Survey of Parasites of Freshwater Snails in Israel

Roni Yizhar[1], Yael Dagan[1], Michal Ucko[2] and Frida Ben-Ami[1],*
[1]Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv 69978, Israel
[2]National Center for Mariculture, Israel Oceanographic and Limnological Research, P.O.B. 1212, Eilat 88112, Israel
*e-mail: Frida@post.tau.ac.il

Introduction

The rapid decline in Earth's biodiversity has spawned considerable interest in studies aimed at elucidating the relationship between biodiversity and ecosystem functioning (Striebel et al. 2009). Processes affecting biodiversity such as biological invasions via international trade (Rixon et al. 2004), eutrophication (Schindler 2006, Brede et al. 2009), pollution by toxicants and other chemicals from the catchment, salinization, and human-driven physical destruction of natural habitats have become real issues for conservation biology, with far reaching implications for agriculture and public health.

Parasites and pathogens also play a decisive role in sustaining the structure and biodiversity in ecosystems, both through their hosts and via other free living species that rely on these hosts (Renaud et al. 1996, Marcogliese 2002, Hudson et al. 2006). However, biodiversity surveys often sample host and parasite richness at different and discrete points in time. Consequently, it is difficult to assess the relative role of biotic vs. abiotic factors in maintaining species richness, and to identify patterns and trends of biodiversity decline. Depending on factors such as host susceptibility and the size of the ecosystem, parasites can be stabilizers or destabilizers of processes as diverse as competition, migration and speciation (Combes 1996, Holmes 1996). Invading parasites and the infectious diseases they transmit have also become a major threat to wildlife conservation and endangered species, by influencing host genetic diversity and altering species composition (Altizer et al. 2003). Yet this very same coevolution-driven genetic diversity may buffer natural populations against widespread epidemics (Poulin and Morand 2004).

Mollusks are among the most notorious invaders, particularly because of their intermediary role in transmitting parasitic diseases to humans (Prenter et al. 2004).

Although comprehensive databases of indigenous (Heller 2009) and non-indigenous (Roll et al. 2009) freshwater gastropods of Israel have been compiled recently, knowledge of their parasites and how they interact with native vs. introduced snails is still lagging behind. This lack of knowledge stands in striking contrast with the important public health and agricultural implications of snail-infecting parasites in freshwater bodies in Israel (see Table 1), because snails usually serve as intermediate hosts and adult stages of many trematodes are non-fastidious in their choice of definitive hosts (Dzikowski et al. 2004). Introduced gastropods can aggravate the situation either by transmitting invasive pathogens or through increased resistance to native parasites which allows them to outcompete native snails (Gerlach 2001, Fromme and Dybdahl 2006, Genner et al. 2008; reviewed in Prenter et al. 2004). For example, in a period of three years, *Thiara scabra* became the most abundant gastropod in the Sea of Galilee, the major source of drinking water in Israel (Mienis and Mienis 2008). After being discovered in proximity to the Jordan River (Ben-Ami 2006), *Tarebia granifera* has also spread to various localities in the Beit She’an Valley (F. Ben-Ami, unpublished data). Both gastropods may serve as intermediate hosts of trematodes that cause parasitic diseases in humans and livestock (Chontananarth and Wongsawad 2010; Jayawardena et al. 2010; McKoy et al. 2011). Once established, exotic trematodes along with their snail host have been suggested to negatively affect the susceptibility to infection of native vertebrate communities (Font 2003). These parasites are pathogenic to vertebrate hosts lacking coevolved defense mechanisms (Taraschewski 2006). For instance, *Transversotrema patialense* was initially found in an aquarium harboring tropical fish (Ben-Ami et al. 2005), but two years later it reappeared in several fish farms in the Beit She'an Valley (Nir David Central Fish Health Laboratory 2007). The purpose of this survey is twofold: (i) to identify freshwater-snail-infecting parasites in Israel using comparative morphology and molecular techniques, and at the same time (ii) to survey both indigenous and introduced freshwater gastropods. Ultimately this study will serve as a basis for future research in conservation biology that may improve our current understanding of the players affecting freshwater gastropod fauna in Israel and assist in developing effective eradication and containment schemes to the benefit of agriculture and public health.

Materials and Methods

Species. Roll et al. (2009) report on 19 introduced freshwater gastropod species, of which three have gone extinct, nine are found in human dominated habitats (Ampullariidae: 4, Lymnaeidae: 3, Physidae: 1 and Planorbidae: 1) and the remaining seven species are from natural habitats (Thiaridae: 2, Lymnaeidae: 2, Physidae: 1 and Planorbidae: 2). The present survey focused on natural habitats, in which indigenous species from the following families are abundant: Thiaridae, Melanopsidae, Neritidae, Physidae and Planorbidae. We surveyed all freshwater snails found within a given habitat.

Geography. Freshwater gastropods are found in the Negev desert, along the Mediterranean coast and in the Jordan Valley, where they occur in a variety of freshwater bodies ranging from small ponds, springs and streams to large lakes and rivers (Ben-Ami and Heller 2005). We surveyed 15-20 locations from each geographic area, with a diverse range of freshwater bodies. Since data on the approximated invasion time and the first dispersal event of some exotic species is available (e.g., *Tarebia granifera* and *Thiara scabra*), we used this information to assess the spreading ability of these species in Israel.

Sampling and Collection. Freshwater gastropods can persist in slow running water, on soft mud and sand substrata, in places where water plants are abundant and in water moderately polluted with organic matter, as is often the case near human habitations. Therefore, in every site we sampled specimens from different sub-habitats: banks, deep water, plants, mud and rocks. Additionally, the presence of parasites in snails is often linked to the presence of the definitive host, i.e., birds and mammals. Hence, each site was sampled twice – once in summer and later on during winter. In each sub-habitat five random squares (20 x 20 cm) were collected. In the field snails from each species were counted and up to 50 individuals transported alive to the laboratory, where they were housed in aquaria. Twenty five out of the 50 snails from each location were put in ethanol and stored as part of the Tel Aviv University molluscs collection for future molecular work.

Morphological Analysis of Trematodes. The remaining 25 snails were checked for trematode infection by examining the gonad and digestive gland of the snail under a light microscope. Initially parasites were identified using comparative morphology. However, since some trematode cercariae extracted from the snail host cannot be reliably identified at the species level (i.e., adult stages from the definitive host are required), we also used molecular identification when necessary (Dzikowski et al. 2004). Representative parasites were placed in ethanol and stored as part of the Tel Aviv University invertebrate collection. Lastly, we also prepared a pamphlet with photographs of cercariae to help with future identification of difficult species.

Molecular Identification of Trematodes. Amplification, sequencing and aligning of the 18S (SSU) and internal transcribed spacer (ITS) of the rDNA gene were carried out as described in Levy et al. (2002) using universal trematodes primer sets, as well as species-specific primers that were developed for identifying and differentiating between various species according to Dzikowski et al. 2004.

Preliminary Results

We found that the invading snail species *T. scabra* and *T. granifera* have extended their distribution to new areas in Israel (Figure 1). *Thiara scabra*, which became the most abundant snail in the Sea of Galilee in just a few years, has also spread to the Bet She'an Valley and the West Coast of Israel, and is now abundant in Ein Afek nature reserve and Taninim stream. *Tarebia granifera*, which was first found in proximity to the Jordan River, has dispersed to a few springs in Bet She'an Valley and established in Bokek stream in the Judean Desert, where it constitutes more than 50% of the freshwater snails' community. Our findings indicate that the presence of these invading snails resulted in a significant reduction in the density of two local snail species: *Melanoides tuberculata* and *Melanopsis saulcyi* (Figure 2).

Examination of the gonad under a light microscope revealed four types of trematodes' cercariae infecting the snails. Three types of cercariae – Parapleurolophocercous, Brevifurcate pharyngate and Virgulate – were found in all *Melanopsis* species. An additional type, gymnocephaluos cercaria from the genus *Philophthalmus*, was found in both invading species *T. scabra* and *T. granifera*, and in the local species *M. tuberculata* and *M. saulcyi*.

Table 1. Known freshwater-gastropod-infecting parasites in Israel

Parasite	**Snail Host**	**Additional Hosts**	**References**
Centrocestus sp.	*Melanoides tuberculata*	Fish, Birds	Heller and Farstey 1990 Paperna 1996 Ben-Ami and Heller 2005 Ben-Ami 2006
Haplochis pumilio	*Melanoides tuberculata*	Fish, Birds	Dzikowski et al. 2004
Phagicola longa	Unknown	Fish, Birds	Dzikowski et al. 2004
Philophthalmus sp.	*Melanoides tuberculata* *Melanopsis praemorsa* *Tarebia granifera*	Humans	Radev et al. 2000 Gold et al. 1993 Ben-Ami 2006
Pygidiopsis genata	*Melanopsis costata*	Fish, Birds	Dzikowski et al. 2004
Transversotrema patialense	*Melanoides tuberculata*	Fish	Ben-Ami et al. 2005

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Figure 1: Spreading of the invading species *Thiara scabra* and *Tarebia granifera* in Israel.

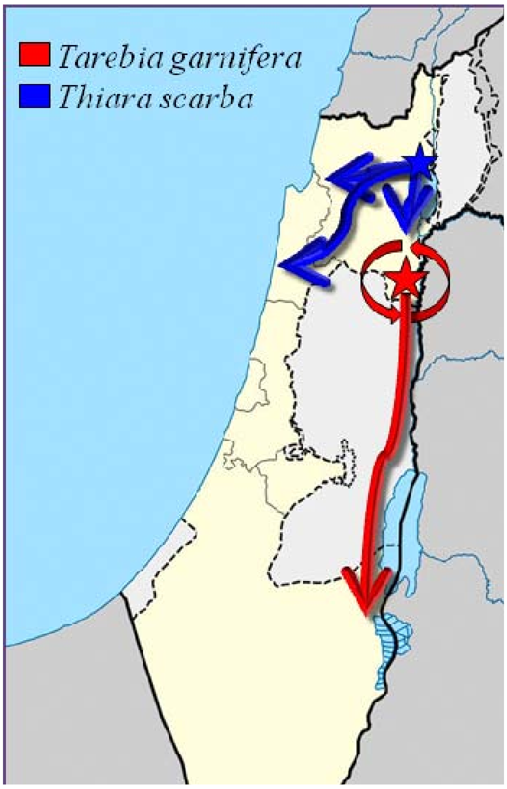

Figure 2: Effects of the invading species *Thiara scabra* and *Tarebia granifera* on local species density.