

Summary:

During the first year of this project relevant aspects of the biology and ecology of the New Zealand flatworm (*Arthurdendyus triangulatus*) have been investigated to provide essential data (not currently available in the literature), which is needed for the development of a comprehensive risk analysis that is acceptable for UK trading partners.

Detailed studies of the effect of temperature on the potential for establishment and widespread population development have been completed. Well established and internationally accepted techniques were used to show that mortality of *A. triangulatus* increases rapidly between -1°C and -2°C, with 100% of individuals dying after 1-3 days exposure to the lower temperature. No substantial differences in this temperature response were recorded for any of the life stages tested (egg cocoons, juveniles, adults). *A. triangulatus* was therefore found to be much less cold hardy than other invasive invertebrates that have been tested using these techniques, indicating less likelihood of establishment in many parts of Europe. High mortality of adult *A. triangulatus* was also recorded after slightly longer exposures to higher temperatures. For example 100% of individuals tested were dead after 12 days exposure to 23°C and 20 days at 22°C. These data have been provided to plant health consultants and already used in negotiations in with European trading partners.

It has been suggested that substrate (soil) moisture may affect *A. triangulatus* survival and therefore establishment potential when introduced into a new geographical area. The results of early experiments conducted at low temperatures suggest that at -1°C and -2°C, no differences were detected between mortality responses obtained from *A. triangulatus* adults kept in substrates with sufficient water added to establish moisture levels of 100% (saturated), 50% or 0% (dry) of their carrying capacity. Work investigating the response of both adults and egg cocoons to substrate moisture levels is ongoing. Similarly, protocols for new work on the effect of substrate pH on establishment potential of *A. triangulatus* have been written, and experiments are scheduled to commence during the next period of the project.

Molecular studies of the mode of reproduction of *A. triangulatus* have been undertaken. Like other flatworms *A. triangulatus* is hermaphroditic. The normal means of reproduction of terrestrial flatworms is sexual, although asexual reproduction has been recorded in some species. If *A. triangulatus* is capable of self-fertilization or some form of parthenogenesis then new populations could establish from a single founder, affecting colonization potential. The majority of base differences between adult and offspring sequences reported at the beginning of this study can mostly be attributed to the differences between two forms of rDNA (type I and type II). In light of the presence of these two types of rDNA, comparisons of both type I and type II rDNA sequences have been carried out on adults and their juveniles. Fewer base differences are evident and in some cases an adult and one particular juvenile may have no differences at all. These new findings have not dismissed the possibility that *A. triangulatus* may reproduce by self-fertilization.

A large manipulative field experiment has been undertaken to investigate the impact of *A. triangulatus* on populations of their earthworm prey. Although conclusions cannot be drawn until the final results of this long-term experiment are available towards the end of the project, data collected to date support the view that anecic species of earthworm (which construct vertical, semi-permanent burrows that allow easy access by flatworms) may be more at risk from *A. triangulatus* predation than epigeic or endogeic species. Secondly, vulnerability to predation may be increased because anecic species are relatively slow to mature and reproduce. Thus the threat posed by *A. triangulatus* may not be a decline in earthworm populations, but rather an alteration of the species profile.

Extensive field monitoring of *A. triangulatus* at seven Scottish sites has been continued in an unbroken run throughout this project, yielding data that provides a basis for the interpretation of results of both the laboratory and manipulative field experiments. Long term trends have emerged at all sites, which can be related to changes in the environmental conditions experienced by flatworms, as predicted by experiments conducted under this project. Observations at these sites have therefore supported the results of the experimental work, indicated which areas of investigation should be given priority during the next project period and provided data that can be used to optimise the design of future experimental series.