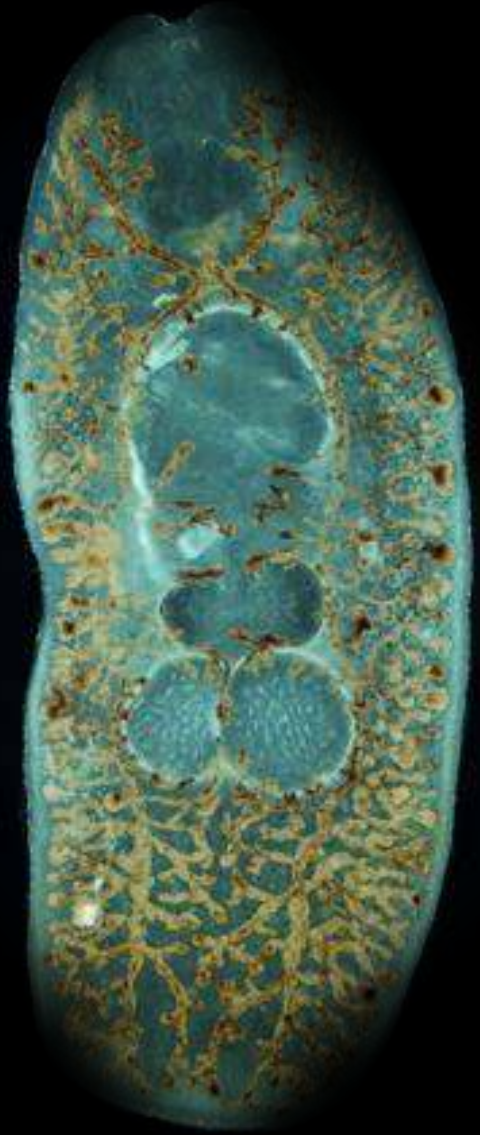
Having a deeper look…

Two Oceans AQUARIUM CAPE TOWN SOUTH AFRICA

MONTEREY BAY AQUARIUM

Introduction: Monogenea

- Parasitic flatworms
- Parasites of teleosts, chondrichthyes, certain aquatic reptiles and amphibians (and 1 mammal host: Hippo)
- Between 4000-5000 described species (Whittington 2005)
- Extremely specialised, two subclasses

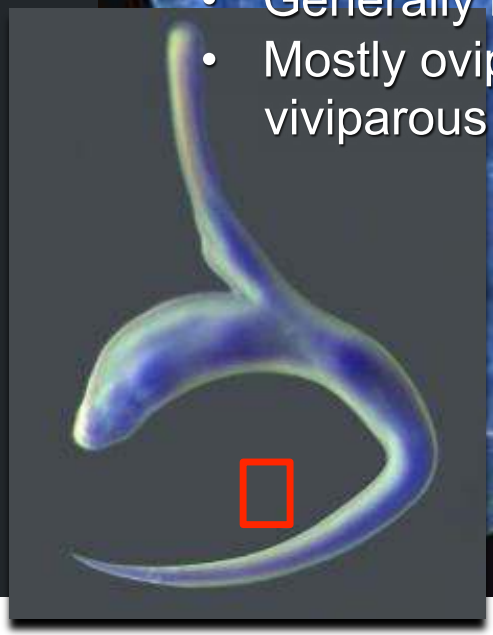


Polyopisthocotylea

- Haptor: multiple attachment units, symmetrical or asymmetrical
- Attachment structures: clamps/suckers with “skeletal” sclerites
- Gills, branchial chamber, buccal cavity
- Sanguinivorous
- Generally sedentary
- Oviparous

Monopisthocotylea

- Haptor: single unit, symmetrical
- Hooks/modified armature
- Skin, gills, branchial chamber, buccal cavity fins, nasal fossae, eyes, urogenital system, body cavity, digestive tract, heart muscle and blood vessels
- Graze on epithelial cells, mucus
- Generally highly motile
- Mostly oviparous, some viviparous



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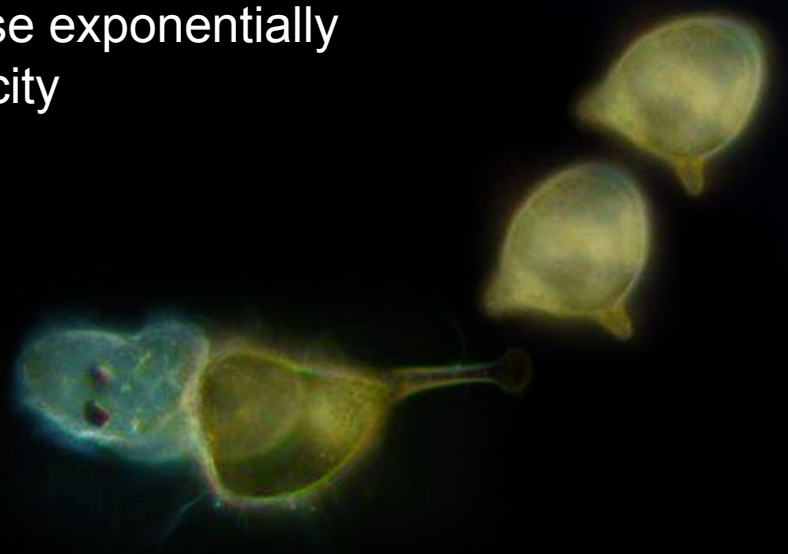
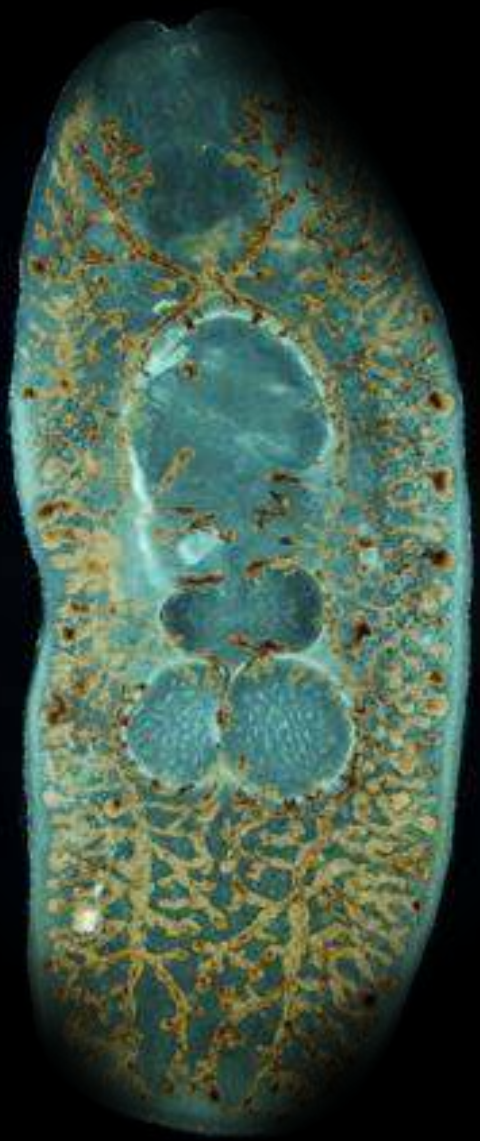
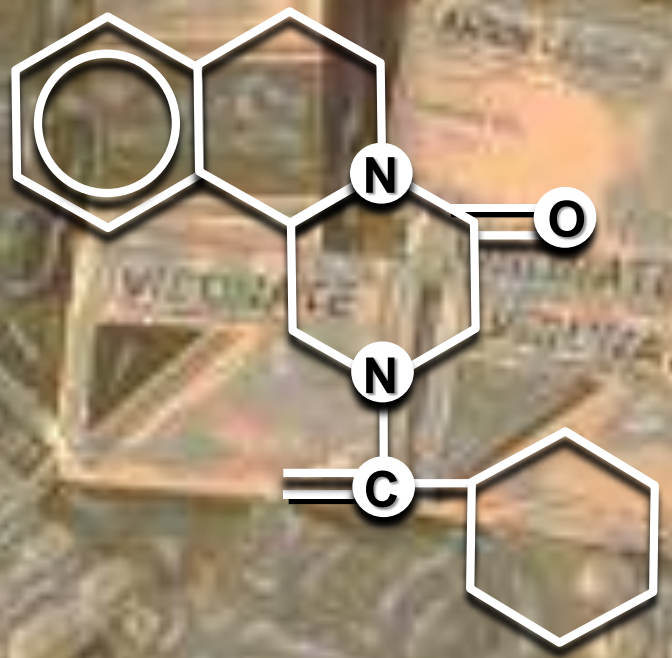
- Between 4000-5000 described species (Whittington 2005)
- Extremely specialised, two subclasses
- Direct life-cycle – reproduce successfully in captivity (Monogenea = one generation)
- Potential to increase exponentially
- Stenoxenic specificity
- Cause disease

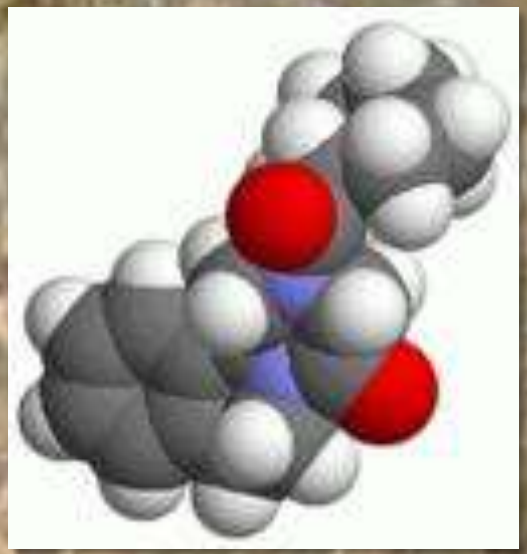




Introduction: Praziquantel

- Anthelmintic developed by Bayer, Germany in 1970s
- Schistosomiasis and cestode infections of human and veterinary importance
- Has been used to treat fishes in public aquaria with varying degrees of success
- Its scientific development in public aquaria has stagnated in comparison to aquaculture





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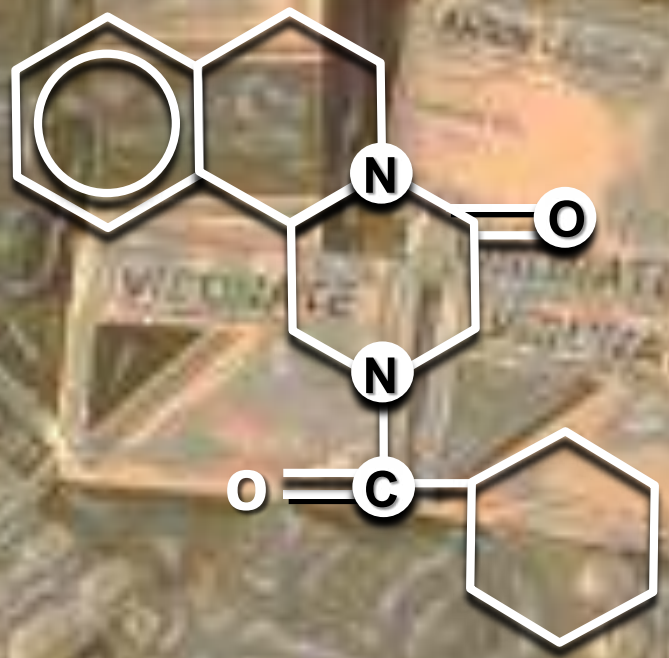
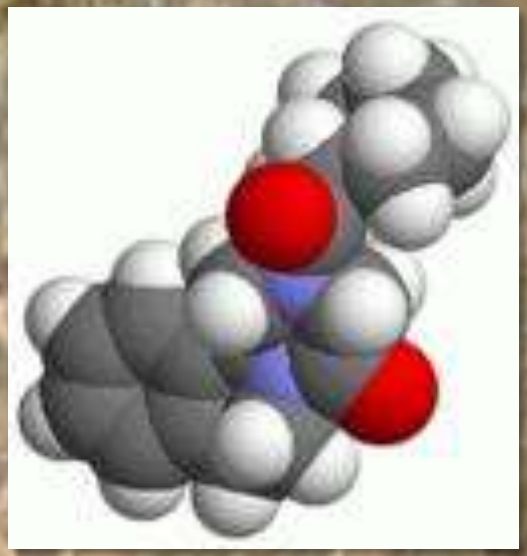
Intr duction: Praziquantel

- Anthelmintic developed by Bayer, Germany in 1970s
- Schistosomiasis and cestode infections of human and veterinary importance
- Has been used to treat fishes in public aquaria with varying degrees of success
- Its scientific development in public aquaria has stagnated in comparison to aquaculture
- Reason is three-fold:
  a) Praziquantel is not registered for use in fish in many countries
  b) Registered products are often registered for specific finfish species based on efficacy and tissue retention times
  c) Research in aquaculture is fuelled by necessity for profit
- Empirical data from research into the drug's efficacy in many fish species is lacking
- Public aquaria rely nearly exclusively on anecdotal information obtained from shared experiences





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VERSITA

Heterocotyle tokoloshei sp. nov. (Monogenea, Monocotylidae) from the gills of *Dasyatis brevicaudata* (Dasyatidae) kept in captivity at Two Oceans Aquarium, Cape Town, South Africa: Description and notes on treatment

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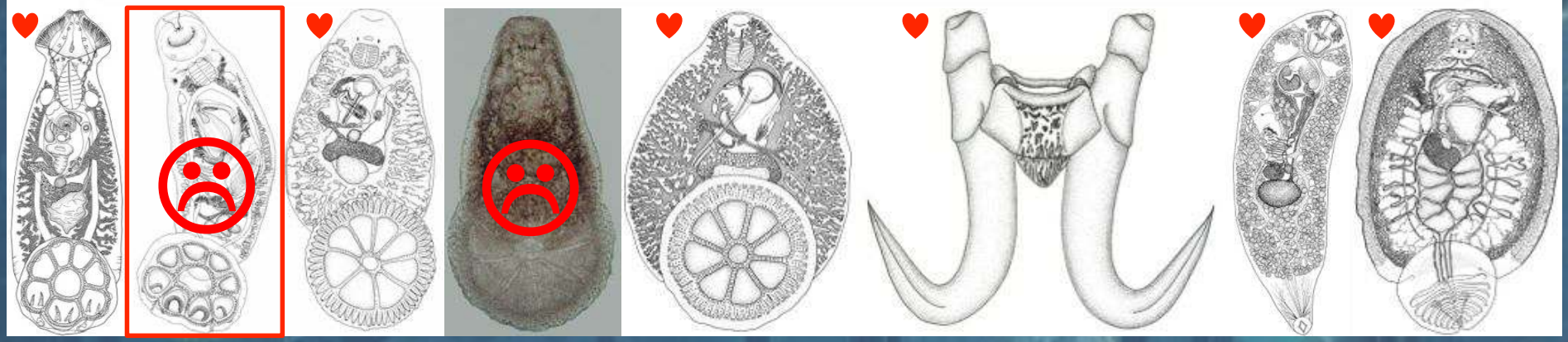


This species made us challenge the thinking behind our approach to treatment

- *Dasyatis brevicaudata*
- Clinical signs: laboured breathing, refusing to feed, lethargy
- Removed to quarantine, checked for the presence of monogeneans
- Confirmed – presence of tetrahedral eggs
- Treated with 20mg/L for 12hrs (Chisholm and Whittington 2002)
- 3084 worms + eggs recovered
- 24hrs later eggs still recovered
- Anaesthetised, intubated with 150mg/kg (Hirazawa *et al*. 2004, Williams *et al*. 2007).
- 12hrs post-treatment, 392 000 worms + eggs recovered
- 24hrs post-treatment, 3383 worms, NO eggs recovered
- 48hrs – 10d post-treatment, NO worms or eggs recovered

Why?

- Monogeneans must be in direct contact with prazi concentration for it to be successful
- Monogeneans can react by withdrawing between tissue (Chisholm and Whittington 2002) and/or stereotypic reaction of gill tissue provides significant protection from exposure to drug:
    - Inflammatory response
    - Cell proliferation, epithelium, mucus cells
    - Fusion of secondary, primary lamellae, entire arch*
- Why 150mg/kg?
    - Poynton *et al*. (1997) – oral prazi 3, 8, 15, 19mg/kg ineffective
    - Kim *et al*.(1998) – oral prazi (intubated) 200mg/kg successful
    - Janse and Borgsteede (2003) – oral prazi 10-40mg/kg ineffective
    - Hirazawa *et al*.(2004) – oral prazi 40mg/kg successful, 150mg/kg successful but palatability issues
    - Williams *et a*l. (2007) – oral prazi 100, 150mg/kg palatability issues, but success with intubation
- It has previously been considered that ineffectiveness related to monogenean feeding biology (sub-class): blood feeders more likely to be affected…

?

Proposal: Success of oral praziquantel is more a function of the relationship between **bioavailability** and **susceptibility**

- These lesser-known interests we took forward in an attempt to gain better understanding on how best to treat monogeneans in public aquaria

- H_0: Uptake and delivery is the same in teleosts and elasmobranchs
  - If true then bioavailability could be comparable
  - Teleost parasite treatment model could be used to generate statistical significance, extrapolated to elasmobranchs

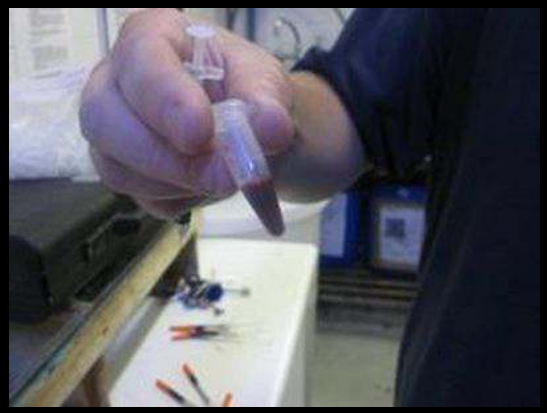
- To test bioavailability (initially in plasma) – employed HPLC (Kim *et al*. 2001a)

Argyrosomus japonicus

Photo: Phil Heemstra

Rhinobatos annulatus

Methods

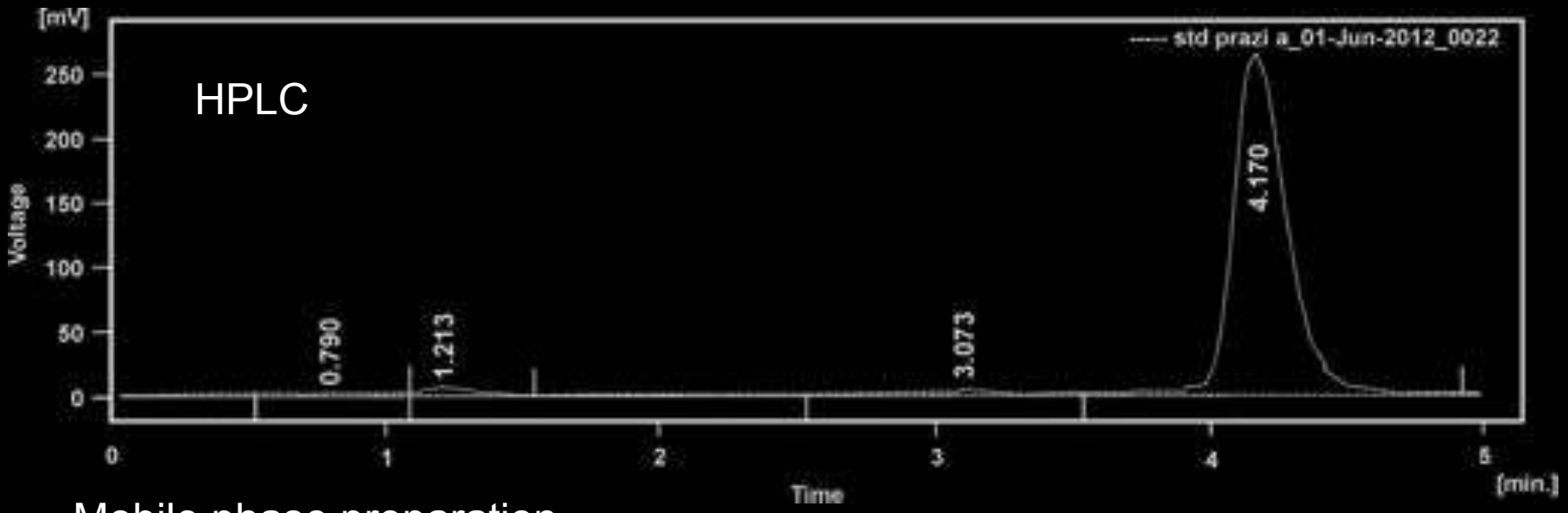


All photos courtesy of Tesria Greenstone

- n = 81 *A. japonicus* into 3 tanks of 27 fish representing 3 replicates
- Anaesthetised (2-phenoxyethanol), weighed and given 150mg/kg (100% prazi) via autopipette
- 3 fish randomly selected from each tank at 8 time intervals: 0, 4, 8, 12, 24, 48, 72 and 96hrs for blood extraction
- Sodium heparin-charged syringes and needles were used to prevent clotting
- Approximately 500µl extracted from each fish
- Centrifuged for 10min to separate
- Plasma supernatant removed and immediately frozen

- Restricted in numbers of *R. annulatus* – n =18
- Tagged with subcutaneous pit tags (inserted into dorsal saddle) to facilitate repeated measures
- All *R. annulatus* were pre-weighed
- Anaesthetised, force-fed 100% prazi compressed into tablets
- Followed the same blood extraction and processing protocol

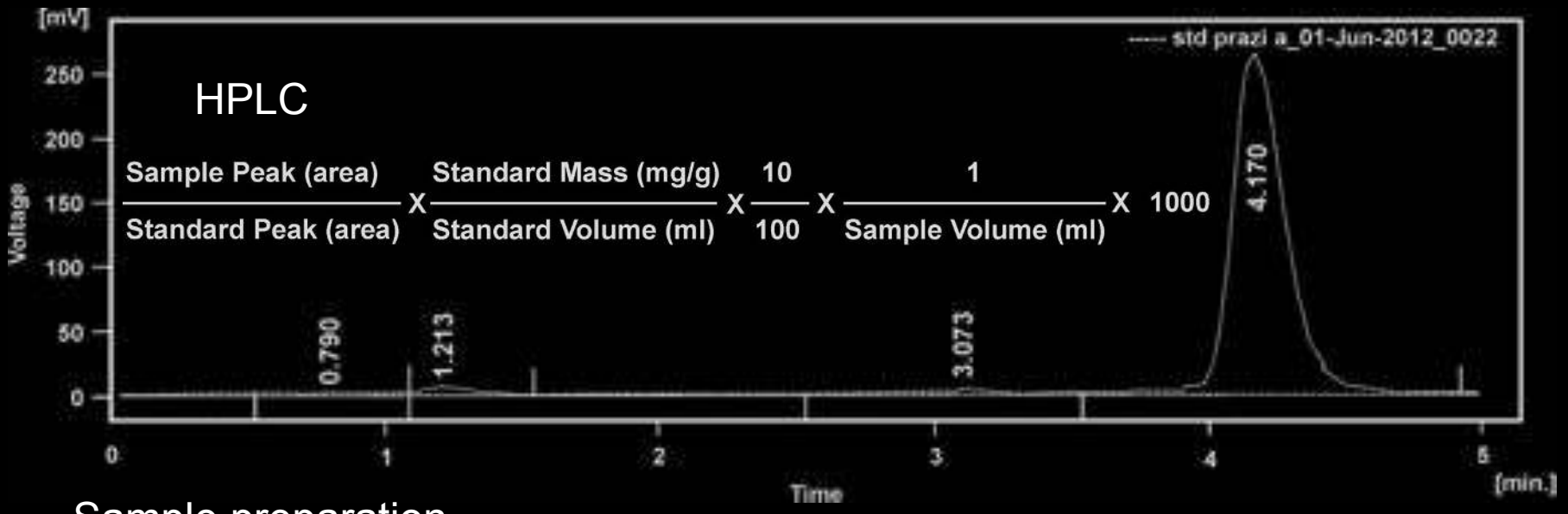
Methods



Mobile phase preparation

- Buffer: 3.4g KH_2PO_4 (Monopotassium phosphate) added to 450ml H_20
- pH adjusted to 3.0 with phosphoric acid
- 500ml buffer added to 500ml acetonitrile

Methods



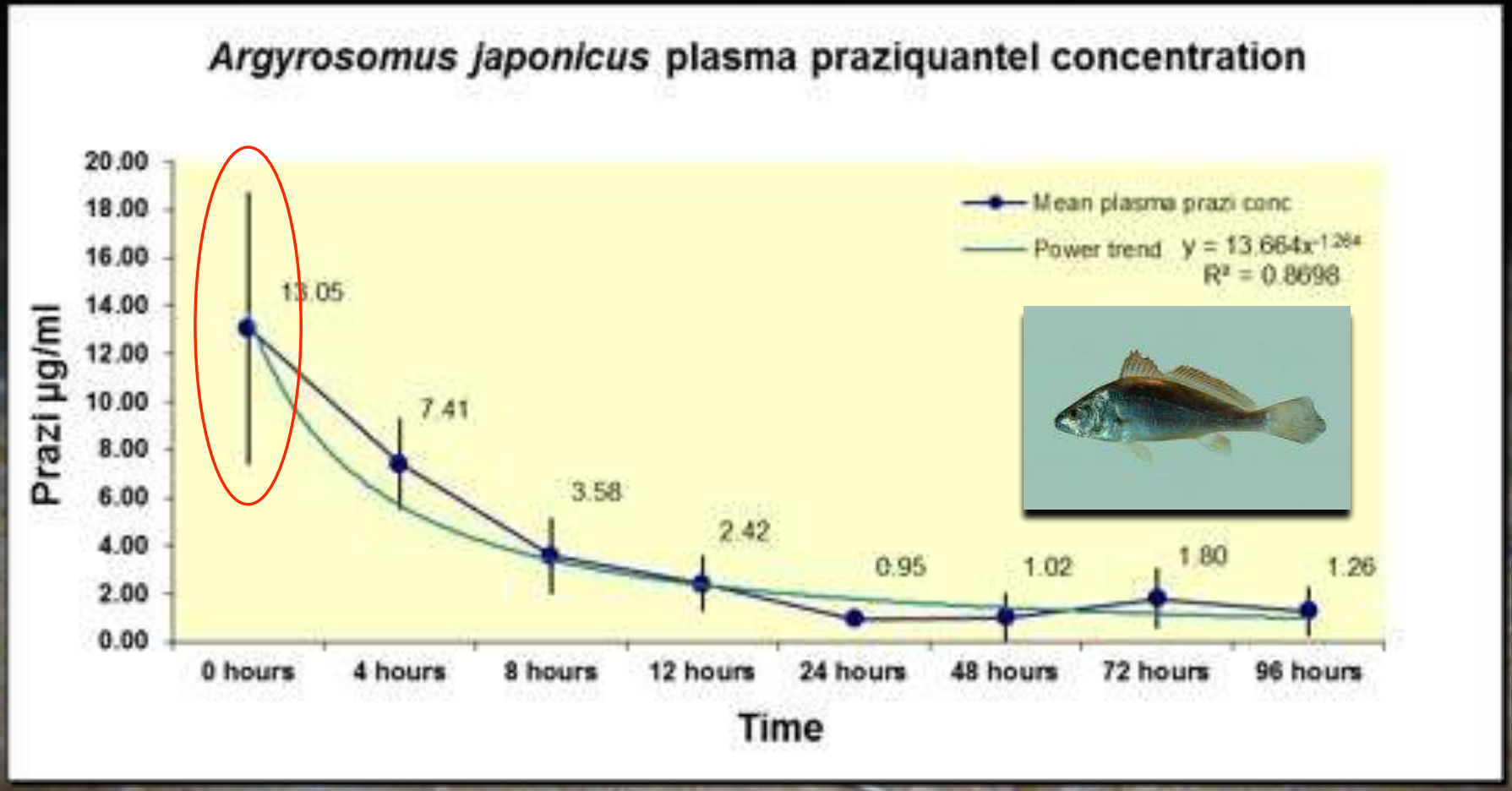
Sample preparation

- 1ml 100% Acetonitrile to volume of plasma sample
- Sample allowed to rest for 10min at 4°C
- Centrifuged at 10 000 x g for 10min
- Supernatant evaporated to dryness
- Residue dissolved in 1ml of mobile phase
- 100µl injected at 1ml/min at 217nm on a C18 column

Standard preparation

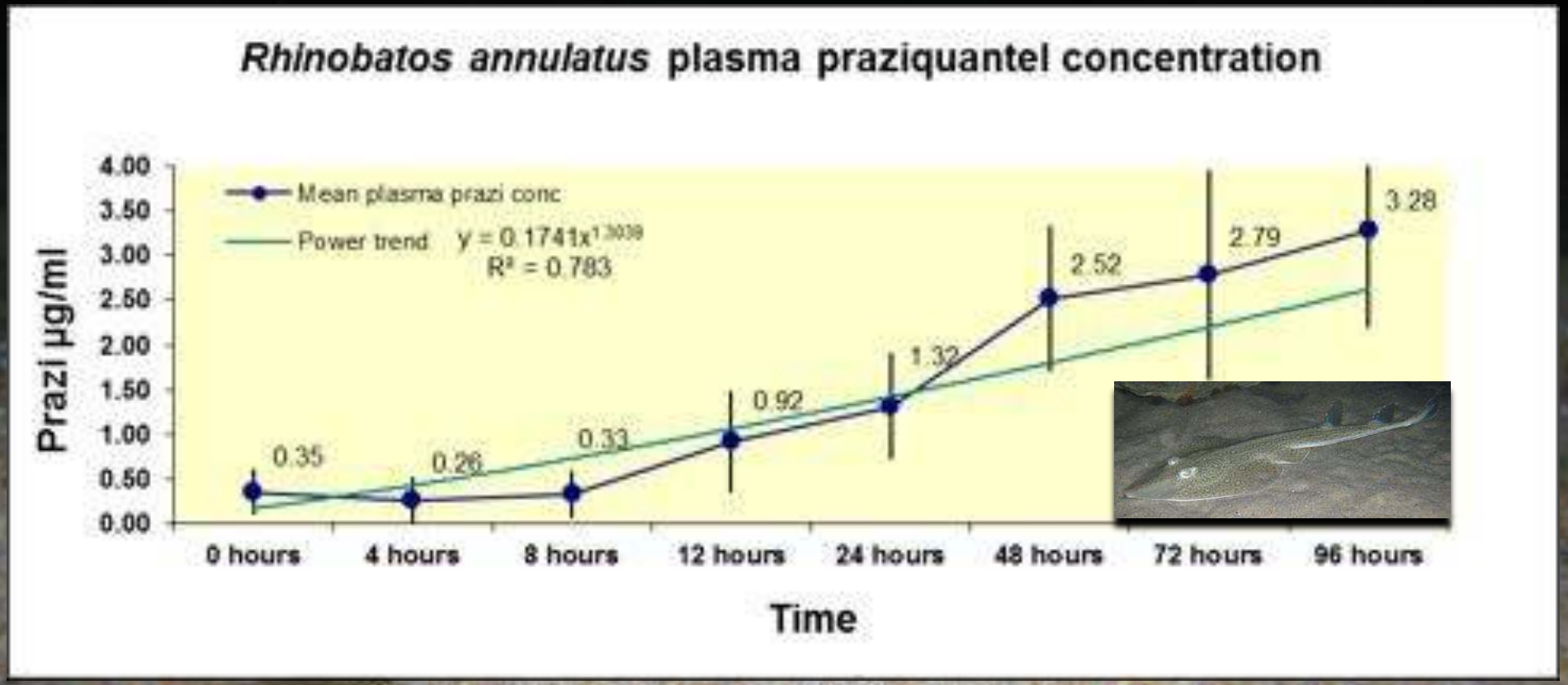
- 15.9mg praziquantel added to 100ml mobile phase, diluted 10:100

Results



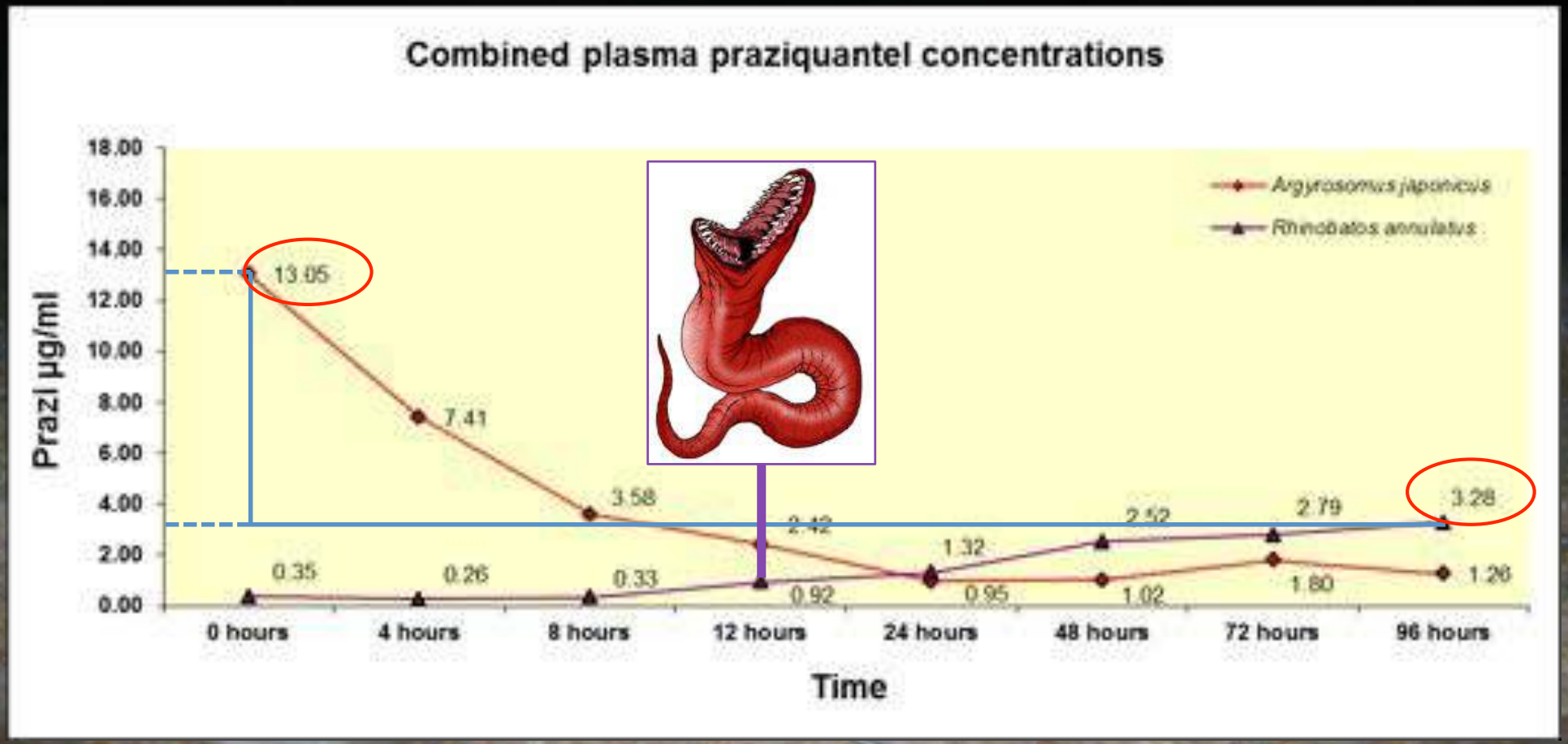
- Initial spike in blood plasma concentration of praziquantel
- Followed by gradual decrease to 96hrs (generally comparable to other studies)
- Large variation (stdev) in initial group – considered to be a combination of sampling time error and individual regurgitation
- Anaesthetics false positive? – HPLC tested 2-Phenoxyethanol, Tricaine-methanesulfonate and Isoeugenol – all non-interfering

Results



- Slow increase in concentration of praziquantel
- Peak concentration somewhere around or after 96hrs?

Results



- If we assume *R. annulatus* peak (at 96hrs), then the delivery is 25% that of *A. japonicus* at same dosage
- Difference in delivery can likely be explained as a function of first-pass metabolism (expected to be different in teleosts and elasmobranchs)
- If we assume relative similarity between *R. annulatus* and *D. brevicaudata*, then at 12hrs post-treatment – significant result before prazi peaked in plasma

What does it mean?

- Reject our hypothesis!
- Elasmobranchs may metabolise praziquantel more efficiently through FPM, therefore it is not surprising that previous workers had no success with comparatively low dosages
- Praziquantel either delivered to muscle and skin in elasmobranchs relatively quickly, or Monopisthocotylean monogeneans could also be feeding on blood?

What we still need to know

- What is the lethal concentration of praziquantel in plasma, skin and mucus for monogeneans? – with this we can optimise oral dosages based on delivery
- If delivery is higher in teleosts, are we using too much? – a reduction would alleviate palatability issue
- Effect of Cimetidine on FPM in elsmobranchs needs to be investigated!
    - Cimetidine is a histamine receptor antagonist which inhibits gastric acid secretion – known to reduce FPM in teleosts (Kim *et al*. 2001b)

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VERSITA

Amendment of *Pseudoleptobothrium* Young, 1967 (Monogenea, Microbothriidae) with the description of *Pseudoleptobothrium christisoni* sp. nov. from the dermal denticles of *Rhinobatos annulatus* (Rhinobatidae) off the southern tip of Africa

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Abstract

Pseudoleptobothrium christisoni sp. nov. is described from the dermal denticles of the dorsal skin surface of a single female *Rhinobatos annulatus* collected off Cape Agulhas, South Africa and destined for public exhibition at the Two Oceans Aquarium in Cape Town. This new species differs from the only other species in the genus, *P. apsychotrematis* Young, 1967, primarily by the morphology of the male copulatory tract. The distal portion of the male copulatory tract is wide and bears muscular papillae internally and externally. In addition, the area of ventral tegument near the vaginal pore has several parallel ridges which appear to serve as the initial attachment site for a bipartite spermatophore. The formation of the spermatophore in *P. christisoni* is discussed. The generic diagnosis of *Pseudoleptobothrium* is revised to accommodate *P. christisoni* and a partial redescription of *P. apsychotrematis* is provided to include characters originally not described or described incorrectly.

Keywords

Monogenea, Microbothriidae, *Pseudoleptobothrium*, *Rhinobatos annulatus*, South Africa

Introduction

There are currently 5 *Rhinobatos* Linck, 1790 species found in South African waters including the common lesser guitarfish *Rhinobatos annulatus* Müller et Henle, 1841 (see Compagno 2001). Recently, the monocotylid monogenean *Neoheterocotyle robii* Vaughan et Chisholm, 2010 was described from the gills of *R. annulatus* (see Vaughan and Chisholm 2010), but there are no other records of monogeneans from South African rhinobatids.

Microbothriid monogeneans are parasites of the Carcharhinidae, Carcharidae, Scyliorhinidae, Squatinidae, Pristidae and Rhinobatidae (see Young 1967). Microbothriids cause significant skin damage to their elasmobranch hosts in captivity leading to disease or even host mortality (Cheung *et al.* 1982, 1983; Cheung and Nigrelli 1983; Cheung and Ruggieri 1983; Rand *et al.* 1986; Poynton *et al.* 1997). Microbothriids are also reported to be pathogenic on wild-caught hosts (see Bullard *et al.* 2008) and are the possible vectors of bacterial infections (see Grimes *et al.* 1985).

Pseudoleptobothrium apsychotrematis Young, 1967 is a monotypic species recorded from the rhinobatids *Apsychotrema rostrata* (Shaw, 1794) and *Trygonorrhina dumerilii* Castelnau, 1873 (reported as *Trygonorrhina fasciata* Müller et Henle, 1841 by Gleeson *et al.* (2006) but was later changed to *T. dumerilii* by Last and Stevens (2009)). We recently discovered microbothriids on the skin of a single *R. annulatus* collected off Cape Agulhas in 2008 that was donated to the Two Oceans Aquarium in Cape Town. We identified it as a new species of *Pseudoleptobothrium* and describe it herein.

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A new species of*Neoheterocotyle*Hargis, 1955 (Monogenea: Monocotylidae) from the gills of *Rhinobatos annulatus* Müller & Henle (Rhinobatidae) off the southern tip of Africa

David B. Vaughan · Leslie A. Chisholm

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Abstract *Neoheterocotyle robiin.* sp. is described from the gills of four female *Rhinobatos annulatus* Müller & Henle caught as bycatch during routine demersal research trawls off the Southern Cape coast of South Africa. The new species can be distinguished from all other members of*Neoheterocotyle* Hargis, 1955 by the morphology of the accessory piece associated with the male copulatory organ, the presence of a distinct horseshoe-shaped muscle associated with the male copulatory organ and the presence of a sclerotised structure in the proximal portion of the vagina. This is the first*Neoheterocotyle* species described from South African waters.

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Introduction

Rhinobatos annulatus(Mu¨ller & Henle) is a common rhinobatid found around the South African coast frequenting the surf zone as well as estuaries (Compagno,2003). This rhinobatid is an excellent candidate for exhibition in public aquaria because of its small size, compared to other rhinobatids, and its relatively simple husbandry requirements. Monocotylid monogeneans are parasites of the skin, gills, nasal tissue, urogenital system and wall of the body-cavity of elasmobranchs (Chisholm et al.,1995). These parasites are generally encountered in low intensities on wild-caught fishes but some monocotylids can be problematic on captive hosts (Janse & Borgsteede, 2003; Chisholm & Whittington,2004; Chisholm et al.,2004; Vaughan et al., 2008; Vaughan & Chisholm, 2009, 2010). Left uncontrolled, large numbers of feeding monocotylids can damage host tissue leading to disease and, in some cases, even death of the host fish (see Chisholm & Whittington, 2004; Vaughan & Chisholm,2010).

Although South Africa has a rich diversity of elasmobranchs, investigations of their associated monogeneans and the potential impact of these parasites on fish kept in local public aquaria are few (Bullard & Dippenaar, 2003; Vaughan et al., 2008; Vaughan & Chisholm,2010). Accurate monogenean identification and knowledge of their specific biology is essential to ensure the selection of the most appropriate management methods for their control

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