Project title: **Serotonin and behavioural alterations in the New Zealand mud crab *Macropthalmus hirtipes* infected with acanthocephalan cystacanths**

Travel dates: **July 2nd- Sept. 22nd**

Location: **Otago University. Dunedin, New Zealand**

Group members: **Katherine Nichol**

Aims: **To gain lab experience through performing novel scientific research**

Introduction

Studies have established that *Macropthalmus hirtipes* (Brachyura: Ocypodidae) harbour *Profilicollis antarcticus* and *P.novaezalandae* that cause changes in the behaviour of the host crab making them more susceptible to predation by birds, the definitive hosts of the parasites (Latham & Poulin 2002 a,b). The purpose of my study was to assess the underlying mechanisms of behaviour alteration caused by acanthocephalan cystacanths living in *M. hirtipes*

Changes in serotonin levels have been implicated in other studies of host behaviour manipulation by Acanthocephalan worms. The main aim of the project was to quantitatively assess the level of neurotransmitters in the brains of crabs with varying levels of parasitic infection. We also aimed to collect haemolymph samples for nutrient analysis to see if there was a difference in composition between infected and uninfected individuals.

Methods

The crabs were all collected during a receding tide on the Otago peninsula, New Zealand. The crabs were immediately wrapped in aluminium foil and frozen in liquid nitrogen. The samples were transported on dry ice and then stored at -70°C. 24 hrs before dissection the crab were moved to a fridge to defrost at 3°C. The crab brains were removed and the remaining tissue assessed for parasitic infection. The brains were immediately returned to the freezer (-70°C).
We had difficulty extracting the haemolymph from the crabs, the brittleness resulting from immersion in liquid nitrogen often resulted in the carapace cracking or limbs falling off allowing haemolymph to drain out during defrosting, thus we were unable to analyse this as planned.
Reverse phase high performance liquid chromatography (HPLC) was performed using electrochemical detection to measure serotonin and dopamine levels in the brain tissue.

Results

In *Macropthalmus hirtipes* a hint of a negative relationship was seen between the level of serotonin and the number of *Profilicollis* found per crab. A similar relationship existed between *maritrema* sp. (a trematode also found in the crab) and serotonin. When pooled the data showed a significant negative relationship between total parasite load and serotonin concentration ($r = -0.35$, $p=0.0394$) independent of crab size.

In a second species analysed, *Hemigrapsus crenulatus*, we measured the size of the Acanthocephalan cystacanths and found mean cystacanth volume is negatively influenced by the number of *Profilicollis* found per crab. This effect did not extend between species e.g. *Profilicollis* size was not affected by the number of *Maritrema* present.

In *H. Crenulatus* a positive association between the number of *Maritrema* and number of *Profilicollis* was seen, independant of host size. This was not observed in *Macropthalmus hirtipes*.

Conclusions

Larval helminths, in spite of their seemingly dormant nature, show population dependent density growth and appear to cooperate to alter the physiology and behaviour of their hosts. It was evident that the parasites were not randomly distributed between hosts with respect to one another. This was unexpected as the parasites have different methods of entry into their crab host, Acanthocephalans are deposited in bird faeces and accidentally ingested by the crab whereas the cercariae of trematodes released by snails penetrate the body at joints in the crab exoskeleton. It is possible that the one parasite preferentially infects a host already harbouring another species e.g. an acanthocephalan infection may alter the behaviour of the host leaving them more susceptible to *maritrema* infection. Acanthocephalan infected individuals are more prone to bird predation (Latham and Poulin, 2002a, b) the trematode could benefit from this increased probability of ingestion by the definitive host.

We observed the mean *Profilicollis* cystacanth volume was smaller in heavily infected hosts and larger in those with lighter infection levels. The size of the cystacanths was not related to the size of the crab and was not affected by large numbers of trematode metacercariae. It is suggested that the size of the acanthocephalan cystacanth in the intermediate hosts has important survival benefits for the establishment of life in the definitive host an energy costly process requiring a large store of glycogen (Taraschewski, 2000). However despite the disadvantages of lower glycogen stores it may be advantageous in terms of host manipulation, to have a high-density infection.

The total number of larval helminths harboured by a crab correlated negatively with the amount of serotonin in the brains of the *M. hirtipes* individuals sampled. In a previous study (Latham and Poulin, 2002a, b)it was shown high numbers of *Profilicollis* influenced the burrowing behaviour of *M. hirtipes* but not *H.crenulatus* leaving the former more regularly exposed at low tide than the latter. It appears that the parasite species are co-operating to manipulate the behaviour of the host as the effect is only seen when the parasite numbers are taken together.

It might be that lower serotonin levels result from the crab stress response to a large infection.

Acknowledgements

For financial assistance I would like to thank the Weir and James Rennie bequest committees for their support. Many thanks must go to Assoc. Prof. R. Poulin for supervising the project and organising lab space/ equipment. Jane Duthie deserves mention for her patience in creating a relatively efficient HPLC operator from a rather wary student. Dave Latham was invaluable in his crab collecting and also in dutifully counting and measuring parasites.

Much of this report is taken from a co- authored paper that has been submitted to the International Journal of Parasitology entitled 'Host sharing and host manipulation by larval helminths in shore crabs: Cooperation or conflict?' (R.Poulin, K. Nichol & A.D.M.Latham 2002.)

References

Latham, A.D.M. & Poulin, R. 2002a Field evidence of the impact of two acanthocephalan parasites on the mortality of three species of New Zealand shore crabs (Brachyura), Marine Biology. (In press)

Latham, A.D.M. & Poulin, R. 2002b Effect of acanthocephalan parasites on hiding behaviour in two species of shore crabs (Brachyura) Journal of helminthology. (In press)

Taraschewski, H. 2000 Host- parasite interactions in Acanthocephalan: a morphological approach. Advances in parasitology. 46, 1-79.

Personally, I gained experience and new skills in the lab giving me confidence I will carry into my studies this year. Seeing another part of the world was fun and exciting and the people I met made my trip a wonderful experience

Appendix 1: Itinerary and financial synopsis

July 4th Arrived Auckland
July 7th Travelled to Dunedin
July 8th Met project collaborators Assoc. Prof. Robert Poulin and Dave Latham
July 9th-12th Collecting Macropthalmus hirtipes from Papanui inlet, Otago peninsula
July 15th - 29th Dissecting samples, collecting brains and counting parasites
July 29th - Aug 10th Cleaning and calibrating HPLC apparatus (Jane)
Aug 14th - Sept 10th Performing HPLC analysis also collecting and dissecting Hemigrapsus crenulatus
Sept 10th- 18th Data analysis and report writing
Sept 19th Travelled back to London via Auckland and Los Angeles arriving back **Sept 22nd**

Expenses:

Flights	£800
Transfers	£200
Accommodation	
10 weeks @ £40/ week	£400
Food	
10 weeks @ £30/ week	£300
Personal expenditure	
10 weeks @ £40/ week	£400
Insurance	£60
Equipment (e.g. Welly's!)	£40
Total	£2200

The accuracy of my financial forecast was relatively good and I am very grateful to my family and the University of Edinburgh for their contributions, enabling the project to go ahead.