Ecological aspects of the ascidian community
along the Israeli coasts

THESIS SUBMITTED FOR THE DEGREE “DOCTOR OF PHILOSOPHY”

BY
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This work was carried out under the supervision of

Prof. Yossi Loya
This work is dedicated with enormous love to Dror & little Ido
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ABSTRACT

Ascidians (Phylum: Chordata, Class: Asciidae), or sea squirts, are the largest and most diverse class of the sub-phylum Tunicata (also known as Urochordata). They comprise approximately 3000 species found in all marine habitats from shallow water to the deep sea. Despite the enormous progress that has been achieved in the field of ascidian research worldwide, only a few studies have focused on the ascidians of the Red Sea and the Eastern Mediterranean. This study is the first to investigate this unique group of invertebrates from the Mediterranean and Red Sea coasts of Israel, from ecological, taxonomical and biological aspects. I first ascertained the dominant species found in the region, and determined whether these include any non-indigenous species. I then assessed the annual appearance and spatial distribution of these ascidians and how this appearance may be related to different a-biotic factors. Finally, I studied the possible impact of anthropogenic activities on ascidian ecology in both coral reef and Mediterranean shore environments.

In section I, I review the available data regarding the ascidian fauna along the Israeli coasts and describe the most dominant species found along the Red Sea coast of Israel. This part of the study led to the discovery and description of a new species, Botryllus eilatensis (Shenkar & Monniot 2006). In addition, two solitary species (Rhopalaea sp. and Boltenia sp.), found in Eilat, are also suspected to be new species. Six non-indigenous species (Phallusia nigra, Herdmania momus, Microcosmus exasperatus, Rhodosoma turcicum, Ascidia canellata, and Symplegma brakenheli) were recorded from the Mediterranean shores of Israel. These species probably reached the
Mediterranean via the Suez Canal, since they have an extra-tropical Indo-Pacific distribution and a restricted distribution in the Eastern Mediterranean.

In section II, in order to obtain a broader view of the ecology of non-indigenous species along the Mediterranean coast of Israel, I have chosen to focus solely on the study of comparative aspects of the ecology of the solitary ascidian *H. momus*, in its native (Red Sea) environment versus its introduced (Mediterranean) environment. During 2005 monthly samples of *H. momus* were collected along the Mediterranean and Red Sea coasts of Israel, in order to investigate possible differences in life history strategies of the two populations. The samples were preserved, dissected, and measurements were taken of length, total weight, gonad weight and oocyte diameter, and the occurrence of symbionts was recorded. In the Mediterranean *H. momus* exclusively inhabits artificial substrates and is common at greater depths than in Eilat (Red Sea). Individuals of *H. momus* in Eilat reproduced year round. Although individuals from the Mediterranean were significantly larger than individuals collected in Eilat, their gonad indices and oocyte diameter measurements indicate that they have a short reproductive season. Copepods were found in 50% of the samples from both sites, while a pontoniine shrimp was found in 14% of the samples from Eilat only. The marked differences between the native vs. non-indigenous populations of *H. momus* are attributed to differential food availability, water temperature, currents and wave exposure.

In section III, I report on the ascidian recruitment patterns on an artificial reef in Eilat. The location and timing of one species’ appearance may be the result of seasonality of reproduction, larval preferences, and effects of different a-biotic factors such as food availability, light intensity, water temperature and more. By identifying the ascidians to
species level, we were able to distinguish between the specific recruitment patterns of the different species, rather than generalizing patterns among groups as done in most studies. The major contributions of the data presented in this section are: 1) the finding that two dominant species, *Herdmania momus* and *Didemnum granulatum*, established the pattern for the entire group; and 2) the finding that the epifaunal assemblage developing on an artificial substrate in a tropical environment is influenced by the season of deployment. These results should be taken into consideration when investigating community development on such structures, especially since in coral reefs, possibly due to the less dramatic changes between seasons, this aspect has not been considered to date.

One of the most striking seasonal phenomena observed in the coral reefs of Eilat during the study was that of the seasonal appearance of a new colonial ascidian, *Botryllus eilatensis*. In order to study overgrowth dynamics and the relationship between coral morphology, size, and orientation and ascidian overgrowth, I conducted monthly monitoring of 50 - 70 tagged coral colonies interacting with *B. eilatensis*. This study is the first to investigate coral-ascidian competitive interactions and of major importance since the environmental conditions that are deleterious for corals (e.g. eutrophication) may create a favorable environment for filter-feeding organisms, such as ascidians, accelerating their growth and spread. The rate of *B. eilatensis* overgrowth on corals was influenced by the coral’s morphology and orientation combined with a seasonal appearance of the ascidian. This seasonality was linked to the vertical mixing phenomenon that occurs in Eilat every winter and results in higher nutrient levels in the water column, and thus in higher food availability. I further demonstrate that *B. eilatensis*
fragments translocated to a nutrient-rich site increased three-fold in size and exhibited higher survivorship rates in comparison to intact fragments in a nutrient-poor area.

The data collected in the above studies demonstrate the significant impact of human activities on ascidian ecology. Since ascidians are able to filter even minute particulate matter, any rise in nutrient levels and organic material in coastal waters will have a direct influence on their appearance. This was evident in the large size of *H. momus* found in the Mediterranean and the seasonal outbreaks of *B. eilatensis* in the coral reefs of Eilat. In addition, being a major component of the fouling community, ascidians play an important role in marine bioinvasion, as demonstrated by the appearance of six non-indigenous ascidian species with an Indo-Pacific origin. These species were particularly abundant on artificial marine structures, which are known to facilitate the spread of non-indigenous species. Thus, another human activity that may contribute to the spread of non-indigenous ascidians is that of the proliferation of artificial structures along the coast line.

Ascidians can be strong spatial competitors, and once they become established they often experience population explosions that can develop into dense mats that overgrow and cover available surfaces. However, although there is a growing awareness that ascidians can become of economic and ecological concern, relatively little is known at present about the ecology of many of these ascidians, unlike those taxa with a direct economic value. It is my hope that the current thesis will create a renewed interest in the field of ascidian ecology and that it will encourage future studies in this field and help in the development of tools for controlling the negative impacts of human activities on coastal regions.
1 GENERAL INTRODUCTION

1.1 Background

Ascidians (Phylum: Chordata, Class: Asciidae), or sea squirts, are the largest and most diverse class of the sub-phylum Tunicata (also known as Urochordata). They comprise approximately 3000 species found in all marine habitats from shallow water to the deep sea (Monniot & Monniot 1978). Adult ascidians bear little resemblance to typical chordates, though their tadpole larvae clearly exhibit the four fundamental characteristics of the phylum: a dorsal tubular nerve cord, notochord, pharyngeal gill slits and a post-anal tail (Ruppert & Barnes 1994). The larvae also possess a sensory vesicle containing two darkly-pigmented sense organs a light-sensitive ocellus and a statolith. Following settlement, the lecithotrophic larvae undergo retrograde metamorphosis during which they lose all of these characteristics except for the pharyngeal gill slits (Millar 1971). The name "tunicate" (sub-phylum Tunicata) comes from the polysaccharide-containing tunic that envelops the animal and forms a somewhat flexible skeleton. Various proteins, some blood cells and spicules occur in the tunic (Monniot et al. 1991). All ascidians are hermaphrodites, having both male and female organs. They generally avoid self-fertilization by developing only eggs or only sperm at any one time (Newlon et al. 2003) or barriers to selfing (Lambert 1997). Most solitary ascidians release their eggs and sperm into the water for external fertilization while colonial ascidians usually retain and brood their eggs (Lambert 2005a). Consequently, under natural conditions, ascidian larvae do not normally disperse very far, often just a few meters or less (Ayre 1997). The majority of ascidians filter their food from the water-column via an oral siphon that brings water into the pharyngeal chamber; a cloacal siphon then expels the water.
Particles suspended in the current are trapped in a mucus net on the gill slits. The net pores range from 0.1 to 0.3 µm, allowing ascidians to filter even very small particulate matter, primarily in the range of 0.5 to 2 µm diameter (Bak et al. 1998, Bone et al. 2003). The substances caught in the mucous net are later transported to the stomach for digestion. Ascidian larvae and juveniles are known to be preyed upon by various gastropods (Young & Chia 1984), by oysters that are able to trap the larvae (Dalby & Young 1992), polychaetes (Goodbody 1962), crabs (Davis 1988) and fish (Olson & McPherson 1987, Hurlbut 1991), especially during their early life stages (Osman & Whitlatch 1995, 2004). Adult ascidians are preyed upon by prosobranchs and by nudibranch gastropods (Thompson 1964, Dalby 1989) and sea urchins (Simoncini & Miller 2007). Several solitary ascidians, such as Halocynthia roretzi (von Drasche, 1884) and Microcosmus sabatieri (Roule, 1885), provide a human food source in Japan, Korea, Europe and Chile (Lambert 2005b).

As stated by Pourquié (2001) “Ascidians are back in the limelight, with a good chance of staying there”. During the past two decades enormous progress has been achieved in the fields of development, evolution, immunology, natural products and ecology of ascidians. The ascidian's small genome, small cell number and short life-cycle make it an attractive model system for developmental biologists (Nishida & Sawada 2001, Pourquié, 2001). Investigating the phylogenetic position of the subphylum Tunicata (Urochordata) in relation to the other subphyla in the phylum Chordata is crucial to the understanding of possible mechanisms of chordate evolution (Swalla et al. 2000, Zeng & Swalla 2005). Moreover, the study of self/non-self recognition in ascidians
provides important information regarding the evolutionary origin of the vertebrate immune system (Khalturin & Bosch 2007).

Some species, mainly from the order Phlebobranchia, are known to accumulate vanadium (Lambert 2005b) and store it in specialized blood cells called vanadocytes, reaching a concentration of $10^7$-fold higher than the concentration of vanadium in seawater (Yamaguchi et al. 2006). Since some ascidian species can survive in highly polluted areas, and accumulate arsenic, cadmium, chromium, cobalt, copper, iron, lead, mercury, selenium, tin (as tributyltin) and zinc (Papadopoulou & Kanias 1977, Philip et al. 2003), they can be used in bioassays for pollutants (Monniot et al. 1986) and as bioindicators (Naranjo et al. 1996).

Similar to sponges and bryozoans, many ascidians avoid predation or fouling by producing noxious secondary metabolites (Teo & Ryland 1994, 1995, Davis 1998, Pisut & Pawlik 2002, McClintock et al. 2004). They therefore provide a fertile ground for studies in the field of natural products. For example, ascidians serve as a potential source of new anti-cancer compounds (Wright et al. 1990, Scotto 2002). Ecteinascidin 743, a highly promising, exceedingly potent antitumor agent, isolated from extracts of the ascidian *Ecteinascidia turbinata* (Herdman, 1880), is currently in phase II/III clinical trials (Liu et al. 2006). In addition, antimalarial compounds have recently been isolated from the solitary ascidians *Microcosmus goanus* (Michaelsen, 1918), *Ascidia sydneiensis* (Stimpson, 1855) and *Phallusia nigra* (Savigny, 1816, Mendiola et al. 2006).

Despite the enormous progress that has been achieved in the field of ascidian research worldwide, only a few studies have focused on the ascidians of the Red Sea and the Eastern Mediterranean (Pérès 1958a,b, 1962, Rinkevich et al. 1993, Koukouras et al.
1995, Oren & Benayahu 1998). The ascidians in these two geographical regions are of particular historical interest since many ascidian species were described for the first time during Red Sea expeditions conducted at the beginning of the 20th century (Hartmeyer 1915, Michaelsen 1918, 1920a,b). Moreover, some ascidians are known to have been introduced into the Eastern Mediterranean following the opening of the Suez Canal (Por 1978, Koukouras et al. 1995), providing a living laboratory from which to study migration and invasion of species.

This study is the first to investigate this unique group of invertebrates from the Mediterranean and Red Sea coasts of Israel from ecological, taxonomical and biological aspects. The information amassed from this research greatly contributes to the understanding of ascidian ecology in general and to processes of marine bio-invasion in particular.

1.2 Ascidian taxonomy

Only in 1866, when Kowalevsky discovered the chordate nature of the ascidian tadpole larva, were the ascidiacea reclassified with chordates rather than with the molluscan invertebrates (Raf & Love 2004). Following the original classification of LaHille (1886), the class ascidiacea is now divided into three orders based on the structure of the adult branchial sac: Aplousobranchia, Phlebobranchia and Stolidobranchia. This is the current designation used by ascidian taxonomists. The Perrier (1898) division was based upon the position of the gonads and other morphological considerations and comprised two orders: Enterogona and Pleurogona, with the suborders Aplousobranchia and Phlebobranchia in the Enterogona and a single suborder, the Stolidobranchia, in the Pleurogona (Monniot et al. 1991). The number of families of ascidians is still uncertain,
since there are many cases of synonymy and misidentification; e.g., whereas the Monniots focused on 13 major families (Monniot et al. 1991), Kott (1992) described 23 families from Australian waters. The interesting peculiarity of ascidian classification is the relatively small number of genera in relation to the number of species. More than half of all known species belong to 10 very large genera, listed here in decreasing order of number of species: *Aplidium, Didemnum, Molgula, Polycarpa, Ascidia, Styela, Eudistoma, Pyura, Cnemidocarpa,* and *Synoicum.* The family Didemnidae is the most diverse, with 288 species out of the 728 species reported from Australian waters (Kott 2007).

Ascidian systematics is the domain of specialists, but a keen observer can recognize the well-described common species. Although you “cannot judge an ascidian by its cover”, underwater photographs can provide much information and assist in distinguishing one species from another (Monniot et al. 1991). The invaluable monographs published by Van Name (1921, 1931, 1945), the Monniots (Monniot et al. 1991, Monniot & Monniot 1996, 2001) and Kott (1985, 1990, 1992, 2001) greatly assist in identifying the ascidian fauna worldwide to species level.

**1.3 Non-indigenous ascidians**

One of the most pervasive, seemingly irreversible and devastating impacts of human activity on natural ecosystems is that of introduction of non-indigenous species. In the past few decades there has been a rapid increase in the number of reports of introductions of non-indigenous ascidians (NIAs) into many parts of the world (Lambert & Lambert 1998, Coles et al. 1999, Lambert 2002). There are several possible modes of introductions into new regions by NIAs: 1) in ballast water, as the ascidian larvae are able
to postpone settlement and survive for several days 2) on the hulls of barges, pleasure craft and other boats 3) as epifauna on shells and shellfish transported between mariculture operations (Lambert 2002) and 4) in sea-chests (Coutts & Doudgshun 2007). Hence, NIAs first records of appearance are usually from harbors and marinas, where the species flourish on artificial surfaces. The harbor ecosystem, with its protected and eutrophic waters is ideal for sessile suspension-feeders such as ascidians (Lambert & Lambert 1998, Lambert 2002, Mastrototaro et al., 2004). Most of the non-indigenous species are able to tolerate wide fluctuations in temperature, salinity, and pollution (Sims 1984; Naranjo et al. 1996; Nomaguchi et al. 1997; Stachowicz et al. 2002b). Stachowicz et al. (2002b) showed that under lab conditions, at high temperature, the growth rate of NIAs was higher compared to that of native species. Consequently, they suggest that global warming may facilitate a shift to dominance by non-native species, accelerating homogenization of the global biota.

In addition, NIAs have a rapid growth rate, a usually short life span of a few months, reach sexual maturity when only a few weeks old, and produce large numbers of short-lived non-feeding planktonic larvae (Lambert 2002). These characters, combined with the lack of significant predators, make the ascidians very successful invaders and result in the establishment of huge populations that can significantly change the benthic community of a region (Castilla et al. 2004, Valentine et al. 2007, Bullard et al. 2007, Dijkstra et al. 2007a) and economically affect commercially important activities, such as fishing and aquaculture (Bourque et al. 2007). For example, the solitary ascidian species *Styela clava* (Herdman 1882) and *Ciona intestinalis* (Linnaeus 1758) have had an adverse effect on the aquaculture and fisheries industry along Canada’s east coast, mainly on mussel
culture activities (Thompson & MacNair 2004, Bourque et al. 2007, Howes et al. 2007). In some areas, *S. clava* abundance is so high that they cause severe problems in crop handling, resulting in increased production costs estimated at $4.5 million damage to the aquaculture industry (Reynolds & Fortune 2003, figure 1.1). Another nuisance species is the colonial ascidian *Didemnum vexillum* which was first recorded in the 1980s or early 1990s along the coasts of North America. Since then it has established large populations at numerous sites and has overgrown sessile plants and animals (Valentine et al. 2007), resulting in considerable ecological and economic damage (Bullard et al. 2007, figure 1.2).

![Figure 1.1](image_url) The solitary ascidian *Styela clava* overgrowing mussel culture ropes at Prince Edward Island, Canada.
The colonial ascidian *Didemnum vexillum* covering the bottom at 35m depth, near Groton CT, USA. Photo: D. Cohen

Along the Mediterranean coasts of Spain and Italy there have been recent reports of the appearance of the solitary ascidian *Microcosmus squamiger* (Michaelsen 1927) (Naranjo et al. 1996, Mastrototaro & Dappiano 2005). This species is able to form dense, monospecific crusts that outcompete native species in shallow water communities (Turon et al. 2007). In addition, nine cosmopolitan and/or pantropical species have become established and colonized some sectors of the Mediterranean (Izquierdo-Muñoz et al. 2007).

Although it is believed that diverse communities are highly competitive and therefore more resistant to invasion (Stachowicz et al. 1999, Kennedy et al. 2002), the rapid spread of several NIA species in various coral reefs has been reported (Lambert 2002). In a study conducted in Guam, it was noted that all the 15 known NIA species in this area were found on artificial surfaces. On the natural reefs only three species were found and they were small, sparse, and formed only a negligible part of the reef fauna (Paulay et al. 2001, Lambert 2002).
Increased recreational sailing and the proliferation of marinas in recent decades, provide additional sites for colonization of non-indigenous ascidians, even those with low dispersal abilities. The accumulating evidence for the negative impact of non-indigenous species on local species and habitats (Cohen et al. 2005, Blum et al. 2007, Dijkstra et al. 2007a) raises the necessity for long-term studies that will combine regular monitoring of natural versus artificial habitats, and the use of molecular genetic tools that will allow the identification of sources, patterns of dispersal, and degree of gene flow with local forms.

1.4 Coral reef ascidians

In general, ascidians constitute a minor benthic component on exposed surfaces of the natural coral reefs. They are often found in cryptic environments such as grottos, crevices and the sides or undersides of rocks and corals. In exposed sites, solitary species frequently protect themselves better than colonial species from the dangers of predation, abrasion and physical damage. In such environments their rigid tunic is often covered by epibionts, which provide camouflage and physical protection (Monniot et al. 1991). Some colonial species of the family *Didemnidae* that host the photosynthetic prokaryote symbiont *Prochloron*, thrive on surfaces exposed to high irradiance on the reef flat (Kühl & Larkum 2002). The *prochloron* symbiont was originally described as a unicellular alga (Zoochlorellae), found hosted in *Trididemnum symbioticum* tissue collected at Marsa Abu-Zabad, Gulf of Aqaba (Pèrès 1962). Since the *prochloron* symbiont has since been found in the larvae of several coral reef Didemnids, it is possible to observe the tadpole larvae and follow their dispersal and settlement patterns (Olson 1985, Stoner 1994). It was found that in coral reef habitats ascidian larvae prefer dead coral skeletons (van Duyl et al. 1981, Stoner 1994) and shaded areas (Hurlbut 1993, Oren & Benayahu 1998) for
settlement. In addition, both colonial and solitary species successfully “foul” various artificial substrates such as jetties and other man-made substrata adjacent to the natural coral reef (Oren & Benayahu 1998, Paulay et al. 2001).

Recent changes in environmental conditions in coral reefs worldwide also affect the ascidian fauna. For example, Bak et al. (1996) reported a 900% increase in densities of *Trididemnum solidum*, a colonial ascidian, along the 84 km of fringing reef in Curacao over 15 years. Witman & Smith (2003) reported a three-fold increase in ascidian biomass over one year in an upwelling site in the Galápagos. In contrast, Kelmo et al. (2006) reported a mass mortality of coral reef ascidians in Brazil following the 1997/1998 El Niño event.

Ascidians control the removal of particulate organic nitrogen from coral reef waters (Ribes et al. 2005). Consequently, it is suggested that increased human activity will result in a marine environment less favorable to corals, in which the ascidian would have a competitive advantage (Bak et al. 1996). Considering the high rate of degradation that coral reefs are exhibiting worldwide (Wilkinson 2002), a rapid spread of ascidian species and the arrival of non-indigenous species is expected in the future, emphasizing the need for additional research focusing on the role of ascidians in coral reef ecosystems.

1.5 Research goals

The present research investigates biological and ecological aspects of the ascidian community along the Israeli coasts of the Red Sea and Mediterranean, relating to the current ascidian fauna found in those regions, the arrival of non-indigenous species to the Mediterranean coast, the recruitment patterns to an artificial marine structure in a coral
reef environment and, finally, the spread of a newly described colonial ascidian in the coral reefs of Eilat.

- Section I of the study is entitled: **The ascidian fauna along the Israeli coasts.** This section describes the dominant ascidian species found along the Red Sea coast of Israel, including a morphological description of the new species *Botryllus eilatensis* (published in *Zootaxa* 1256: 11-19, appendix I) and an up-to-date species list of non-indigenous ascidians found along the Mediterranean coast of Israel.

- Section II of the study is entitled: **The solitary ascidian Herdmania momus: native (Red Sea) vs. non-indigenous (Mediterranean) populations.** This section offers a comparative study of the spatial distribution, reproduction strategy and physiological parameters of the solitary ascidian *Herdmania momus* (published in *Biological Invasion* online 30 January 2008), including molecular data analysis of Mediterranean and Red Sea populations.

- Section III of the study is entitled: **Ascidian recruitment patterns on an artificial reef in Eilat (Red Sea).** This section examines the effects of season and spatial distribution on ascidian recruitment to artificial marine structures in a coral reef environment (published in *Biofouling* 24(2): 119-128).

- Section IV of the study is entitled: **Population dynamics of a coral reef ascidian in a deteriorating environment.** This section focuses on the seasonal appearance, reproductive strategy and effect of high nutrient levels on the population of the newly-described colonial ascidian *Botryllus eilatensis* in the coral reefs of Eilat (accepted to *Marine Ecology Progress Series*).
The ascidian fauna along the Israeli coasts

2.1 Introduction

For the past few decades, scientists and environmental organizations around the world have been trying to find a way to slow the rate of loss of animal species. Although extinction may be a natural process, human-based activities such as pollution, global warming and the introduction of non-indigenous species are dramatically accelerating the species loss. One of the major caveats in ascertaining whether species loss is indeed occurring is the lack of knowledge regarding how many species actually exist. Nowadays, only a few professional taxonomists are able to identify a new species. This is especially true in the field of ascidian taxonomy, which has remained the domain of very few experts.

Thus, the ascidian fauna (Phylum: Chordata, Class: Asciidiacea) found along the Gulf of Eilat, and the Mediterranean coast of Israel is poorly known. The sole list of ascidians in these regions was compiled by Pérès over 40 years ago (1958a,b, 1962), and many questions remain open concerning the validity of some species, the geographical distribution of others, and the arrival of invasive species. For example, Pérès (1962) describes only 19 species from Eilat (Table I). This list is certainly incomplete considering the number of ascidian species (approximately 40) reported prior to then from the Red Sea (Michaelsen 1918, 1920a,b). The relatively low number of ascidian species described from Eilat thus appears to be a result of insufficient research and sampling efforts.
On the other hand, the study of the ascidian fauna of the Mediterranean began at the end of the 19th century. However, due to synonymies and the description of new species the actual number of ascidian species in the Mediterranean is still uncertain. According to Fredj & Laubier (1985), of the 200 recorded species probably no more than 160 correspond to true or valid taxa. The ascidian fauna of the Eastern Mediterranean has recently been updated (Koukouras et al. 1995). Only 45 species (which according to Koukouras et al. comprise 24.1% of the total Mediterranean ascidians) are known from the Levantine Basin (Seeliger & Hartmeyer 1906-11, Harant 1939, Harant & Verniers 1933, Pérès 1958a,b,c, Monniot 1965,1969, Lafargue 1974a). All these species have been found only along the coasts of Israel and Egypt (Table II). Pérès (1958b) describes 33 species distributed among nine families found along the Mediterranean coasts of Israel. He particularly stated the extreme scarcity of species from the family *Polyclinidae*, the absence of species from the family *Molgulidae* and the presence of four introduced species from the Red Sea.

Since ascidians thrive on both stationary and moving artificial marine structures, such as marina floats, pilings, buoys, and boat bottoms, they have a high potential for introduction into new regions. Por (1978) listed seven ascidian species as Lessepsian migrants (i.e. from Red Sea to the Mediterranean). However, there is a controversy regarding this list since some of the species are cosmopolitan, and some are widely distributed in the western Mediterranean (Zibrowius 1991, Koukouras et al. 1995). Recently Izquierdo-Muñoz et al. (2007) listed five non-indigenous species from the Mediterranean coast of Israel but did not refer to their origin. Nevertheless, considering the low indigenous biodiversity along the Mediterranean coast of Israel (Fishelson 2000),
and the ongoing changes such as rise in seawater temperature and salinity, the arrival and proliferation of non-indigenous ascidians is expected in the future. In light of the above, in order to be able to document in the future the arrival and spread of non-indigenous species, it is imperative to continue to investigate the ascidian fauna currently found along the coasts of Israel and to create an up-to-date species list of the region.

2.2 Materials and Methods

Ascidian samples were collected from different sites along the Israeli coast (Mediterranean and Red Sea) using SCUBA and snorkeling. Upon sampling, the ascidians were narcotized with menthol crystals in a closed jar in order to prevent evaporation of the menthol. Only after the samples were fully relaxed (determined by inserting a sharp probe into an open siphon and getting no response), the samples were transferred to a jar with seawater/formalin fixative after rinsing the menthol crystals from the animals’ body. The fixative was prepared according to the following formula for 1 liter of fixative use: 100 ml of full-strength formaldehyde, 850 ml of seawater, and 50 ml of distilled water. One gram of sodium borate was added to the mixture and the solution was then mixed with a magnetic stirrer. In order to allow DNA analysis a small portion of each sample was directly preserved in absolute ethanol.

Dissections were stained with hemalum and mounted on permanent slides. Taxonomic identification was carried out using authoritative keys and texts (e.g. Van name 1921, 1931, 1945, Kott 1985, 1990, Monniot et al. 1991, Kott 1992, 2001, Monniot & Monniot 2001). The entire collection is part of the National Collections of Natural History, Tel Aviv University, Israel. In addition, other preserved material available from museum collections in Israel was studied (Table 1.1,1.2).
2.3 Results

A survey of the available preserved material from the Tel Aviv Zoological Museum, combined with the number of species described from the Israeli coasts in the literature, results in an estimation of 27 species from the Gulf of Eilat and 58 species from the Mediterranean coast (Table I,II).

Table 1.1- Ascidians of the Gulf of Eilat (27 species, including the Sinai Peninsula)

<table>
<thead>
<tr>
<th>Species name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaroucium (Aplidium) lobatum</em></td>
<td>Pérès 1962, Fishelson 1971, Tel Aviv Zoological Museum</td>
</tr>
<tr>
<td><em>Ascidia cannelata</em></td>
<td>Tel Aviv Zoological Museum</td>
</tr>
<tr>
<td><em>Ascidia savignyi</em></td>
<td>Pérès 1962</td>
</tr>
<tr>
<td><em>Botryllus rosaceus</em></td>
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</tr>
<tr>
<td><em>Cnemidocarpa mitiligera</em></td>
<td>Tel Aviv Zoological Museum</td>
</tr>
<tr>
<td><em>Didemnum candidum</em></td>
<td>Pérès 1962, Fishelson 1971</td>
</tr>
<tr>
<td><em>Didemnum granulatum</em></td>
<td>Oren &amp; Benayahu 1998</td>
</tr>
<tr>
<td><em>Diplosoma listerianum</em></td>
<td>Pérès 1962</td>
</tr>
<tr>
<td><em>Distapliastylifera</em></td>
<td>Pérès 1962</td>
</tr>
<tr>
<td><em>Ecteinascidia conchlini f. minuta</em></td>
<td>Pérès 1962, Fishelson 1971</td>
</tr>
<tr>
<td><em>Ecteinascidia thursoni</em></td>
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<tr>
<td><em>Halocynthia spinosa</em></td>
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<tr>
<td><em>Herdmania momus</em></td>
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</tr>
<tr>
<td><em>Metrocarpa magnicoecum</em></td>
<td>Pérès 1962</td>
</tr>
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<td><em>Metrocarpa nigrum</em></td>
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<td><em>Phallusia arabica</em></td>
<td>Pérès 1962, Fishelson 1971</td>
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<td><em>Phallusia nigra</em></td>
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<td><em>Polycarpa mytiligera</em></td>
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<td><em>Pyura gangelion</em></td>
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<td><em>Pyura pantex</em></td>
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<td><em>Rhodosoma verecundum</em></td>
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<td><em>Styela canopus</em></td>
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<td><em>Symplegma viridis</em></td>
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<td><em>Trididemnum savignyi</em></td>
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Table 1.2- Ascidians of the Mediterranean coast of Israel. “*” Species that were listed as Lessepsian migrants (Por 1978)

<table>
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<table>
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<td>Aplidium conicum</td>
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<td>Aplidium fuscum</td>
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<tr>
<td>Aplidium griseum</td>
<td>Koukouras et al. 1995</td>
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<td><em>Amaroucium (Aplidium) lobatum</em></td>
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</tr>
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<td>Ascidia aspersa</td>
<td>Pérès 1958</td>
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<td>Ascidia cannelata *</td>
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<td>Ascidia conchilega</td>
<td>Fishelson 2000</td>
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<td>Ascidia malaca</td>
<td>Pérès 1958</td>
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<td>Ascidia mentula</td>
<td>Pérès 1958, Koukouras et al 1995</td>
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<td>Ascidia aspersa</td>
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<td>Asciella scabra</td>
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<td>Corella parallelogramma</td>
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<td>Cystodytes dellechiaei</td>
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<td>Didemnum candidum</td>
<td>Pérès 1958, Koukouras et al. 1995</td>
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<td>Didemnum fulgens</td>
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<td>Didemnum maculasum</td>
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<td>Distaplia magnilarva</td>
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<td>Ecteinascidia turbinata (=moorei)*</td>
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<td>Eudistoma rubrum</td>
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<td><em>Eusynstyela hertmeyeri</em></td>
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<td>Halocynthia papillosa</td>
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<td><em>Molgula helleri</em></td>
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<td><em>Polysyncraton lacazei</em></td>
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<td><em>Pyura dura</em></td>
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<td><em>Rhopalopsis hartmeyeri</em></td>
<td>Pérès 1958, Koukouras et al 1995</td>
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<td><em>Rhopalopsis orientalis</em></td>
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<td><em>Styela partita</em></td>
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<td><em>Styela plicata</em></td>
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<td><em>Trididemnum fallax</em></td>
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<td><em>Trididemnum graphicum</em></td>
<td>Koukouras et al. 1995</td>
</tr>
<tr>
<td><em>Trididemnum tenerum</em></td>
<td>Pérès 1958</td>
</tr>
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</table>

### 2.3.1 Red Sea Ascidians

Out of the 250 individuals collected from the Red Sea, 13 dominant species were identified, including a new species *Botryllus eilatensis* (Figure 2.1 a,b). A field survey
revealed that ascidians are mostly common on artificial substrates such as jetties, buoys and ropes, and can also be found in the natural reef overgrowing dead corals and under rock pebbles. All the species were recorded from both natural and artificial substrates and at a depth range of 0 – 30 m.

A. Order Aplousobranchia

Family Didemnidae

Didemnum candidum (Savigny, 1816)

Material examined: AS25248, Figure 2.1c

Literature for identification: Lafargue 1974b

External appearance: This species was described originally from the Gulf of Suez and later on from the Gulf of Aqaba (Lafargue 1974b). Colonies are thin (1mm thick) with a smooth surface. The color of living colonies varies from white to yellow and orange, always white in preservative. The white spicules are diverse and are sometimes crowded into clumps that form opaque patches on the surface.

Distribution in the Gulf of Eilat: Most common on corals and rock pebbles in the natural reef at a depth range of 0-30m.

Didemnum granulatum (Tokioka, 1954)

Material examined: AS25249, Figure 2.1d

Literature for identification: Kott 2001

External appearance: Colonies are hard and thin with a dense layer of spicules. Living colonies are light pink or orange-red and may create large mats especially over artificial substrate. The relatively small white spicules can be seen through the tunic. Small
spicule-filled papillae protrude from the surface of the colony, which can be hard and brittle.

Distribution in the Gulf of Eilat: Most common on artificial substrates such as supporting pillars and floating buoys at a depth range of 5-30 m.

Family Diazonidae

*Rhopalaea sp.*

Material examined: AS25230, Figure 2.2a, 2.3

Literature for identification: Monniot & Monniot 2001

External appearance: This solitary species is distinguished by its large transparent pharynx through which the longitudinal vessels and rows of stigmata can be seen. The abdomen is separated from the thorax by a narrow oesophageal neck and is attached by a root-like test to the substrate (Figure 2.3). The siphons are very short and close together.

Remarks: This species differs from any Pacific species by the small number of thoracic muscles (6) that are not ramified (as in *R. crassa*).

Distribution in the Gulf of Eilat: This species is often found at depths greater than 20 m, under overhangs and coral bases.
**Figure 2.1** *Rhopalaea sp.* from Eilat, Red Sea.

**B. Order Phlebobranchia**

Family Ascidiiidae

*Ascidia cannelata* (Oken, 1820)

Material examined: AS25229, Figure 2.2b

Literature for identification: Oken, 1820, Pérès 1958b

External appearance: Solitary species with a transparent-gelatinous tunic. The body is oval with siphons parallel and darker than the rest of the tunic. One side of the body is completely attached to the substrate.

Distribution in the Gulf of Eilat: Common on the undersides of rocks in shallow (0-10m) waters.

*Phallusia nigra* (Savigny, 1816)

Material examined: AS25250, Figure 2.2c

Literature for identification: Van Name 1921
External appearance: Individuals are easily recognized by the shiny black dark-blue tunic that is free from epibionts. The body is oval with elongated siphons. Specimens can reach 10 cm in length.

Distribution in the Gulf of Eilat: Widely distributed and common in shallow depths overgrowing both corals and artificial substrates.

*Phallusia arabica* (Savigny, 1816)

Material examined: AS25232, Figure 2.2d

Literature for identification: Kott 1985

External appearance: This species was described originally from the Red Sea. Individuals are robust, up to 15 cm long. They are often narrow and almost cylindrical. Living specimens are translucent grey or light brown with shining yellow ocelli between the branchial and atrial lobes. The test is thinner where the body adheres to the substrate, but everywhere else it is thick, firm and rigid with a smooth surface.

Distribution in the Gulf of Eilat: Found on shaded areas such as coral basis, overhangs and crevices at depth of 10-50 m.

Family Corellidae

*Rhodosoma turcicum* (Savigny, 1816)

Material examined: AS25233, Figure 2.2e

Literature for identification: Kott 1985

External appearance: This species is characterized by the remarkable horizontal fold of the body that acts as a lid over the apertures. It is operated by the highly adapted body
musculature. Individuals are upright, oval in section, slightly laterally flattened. The test is firm, gelatinous and translucent. Individuals are up to 5 cm high and 2 cm wide. Distribution in the Gulf of Eilat: Can be found in cryptic areas on both artificial and natural substrates such as the underside of rocks at a depth range of 10 – 50 m.

**C. Order Stolidobranchia**

Family Styelidae

*Botryllus eilatensis* (Shenkar and Monniot, 2006)

Nov. sp.

Full description is given in Appendix I.

*Eusynstyela latericius* (Sluiter, 1904)

Material examined: AS25244, AS25245, AS25247, Figure 2.1e,f

Literature for identification: Kott 1985

External description: The colonies form flat mats up to 3 mm thick with zooids opening on the upper surface. The zooids can be either embedded entirely in a common test (figure 2.1 e) or connected only at their base when the colony is juvenile (figure 2.1f). The test is always tough and bare, and color may vary from red, orange to pink. A pale stripe along each side of the mid-line between the apertures sometimes creates a symmetrical pattern on the surface.

Distribution in the Gulf of Eilat: Colonies are common on both natural and artificial substrate on exposed surfaces from shallow to 20 m depth.

*Polycarpa mytiligera* (Savigny, 1816)

Material examined: AS25235, Figure 2.2h
Literature for identification: Monniot & Monniot 1987

External description: Solitary globular body, 5 to 10 cm across. The tunic is brown, often covered by epibionts. The interior surface of the siphon is white.

Distribution in the Gulf of Eilat: Very common in the natural environment on coral heads at shallow depths (5-10m).

Family Pyuridae

*Herdmania momus* (Savigny, 1816)

Material examined: AS25253, Figure 2.2f

Literature for identification: Nishikawa 2002

External appearance: Individuals are almost spherical with cylindrical or trumpet-shaped siphons that are lined with red-pink and white bands in living specimens. The test is smooth, rosy-peach in colour. The siphons are turned away from each other.

Distribution in the Gulf of Eilat: This solitary species can be found in the natural environment under rocks and on the basis of corals and is most common on artificial substrate from very shallow to 15 m depth.

*Boltenia* sp.

Material examined: AS25231, Figure 2.2g

Literature for identification: Monniot et al. 1991

External description: Small individuals (3 cm) with a dark brown tunic covered with epibionts. The body is globular and the siphons short.
Distribution in the Gulf of Eilat: This species was collected only from very shallow waters (less than 1 m) overgrowing bouys in the north beach area, often found in dense aggregations.

*Halocynthia spinosa* (Sluiter, 1905)

Material examined: AS25230, Figure 2.2i

Literature for identification: Monniot 2002

External description: This solitary species is characterized by the stiff spines that are especially long around the apertures. The body is rounded or oval to spherical with small-cylindrical or barrel-shaped siphons. Color of living specimens is usually white or light-orange.

Distribution in the Gulf of Eilat: This species is most common in the natural environment growing among live coral branches.

*Pyura gangelion* (Savigny, 1816)

Material examined: AS25234, Figure 2.2j

Literature for identification: Monniot 2002

External description: Individuals are dark brown with a rough rigid tunic often covered with epibionts. The body is very musculature and when disturbed the animal is able to strongly contract, making it very difficult to separate it from the substrate. The siphons are short, well separated by four lobes.

Distribution in the Gulf of Eilat: Common in the natural environment and on artificial substrate at depths of 5-20 m.
Figure 2.2 Common colonial ascidian species found along the Red Sea coast of Israel at a depth range of 0 – 30 meters. a,b) Both color morphs of *Botryllus eilatensis* (Shenkar and Monniot, 2006) c) *Didemnum candidum* (Savigny, 1816) d) *Didemnum granulatum* (Tokioka, 1954) e) *Eusynstyela latericius* (Sluiter, 1904) morph I (photo: A. Gur) f) *Eusynstyela latericius* morph II.
Figure 2.3 Common solitary ascidian species found along the Red Sea coast of Israel at a depth range of 0 – 30 meters. a) *Rhopalaea sp.* b) *Ascidia cannelata* (Oken, 1820, circled
in red) c) *Phallusia nigra* (Savigny, 1816) d) *Phallusia arabica* (Savigny, 1816) e) *Rhodosoma turcicum* (Savigny, 1816) f) *Herdmania momus* (Savigny, 1816) g) *Boltenia* sp. h) *Polycarpa mytiligera* (Savigny, 1816) i) *Halocynthia spinosa* (Sloiter, 1905) j) *Pyura gangelion* (Savigny, 1816).

### 2.3.2 Non-indigenous ascidians along the Mediterranean coast of Israel

Field surveys recorded the occurrence of six non-indigenous ascidians along the Mediterranean coast of Israel. From the order Phlebobranchia the solitary species *Ascidia cannelata*, *Phallusia nigra* and *Rhodosoma turcicum* have been recorded. From the order Stolidobranchia *Symplegma brakenhielmi*, *Herdmania momus* and *Microcosmus exasperatus* have been recorded, *S. brakenhielmi* is the only colonial species from the non-indigenous fauna. *H. momus* and *M. exasperatus* were found only on artificial substrate such as along the Electric Company pier at Hadera, while the other species were also sampled from the natural environment.

*Microcosmus exasperatus* (Heller 1878)

Material examined: AS25237

Literature for identification: Kott 1985, Turon et al. 2007

External appearance: Solitary globular species with long siphons and leathery bright orange tunic with a few encrustations around the base. Characterized by unique siphonal spines.

Distribution along the Mediterranean coast of Israel: Typical to artificial substrates, forming dense aggregations at the Electric Company pier at Hadera.
Symplegma brakenhielmi (Michaelsen 1904)

Material examined: AS25251, AS25252, Figure 2.3

Literature for identification: Monniot & Monniot 1997, Çinar et al. 2006

External appearance: Red colonies about 5 cm in diameter. The zooids are embedded in a common tunic, with the two very short siphons opening for each zooid creating a beehive appearance.

Distribution along the Mediterranean coast of Israel: Common on artificial substrate at the Electric Company pier at Hadera and also in the natural environment at shallow depth (< 5m) along the northern shores of Israel (Achziv) and in the south (Palmahim).

Figure 2.4 Symplegma brakenhielmi from Achziv, Mediterranean coast of Israel, 3 m depth
2.4 Discussion

The current study is the first attempt to compile an up-to-date species list of the ascidian fauna along the coasts of Israel. However, the present list, which includes 14 species from the Red Sea and six non-indigenous species from the Mediterranean, provides but partial information as to the overall species composition along the coasts of Israel. The species currently described are only those species that can be sampled using SCUBA, and as such come from a limited depth distribution and from limited sites that can be easily accessed and are not polluted. Nevertheless, the study emphasizes the need for an essential and basic revision of the ascidian fauna of this region in the future, using different sampling methods (benthic sledges, grabs) and at various sites (including polluted sites).

The little attention that the ascidian fauna has drawn in past years is especially evident in the coral reefs of Eilat, where one of the most common colonial species was found to be a new species, *Botryllus eilatensis*, described for the first time in the current study (App. I). This species has been observed in the coral reefs of Eilat by marine biologists for many years but had remained unidentified until now (L. Fishelson personal communication). Interestingly, two solitary species, *Rhopalaea* sp. and *Boltenia* sp., found in Eilat are also suspected to be new species (F. Monniot personal communication). In addition, the colonial species *Eusynstyela latericius* has been recorded for the first time from this region.

The disparity between the current species list and the list published by Pérès (1962) from the coral reefs of Eilat may be due to several reasons: 1) Pérès’s list includes six species collected from the mangroves of Abu Zabad (Sinai Peninsula), an area with different environmental parameters from those of the northern Gulf; 2) the ongoing environmental
changes taking place in this region over the past 30 years, such as increased
eutrophication (Loya 2007), may have altered the ascidian fauna of the region; and 3)
inaccurate identification of some of the species in the past.

The only species collected solely at a very shallow depth from the marine sport jetty in
the north beach area, and absent completely from the natural reefs of Eilat, was the
solitary species *Boltenia* sp.. This genus is common in cold waters and only one
representative occurs in warm water and not in a coral reef environment (Nishikawa
1991). This is the first record of this genus from the Red Sea. Recent publications suggest
that, in coral reef areas, non-indigenous species are more common on artificial substrates
and harbors (to which they are often introduced as a part of the fouling community) than
are believed to be less susceptible to marine pest invasions, due to the high diversity
found in tropical communities that offer fewer opportunities for introduced organisms to
occupy and compete in their new habitats on arrival (Coles et al. 1999, Hutchings 2002).
However, a recent arrival of non-indigenous species to deteriorated coral reefs in Hawaii
has been reported by Coles and Eldredge (2002). Hence, although non-indigenous species
are relatively rare in tropical areas in comparison to temperate areas, coral reefs are not
immune to species bioinvasions, and under appropriate environmental conditions
introductions of non-indigenous species can and do occur on or near coral reefs (Coles &
Eldredge 2002, Lambert 2002). Therefore, it is possible that *Boltenia* sp. reported in this
study for the first time in the Gulf of Eilat is an introduced species.

Non-indigenous ascidians were more common along the Mediterranean coast of Israel.
Six non-indigenous species were recorded during the current study: *Phallusia nigra,*
Herdmania momus, Microcosmus exasperatus, Rhodosoma turcicum, Ascidia canellata, and Symplegma brakenhielmi. This list corresponds to studies of non-indigenous ascidians elsewhere in the Mediterranean Sea (Streftaris et al. 2005, Çinar et al. 2006, Izquierdo-Muñoz et al. 2007, Turon et al. 2007). These species probably arrived into the Mediterranean via the Suez Canal, since all have an extra-tropical Indo-Pacific distribution and a restricted distribution in the Eastern Mediterranean. The current list partly corresponds to Por’s (1978) original description of “Lessepsian ascidians” (based on Pérès 1958), as only those species listed as “High probability Lessepsian migrants” (P. nigra, H. momus, A. canellata and S. brakenhielmi= misidentified as S. viride) were also collected in the current study.

Interestingly, the solitary species H. momus and M. exasperatus were recorded from the Mediterranean coast on artificial substrates only, which supports the hypothesis that non-indigenous ascidians thrive on artificial substrates but often fail to establish communities on natural substrates (Lambert 2002). Records of M. exasperatus in the Mediterranean have been recently revised by Turon et al. (2007), revealing a very restricted distribution in the Eastern Mediterranean of this species, suggesting that it is a Lessepsian migrant. M. exasperatus was recorded in the Mediterranean only from the coasts of Lebanon and Tunis and has been found in Suez and the Gulf of Aden (Monniot 2002).

Another peculiarity in the non-indigenous species found along the Mediterranean coast of Israel is the occurrence of only one colonial species, S. brakenhielmi. This species is distributed worldwide in warmer seas, especially in harbors where it grows on man-made structures (Lambert & Lambert 1998). In the Levantine basin it was reported from the Lebanon coast (Bitar & Kouli-Bitar 1995) and Turkey (Çinar et al. 2006). It is possible
that since in solitary ascidians fertilization and larval development usually occurs in the water (in contrast to colonial species which are usually brooders), they have a higher potential for dispersal to more distant locations. Nevertheless, it has been suggested that several colonial species have been introduced worldwide by hull fouling and aquaculture (Lambert 2002, Dijkstra et al. 2007b). Considering the increased recreational sailing in the Mediterranean, along with aquaculture development, the continued arrival of non-indigenous ascidians, even with low dispersal abilities, is anticipated.

During the past two decades enormous progress has been achieved in the fields of development, evolution, immunology, and ecology of ascidians. However, taxonomic studies of the ascidian fauna around the world are rare. The management and use of these organisms is absolutely dependent on understanding their taxonomy, the integrative basis of biology. It is taxonomic investigation that will reveal their characteristics, life history and relationships, their geographic range and the selective pressures affecting each taxon. Their significance in the Mediterranean and Red Sea waters in particular, is amplified by the fact that they are especially diverse in warm temperate and tropical locations, providing an exceptional opportunity for future taxonomic studies.
The solitary ascidian *Herdmania momus*: native (Red Sea) vs. non-indigenous (Mediterranean) populations

3.1 Introduction

One of the most extensively documented marine bioinvasion phenomena is the invasion of Red Sea species to the Mediterranean Sea through the Suez Canal (Lessepsian migration, Por 1978 also referred to as Erythrean invasion, Galil 2000). Since the opening of the Suez Canal in 1869, more than 300 Red Sea species of algae, invertebrates and fish have entered the Mediterranean through the canal (Safriel & Ritte 1986, Spanier & Galil 1991, Barash & Denin 1992, Lotan et al. 1994, Golani 1998, Galil 2000). For example, the invasive jellyfish *Rhopilema nomadica* appears in mass swarms along the Mediterranean coasts of Israel every summer, causing severe damage to coastal fishing, tourism and coastal installations (Spanier & Galil 1991). The Red Sea mussel *Brachidontes pharaonis* forms massive beds and is believed to have displaced the local species *Mytilaster minimus* (Rilov et al. 2004). Other Lessepsian migrants, mostly fish, may be of economic importance, as they may be exploited commercially (Goren & Galil 2005).

While invasions of groups such as molluscs, crustaceans and fish have been studied extensively, little is known on Lessepsian migration of ascidians. Ascidians are sessile marine filter feeders with a short lived non-feeding motile larva (Berrill 1950, Millar 1971). They are rapid colonizers of artificial substrates such as marina floats, pilings, buoys, and boat bottoms in protected harbors, where there is reduced wave action and enhanced nutrients from anthropogenic activities (Naranjo et al. 1996, Oren &

Introductions of non-indigenous ascidians into harbors in both tropical and temperate waters are now commonplace, with the rate of introductions increasing yearly (Monniot et al. 1991, Lambert & Lambert 1998, Coles et al. 1999, Lambert 2002). Most of these non-indigenous species tolerate wide fluctuations in temperature, salinity, and even pollution (Sims 1984, Naranjo et al. 1996, Nomaguchi et al. 1997, Stachowicz et al. 2002b). The impact of non-indigenous ascidians on local species and habitats is currently being studied in several sites around the world (Lambert 2002, 2003, Stachowicz et al. 2002a). Despite this, to date there are only few studies in which life-history parameters have been compared between two geographically separated populations of the same species (Rocha et al. 1999) and no comparative study has been conducted between the source and the invasive populations. Such data is of great importance since it will provide tools for estimating invasion rates and possible effects on the natural fauna in the “invaded” site. For instance, higher winter temperatures may result in higher recruitment
rates and faster growth rates of non-indigenous species (Stachowicz et al. 2002b). In addition, since it is common to find in the branchial cavities of many solitary ascidians different crustaceans (Millar 1971, Monniot et al. 1991, Dalby 1996) the study of these “hitchhikers” in their new environment may provide unique information on the mode of invasion and the ecology of the immigrant's host.

Analysis of mitochondrial DNA (mtDNA) sequence data has proven to be a useful tool for tracing recent evolutionary history such as founder events, population bottlenecks and phylogeography in marine organisms (Gopurenko et al. 1999, Shefer et al. 2004, López-Legentil & Turon 2007). The mitochondrial gene cytochrome oxidase subunit I (COI) has been used in many phylogeographic studies of ascidians due to its high degree of variability (Tarjuelo et al. 2001, 2004, Turon et al. 2003, Turon & López-Legentil 2004, López-Legentil & Turon 2006). According to Por (1978) Lessepsian migrants most probably constitute those species that have a continuous distribution from the Gulf of Suez to the Mediterranean. On the other hand, the presence of a species in the Red Sea, the Suez Canal and the Mediterranean coast does not necessarily imply a sequential path of introduction. A natural northbound dispersal would result in Mediterranean populations comprised of haplotypes from the Gulf of Suez and the Red Sea. In addition, genetic distance should correlate with the geographical distance (Shefer et al. 2004). Knowledge of the genetic structure and the phylogeographic patterns of an introduced species may help track human-mediated dispersal pathways and understand the modes of arrival and future dispersal of a non-indigenous species.

The solitary ascidian *Herdmania momus* (Savigny 1816) is common in the Red Sea including the Gulf of Suez, Aqaba and Aden. In the eastern Mediterranean it has been
documented from Israel, Lebanon and Cyprus (Nishikawa 2002). It was first recorded in the Suez Canal at 1924 by Harant (1927) and in the eastern Mediterranean in 1958 (Pérès 1958) and is considered as a Lessepsian migrant (Por 1978). The proximity of the Red Sea coral reef environment and the Israeli Mediterranean rocky shore, together with the recent (in evolutionary terms) opening of the Suez Canal, provides fertile ground for comparative studies of ascidian distribution and migration. The aim of this study is to investigate possible differences in life history strategies of two populations of the ascidian *H. momus* - the indigenous population in Eilat, Red Sea and an invasive population in Hadera, Mediterranean Sea. We hypothesize that differences in environmental factors such as, food availability, water temperature ranges, currents and differential exposure to wave action will have a strong effect on the spatial distribution, body size, reproductive cycles and symbiotic relationships of *H. momus* in the Mediterranean Sea. In addition, we used mtDNA data to explore genetic diversity and structure in order to understand the arrival modes of *H. momus* into the eastern basin of the Mediterranean Sea.

### 3.2 Materials and Methods

The study was conducted during 2005-2006 in two study sites: 1. the pier of the Electric Company in Hadera, Mediterranean coast of Israel: 32°28’16’’N, 34°53’06’’E and 2. the Eilat-Ashkelon pipeline pier in Eilat, Red Sea 29°31’ N 34°55’ E (Figure 3.1a). In both sites supporting pillars distributed from the shore to a 25m depth inhabited by the solitary ascidian *Herdmania momus*. Water temperature in Eilat varies from 20 to 27ºC, and salinity is ca.40.5 ‰ throughout the year (Loya 2004). In Hadera, water temperature varies from 17 to 30ºC and salinity is ca. 39.0 ‰ throughout the year (Por 1978). During
the study sea water temperature for the Hadera site was obtained from the Geology and Coastal Processes Department, Israel Oceanographic & Limnological Research (IOLR) and for the Eilat site from The National Monitoring Program (NMP, http://www.iui-eilat.ac.il).

In addition to taxonomic identification of the ascidians based on morphological characters, a successful cross fertilization experiment was conducted between six pairs of *H. momus* individuals from the Mediterranean and Red Sea sites following Degnan et al. (1996) suggesting the populations represent the same species.

Between March 2005 and May 2006, monthly samples (n=5-9) were randomly collected from each site. The samples were narcotized with menthol crystals for up to three hours and fixed in 4% formalin in filtered sea water. Each of the samples was lightly blotted on absorbent paper and wet weights of the whole animal, its tunic and gonads were weighed to the nearest 0.001g. The gonad index (in percentage, GI) was calculated as the ratio of gonad weight to the whole animal weight. In addition, the samples were photographed with Sony T-1 camera and the “length” of the animal was measured as the distance between the siphons nearest edge along the mid-ventral line (Figure 3.1b) using UTHSCSA Image Tool for Windows version 3. In order to observe the symbionts inhabiting the branchial sac the samples were opened along the endostyle. The branchial sac was examined under a dissecting microscope and the presence of crustaceans was recorded.

To assess reproductive state of the studied population, each month gonads from three individuals from each site were further processed for histology. The gonads were repeatedly washed with running fresh water and maintained in 70% Ethanol. They were
prepared for histological examination using a Citadel 2000 Tissues Processor, embedded in paraffin, sectioned and stained in Hematoxylin-Eosin. The slides were photographed using Nikon Digital Sight DS-L1 and scanned to JPG format. The diameter of each oocyte which appeared sectioned at the nucleolus level was measured using CPCe (Kohler & Gill 2006) program. Whenever possible, a total of one hundred oocytes from each individual were measured.

In order to study the distribution of *H. momus*, field surveys were conducted using SCUBA along the Mediterranean and Red Sea coasts of Israel (Figure 3.1a). The presence of *H. momus* was documented along with the substrate type. To ascertain the recruitment density of *H. momus* along a depth gradient, 12 ceramic plates 20 X 20 cm each, were attached in August 2004 at 5, 10 and 15m depth to a supporting pillar at each site. The plates in Eilat were removed after 4 months of submersion and in Hadera after 6 months. After removal the panels were immediately transported in seawater to the lab and examined under a dissecting microscope. The number of *H. momus* individuals was recorded on each plate.

During August 2005 ascidian density was examined in Hadera using a 50 X 50 cm quadrat. Twenty quadrats were placed around the pillars at 5, 10, 15 and 20m depth. The number of *H. momus* and other dominant species was recorded in each quadrat. This method could not be used in Eilat due to a potential damage to the corals overgrowing the pillars.

All statistical analyses were carried out using Statistica 7. The data were tested for normality and homogeneity of variances. Statistics of gonad indices were carried out after arcsin transformations. In order to ascertain if there are significant differences in GI
throughout the study period a one way ANOVA was carried out for each of the sites. A nested design ANOVA (oocyte diameters nested within individuals) was used in order to test possible differences in oocyte diameter during the study for each site. Fisher’s least significant differences (LSD) tests were used as post hoc comparisons when significant differences were detected. Separate t-tests were performed in order to test differences in GI measurements between the sites at each month. Results are presented as averages ± standard errors throughout the text unless denoted otherwise.

DNA analysis

A total of six populations of *H. momus* were collected from the Mediterranean and Red Sea coasts of Israel and from Cyprus. Two were collected from the Red Sea coast of Israel (Eilat-Ashkelon pipeline pier in Eilat and fish cages 29°30.23’ N 34°55.14’ E), three from the Mediterranean coast of Israel (artificial reef in Nahariya  33°03’ N 35°06’ E, artificial reef in Haifa 32°49’ N 34°50’ E and Hadera electric company pier) and Famagusta Harbor, Cyprus (figure 3.1a). Sampling was undertaken in 2006 by SCUBA diving or snorkeling. All samples were kept in absolute ethanol at -4°C until used. In order to optimize DNA extractions, the gonads were separated from the body under a binocular microscope. DNA extraction was carried out with Biosprint 96 (Qiagen) and DNA was eluted in 200ul Qiagen elution buffer and stored in freezer. COI amplification was carried out on all samples in a 96 well PCR plate using dgCO1 Folmer primers (Folmer et al. 1994), 2mM MgCl₂ and 0.25 ug/ul BSA,. 1µl undiluted extracts were amplified using 48°C annealing temperature with 34 cycles. For sequencing, "Exosap cleanup" was carried out on the samples with 2µl of four fold diluted exosapit. Sequencing reaction was performed with 0.5µl big dye, dgCO1 primers and 1µl template
on CS 5025 PCR program. Reactions were cleaned using Millipore 384-well sequencing reaction clean up plate (Matrix automated pipettor) and were then sequenced. Data analyses were performed using ARLEQUIN version 2.000 (Scneider et al. 2000). Analysis of molecular variance (AMOVA, Excoffier et al. 1992) was performed by pooling the Nahariya, Haifa, Hadera samples (Mediterranean) together, and the fish cages and pipeline pier samples (Red Sea) together. The Cyprus samples were a separate group. Mantel test was performed using XLSTAT software.
Figure 3.1 (a) The study area, showing sampling location in Eilat (Red Sea) and Hadera (Mediterranean Sea) coasts of Israel. (b) Animal “length” measurements between the siphons along the mid-ventral line.
3.3 Results

3.3.1 Geographic and depth distribution of *Herdmania momus* along the Israeli coast

Field surveys revealed that *Herdmania momus* in Eilat (Red Sea) is common along the entire coast on artificial substrates (piers, artificial reefs) and in the natural environment, on the base of stony corals and underneath rock pebbles. In contrast, along the Mediterranean coast of Israel *H. momus* is restricted to artificial substrates and only few individuals were found on natural substrate.

In Eilat *H. momus* is common from shallow (< 1m) to 15m depth. There was no significant association ($r^2 = 0.06$, $y = -0.113x + 2.583$, $p > 0.05$) between *H. momus* recruits density to the settlement plates and water depth, although a higher density was found in 5 and 10 m depth in comparison to 15 m depth (Figure 3.2a). In contrast, in Hadera (Mediterranean Sea), no *H. momus* recruits were found in 5m depth. As water depth increases the density of *H. momus* recruits increases significantly ($r^2 = 0.88$, $y = 0.917x - 1.11$, $p < 0.05$) to $1.83 \pm 0.8$ individuals per plate at 15m depth and to $2.85 \pm 0.4$ individuals per quadrat at 20m depth ($r^2 = 0.81$, $y = 0.185x - 1.37$, $p < 0.05$, Figure 3.2b).

3.3.2 Size measurements

*Herdmania momus* measured from monthly samples at Hadera are significantly larger than at Eilat (t-test, $p < 0.01$, Figure 3.3). Thus, the total wet weight of the Mediterranean ascidians was $11.07 \pm 1.0$ g (n=93) in comparison to $3.9 \pm 0.3$ g (n=114) from the Red Sea, and the average *H. momus* length was $9.1 \pm 0.3$ cm in Hadera compared to $6.87 \pm 0.2$ cm in Eilat (Figure 3.3).
The maximum total wet weight of *H. momus* from Eilat was 17.5 g and 12 cm in length compared to *H. momus* from Hadera that reached 50.2 g total wet weight and 18 cm in length.

**Figure 3.2** Depth distribution of ascidians in Eilat (Red Sea) and Hadera (Mediterranean Sea). (a) Average number of *Herdmania momus* individuals (+ SE) per plate (20 x 20 cm, n = 12) at 5, 10, 15 m depth in Eilat (black bars) and Hadera (blank bars). (b) Average number of individuals for ascidian species (+ SE) per quadrat (50 x 50 cm, n = 20) at 5, 10, 15, 20 m depth in Hadera.
3.3.3 Occurrence of symbionts

In 38% of the samples from Hadera (n = 95) and 30% of the samples from Eilat (n = 115) copepods were found inhabiting the branchial sac. In 14% of Eilat samples a pontoniine shrimp, identified as *Odontonia sibogae* (Bruce, 1972) was found in the branchial sac and in the atrial cavity of *H. momus* ranging from 4.2 – 12 cm (0.54 – 17.5 g, respectively). This is the first record of this species in the Red Sea. It was absent from the Mediterranean samples.

3.3.4 Seasonality of reproduction

Both GI and average oocyte diameters showed similar trends throughout the study period (Figure 3.4b,c). High values were obtained for the gonad indices (above 2%) of *H. momus* in Eilat throughout the year (excluding April 2005, January and June 2006). Although a strong peak (5.7%) was measured during February, no significant differences (one-way ANOVA, transformed data, p > 0.05) were found in GI of the Eilat *H. momus* samples throughout the study period. This suggests that in the northern Red Sea this species reproduces year round (Figure 3.4b). This data concur with personal observations.
of monthly recruitment panels on which *H. momus* were recorded throughout the year (Shenkar et al. in prep). On the other hand, the low GI values (1.2 ± 0.1%, n=26) measured in the Mediterranean (Hadera) during summer months (July - September 2005) and winter months (January-May 2006, 1.24 ± 0.6%, n=35) indicate that the reproduction of *H. momus* along the Mediterranean coast of Israel is seasonally limited. Significantly higher values of gonad indices (one-way ANOVA, transformed data followed by Fisher LSD, p < 0.05) in the Mediterranean were obtained twice a year, when the water temperature conditions in the Mediterranean were similar to those measured in the Red Sea (22 – 25 º C). This similarity occurred in May 2005, in November-December 2005 and once again in June 2006 (Figure 3.4a). A monthly comparison of the GI recorded in both sites showed significantly higher values in Eilat than in Hadera in July 2005, February and March 2006 (t-tests, transformed data, p < 0.05). Correspondingly, a significant difference in mean oocyte diameter between months was found only in the Mediterranean population (nested design ANOVA, p < 0.05, Figure 3.4c). Mature oocytes (> 120 µm) appeared in high numbers (> 50 %) in the Eilat samples throughout the study period (excluding May 2005), whereas in Hadera, gonads with high numbers of mature oocytes were measured only in June 2005, September through January 2006 and once again in June 2006.
Figure 3.4 *Herdmania momus*. (a) Temperature data at both sites (means ± std). (b,c) Seasonal variations (means ± SEs) of GI (n= 5-9) and oocyte diameter (n=3) in Eilat, Red Sea (black bars) and Hadera, Mediterranean Sea (blank bars).
### 3.3.5 Population genetics

The COI gene of 90 individuals was analyzed and resulted in nucleotide sequences of 545 bp in length after alignment and trimming. Forty three haplotypes were found in the 90 specimens: twenty seven from the Mediterranean, twelve from the Red Sea and four haplotypes were shared between sites (Figure 3.5). A total of 50 (9.17%) variable sites were found. An association of genetic differences with geographic distance was corroborated by the results of Mantel test ($p = 0.007$).

The hierarchical AMOVA analysis revealed that up to 81% of the genetic variance was found within populations (Table 3.1). A smaller amount of variance (18.5% and 20%) was explained by comparisons among populations without grouping and between population after grouping the Mediterranean coast of Israel samples versus the Red Sea and Cyprus ones. All components were significant in permutation test ($p < 0.01$). The comparison between the Red Sea populations and the Mediterranean Sea populations provide no source of variation (-1.08%). Results of exact test between populations revealed that the Cyprus population is significantly different ($p < 0.001$) from all the other populations, the Naharyia population was significantly different from the Red Sea populations ($p < 0.001$), and the Haifa population was significantly different from one of the population from the Red Sea (oil company pier, $p < 0.01$, table 3.2). The high level of diversity within populations is well illustrated by the minimum-spanning tree, where the haplotypes from Hadera are distributed across the whole tree. In addition, four possible clusters were found. These included 1.) Mediterranean harbors (Hadera, Israel and Famagusta, Cyprus) and Eilat 2.) Mediterranean coast of Israel 3.) Hadera and Eilat and 4.) A mixture of Mediterranean and Red Sea haplotypes (Fig. 3.5).
Table 3.1 Analysis of molecular variance for the cytochrome c oxidase subunit I (COI) sequences of *Herdmania momus*. Analyses are presented for the whole area studied without grouping, pooling populations in Mediterranean and Red Sea groups, and pooling populations along the Mediterranean coast of Israel, Red Sea coast of Israel and Cyprus. Significant values at $p < 0.05$ are indicated with an asterisk. $V_a$, $V_b$ and $V_c$ are the associate covariance components. $F_{ct}$, $F_{sc}$, $F_{st}$ are the $F$-statistics.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>Sum of squares</th>
<th>Variance components</th>
<th>Percentage of variation</th>
<th>Fixation indices</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among populations without grouping</td>
<td>5</td>
<td>6.61</td>
<td>0.69216 $V_a$</td>
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<tr>
<td>Within populations</td>
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<td>256.747</td>
<td>3.05651 $V_b$</td>
<td>81.54</td>
<td>$F_{st}$: 0.18464*</td>
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<tr>
<td>Between Red Sea and Mediterranean Sea</td>
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<td>1.138</td>
<td>-0.00503 $V_a$</td>
<td>-1.08</td>
<td>$F_{ct}$: -0.01083</td>
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<tr>
<td>Among populations without grouping</td>
<td>4</td>
<td>5.251</td>
<td>0.06120 $V_b$</td>
<td>13.18</td>
<td>$F_{sc}$: 0.13042*</td>
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<tr>
<td>Within populations</td>
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<td>87.9</td>
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<tr>
<td>Between Mediterranean coast of Israel, Red Sea and Cyprus</td>
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<td>0.78376 $V_a$</td>
<td>19.94</td>
<td>$F_{sc}$: 0.19938*</td>
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<tr>
<td>Among populations without grouping</td>
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<td>$F_{st}$: 0.02881</td>
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<tr>
<td>Within populations</td>
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<td>3.05651 $V_c$</td>
<td>77.76</td>
<td>$F_{st}$: 0.22245*</td>
</tr>
</tbody>
</table>

Table 3.2 p-values of population average pairwise differences within populations of *Herdmania momus* (distance method: Tamura & Nei).

<table>
<thead>
<tr>
<th></th>
<th>Cyprus</th>
<th>Naharia</th>
<th>Haifa</th>
<th>Hadera</th>
<th>Fish cages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naharia</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haifa</td>
<td>0</td>
<td>0.5</td>
<td></td>
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<tr>
<td>Hadera</td>
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<td>0.26364</td>
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<td></td>
</tr>
<tr>
<td>Fish cages</td>
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<td>0.07273</td>
<td>0.09091</td>
<td></td>
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<tr>
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<td>0</td>
<td>0.00909</td>
<td>0.11818</td>
<td>0.49091</td>
</tr>
</tbody>
</table>
Figure 3.5 Minimum-spanning tree for *Herdmania momus* haplotypes based on COI sequences (n = 90).

3.4 Discussion

Introduction of non-indigenous species into new regions, accidentally or deliberately, is now commonplace causing severe threats to terrestrial and marine
biodiversity (Grosholz 2002). In general, increasing diversity of the recipient assemblage is associated with a lower recruitment of non-native species (Stachowicz et al. 1999). The nearly tropical temperature and salinity conditions along the Mediterranean coasts of Israel measured during the summer combined with the relatively low species diversity in this region (Spanier & Galil 1991, Fishelson 2000) results in thriving populations of Red Sea species along the Levantine coasts. The current work is the first to conduct a simultaneous comparative study of native versus non-indigenous ascidian populations revealing differences in their distribution, occurrence of symbionts and reproductive activity.

Lambert (2002) suggests two criteria for the designation of introduced ascidians species in Guam (1) be restricted to artificial surfaces and (2) have an extra Indo-West Pacific distribution. Using similar criteria; since *Herdmania momus* was found along the Mediterranean coast only on artificial substrates, has an Indo-Pacific origin (Kott 2002), is recorded through the Suez Canal (Ghobashy & Adbel Messeih 1991), and so far has been recorded in the Mediterranean only from the Levant basin (Nishikawa 2002) it should be considered a non indigenous species, which most likely arrived to the Mediterranean through the Suez Canal. In addition, the absence of *H. momus* from the natural environment along the Mediterranean coast may indicate it is currently in its primary stages of establishment and in the future it may possibly be found in the natural environment as well.

The molecular data analysis supports these conclusions with evidence for geographic structure of the introduced populations. First of all, the populations are significantly isolated by distance which may apply to ‘stepping stone’ invasion in which a species can
move from one environment, via a noncolonizable environment, to a new and potentially hospitable environment (Apte et al. 2000). Second, the minimum-spanning tree revealed that the Mediterranean harbors and Red Sea haplotypes are joined by one clade (Fig. 3.5). Harbours and marinas are often the entrance gates for new colonizers traveling on ship’s hulls, in ballast water and sediment, or in biofilms on their hulls (Carlton and Geller 1993) or sea chests (Coutts & Doudgshun 2007). Harbor communities generally present low species richness, strong environmental stress and high level of pollution. These factors make them more vulnerable to the introduction of new genetic variants or species which may be opportunistic forms (López-Legentil & Turon 2006). Indeed, AMOVA results showed no significant difference between the Hadera population and the Red Sea populations (table 3.1, 3.2) which may imply a continuous introduction and/or several introductions of H. momus to this site. In contrast, the significant difference of the Cyprus population (Famagusta harbor) may indicate a lower gene flow to this site. However, the high number of haplotypes unique to the Mediterranean (twenty seven out of forty three, figure 3.5) and the high genetic variation within populations (78%) rejects a ‘bottleneck scenario’ with a founding population undergoing reduction in genetic diversity (Holland 2000). The results of the current molecular analysis are only partial since samples from the Red Sea were collected from only one region (Gulf of Eilat). Future studies with a wider geographic sampling effort which will include the Suez Canal may contribute to a better understanding of the arrival and dispersal modes of this species.

The current study revealed marked differences in the spatial distribution of H. momus. In Eilat (Red Sea) H. momus was found on settlement plates from shallow (< 1m) to 15m depth (Figure 3.2a) while in Hadera (Mediterranean Sea) no H. momus were
found in shallow depths. Additionally, as water depth increased the density of *H. momus* increased significantly on settlement plates and on the nearby pillars (Figure 3.2a,b). This disparity might be a result of the strong wave exposure that is typical of the Mediterranean site (1-2m waves, IOLR Mediterranean Wave Forcast, http://isramar.ocean.org.il) which prevents the attachment of *H. momus* to the substrate in shallow depths and causes detachment in greater depths following strong winter storms (N.S. personal observations).

A few studies have demonstrated that ascidians can show differences in life history traits between subpopulations in different seas (Millar 1952, 1954, Davis 1989). Rocha et al. (1999) compared the body size of the solitary ascidian *Phallusia nigra* at São Sebastião, Brazil in a subtropical environment with a population studied in a Caribbean coral reef at Jamaica (Goodbody 1962). The largest animals in Brazil were 7-8 cm whereas in the Caribbean they reach 10 to 12 cm in length. In the present study we also showed a significant difference in body length and weight (Figure 3.3) between *H. momus* populations from Eilat and Hadera. However, in this case the animals from the temperate environment (Hadera) were significantly larger than the animals from the coral reef environment (Eilat) in both parameters. Since ascidians are filter feeding organisms, it is most likely that this is a result of the higher organic material (~0.35 µg/l chlorophyll *a*, IOLR database, http://www.ocean.org.il) in the coastal waters of the Mediterranean in contrast to the oligotrophic conditions in the coral reefs of Eilat (~0.16 µg/l chlorophyll *a*, NMP database, http://www.iui-eilat.ac.il). Another disparity between the two populations was the occurrence of symbionts. The study of invasive species of symbionts may indicate if a given species invaded as a larva or as an adult. For instance, based on
parasitological evidence, Diamant (1998) claims that rabbitfish invaded the Mediterranean through the Suez Canal as active adult fish and not as passively swept planktonic larvae, since they were found containing parasites that occur only on adult forms and are not known from the Mediterranean. In the current study, approximately one third of the samples from both localities had copepods in the branchial cavity. However, we found the pontoniine shrimp *Odontonia sibogae* (Bruce 1972) only in the samples of *H. momus* from Eilat. This species can also be found in the ascidians *Styela whiteleggei*, *Rhopalaea crassa*, and *Polycarpa* sp. (Fransen 2002). They often live in pairs insuring sexual reproduction, and have colors to match those of their ascidian hosts (Monniot et al. 1991). Eilat is the most northern locality where *O. sibogae* is recorded (AJ Bruce, personal communication). The absence of *O. sibogae* from Mediterranean samples may be either because *H. momus* arrived to the Mediterranean coasts as small juveniles (less than 4.2 cm) or it arrived as adult with symbionts, but they failed to survive and establish a population in the Mediterranean. Since *O. sibogae* is recorded only from coral reef environments, it is possible that the wide water temperature fluctuations along the Mediterranean shores (16-31 ºC) inhibit the establishment of a population of this pontoniine shrimp.

Temperature has been suggested as the main factor regulating the sexual reproduction of ascidians (Millar 1971). However, other parameters such as food availability (Yamaguchi 1975, Sahade et al. 2004), turbidity (Millar 1974) and depth (Svane 1984) may also influence gametogenesis. The results of the present study (Figure 4b,c) show that the two populations of *H. momus* have different reproductive peaks. The population in Eilat reproduces year-round with a strong peak in February. Year-round
reproduction is typical to tropical coral reef ascidians (Goodbody 1961, van Duyl et al. 1981). In more fluctuating environments, ascidians usually exhibit seasonal breeding (Becerro & Turon 1992, Durante & Sebens 1994, Sahade et al. 2004) as we found for *H. momus* in the Mediterranean. Our results show that the Mediterranean population reproduces only when the water temperature conditions are similar to those measured in the Red Sea (22 – 25 °C). The ability to change reproductive periods is known from other Lessepsian migrants, such as molluscs (Atad 2005) and fish (Golani 1990). However, these species had a wider reproductive period (4-5 months during the year) in comparison to *H. momus* that is limited to approximately two months a year (May-June and November) when the GI is high and the oocytes are mature. We suggest that the limited reproductive period of *H. momus* in the Mediterranean is preventing the dispersal of this species to colder areas in the region. Nevertheless, taking into account the anticipated rise in sea-water temperature, due to global warming (Rahmstorf & Ganopolski 1999), it is possible that *H. momus* will spread farther in the Mediterranean in the future.

We conclude that *H. momus* in the Mediterranean compared to the Red Sea is restricted to artificial substrates, found in greater depths, larger in size, has different symbionts and has a limited reproductive season. These differences are attributed to differential temperature ranges, food availability and wave exposure. The increasing evidence of negative effects of non-indigenous ascidians on natural fauna (Cohen et al. 2005) emphasizes the need for additional research regarding the ecology of ascidians along the coasts of Israel.
4.1 Introduction

Artificial marine reefs play an important role in different ecosystems by significantly enhancing professional and recreational fishing, controlling beach erosion, contributing to conservation biology and creating sport-diving opportunities (reviewed in Baine 2001). Community development on artificial structures has been extensively studied in past years (Osman 1977, Bailey-Brock 1989, Qiu et al. 2003, Perkol-Finkel & Benayahu 2005), including investigation of colonization processes by means of periodical examination of various types of settlement plates (Hatcher 1998, Bowden et al. 2006, Perkol-Finkel et al. 2006). However, only a few studies have examined the influence of seasonality (period deployed in water) on the epifaunal assemblage (Brown & Swearingen 1998, Qiu et al. 2003, Watson & Barnes 2004, Brown 2005). Thus, for example, if larval availability varies seasonally, the time of year at which the settlement plates are immersed could greatly influence the eventual community composition. Although several studies have investigated various parameters of community development on artificial substrates along the coral reefs of Eilat (Red Sea) (Goren 1979, Angel & Spanier 2002, Perkol-Finkel et al. 2006), none of them refer to the effect of time of deployment on recruitment.

Marine invertebrate recruitment is generally defined as the number of organisms surviving when encountered by an observer (Keough & Downes 1982). Recruitment is strongly influenced by reproductive effort (Benayahu & Loya 1985, Gotelli 1987), mortality in the plankton (Thorson 1950, Olson 1985), larval behavior (Bingham &
Young 1991), settlement (Gaines et al. 1985) metamorphosis (Cloney 1978), and early juvenile mortality (Young & Chia 1984, Davis 1987, Osman & Whitlatch 2004). Since all the above factors change with time, they will be reflected during the periodic examination of the settlement plates (Fitzhardinge & Bailey-Brock 1989, Qiu et al. 2003, Brown 2005).

Ascidians play an important role in altering biodiversity in marine fouling communities in both cold-water and temperate regions (Bullard et al. 2007, Clynick et al. 2007, Dijkstra et al. 2007a). In tropical environments, although ascidians constitute only a minor benthic component on the natural coral reef they successfully foul various artificial substrates such as jetties and other man-made substrata (Goren 1979, Oren & Benayahu 1998, Lambert et al. 2002, Perkol-Finkel & Benayahu 2007). Nevertheless, accurate data regarding recruitment patterns of ascidians in the Red Sea are restricted to date to one study on didemnid ascidians only, which did not consider the seasonality effect (Oren & Benayahu 1998). Moreover, reproductive cycles, which are a major factor in larval supply of coral reef ascidians, have been poorly studied in these organisms and limited to the dominant, well-identified species (Goodbody 1961, van Duyl et al. 1981).

Spatial orientation of surfaces (vertical versus horizontal, and/or overhang surfaces) appears to be another important factor influencing ascidian recruitment patterns on both artificial structures (Oren & Benayahu 1998, Glasby 2000, Knott et al. 2004) and natural ones (Gotelli 1987). Generally, it appears that invertebrates cover a larger area on vertical than on horizontal surfaces (Wendt et al. 1989, Baynes 1999). These differences are attributed to several physical and biological factors, such as light (Young & Chia 1984, Glasby 1999), sedimentation (Balata et al. 2005), water-flow (Glasby 2000, Perkol-
Finkel et al. 2006), competition and grazing (Baynes 1999). These parameters may also affect depth zonation of sessile invertebrates on artificial reefs (Mundy & Babcock 1998, Moura et al. 2006, Perkol-Finkel et al. 2006).

Since ascidians are characterized by fast growth rates (Millar 1971, Bak et al. 1981, Lambert 2002), accurate identification to species level can be achieved over a short period. We hypothesized that ascidian recruitment patterns to an artificial marine structure in the Red Sea would vary among species according to season, orientation and depth conditions.

4.2 Materials and Methods

4.2.1 Study site and sampling procedure. As part of a comprehensive research on artificial marine structures (AMS) as a multifunctional tool for research and environmental management in the Red Sea, four experimental AMS were deployed in the area of the oil port at Eilat, 29°31’ N 34°56’ E in June 2004. Eilat is located at the northern tip of the Red Sea, with water temperature between 20 to 27°C, and salinity of ca.40.5 ‰ throughout the year (Loya 2004). Ambient seawater temperature and visibility (measured as secchi disk depth) data were obtained from The Israel National Monitoring Program (NMP, http://www.iui-eilat.ac.il).

The AMS were deployed at 20 m depth on sandy substrate adjacent (less than ten meters) to the reef, approximately 50 m from each other. Each AMS (130 x 120 x 130 cm, weight: 2.5 tons) was constructed of reinforced concrete and contained 16 polyethylene tubes, each 25 cm in diameter. A floating AMS (100 x 100 x100 cm) was attached to each of the four benthic AMS at 12 m depth with ropes at each corner. The floating devices constituted a 1 m steel cube (weight: 52 kg) containing 16 similar polyethylene
pipes which are neutrally buoyant. Although this construction prevented the rotation of the floating units, these units were subjected to significantly stronger water flow in comparison to the seabed units (~75 mm/sec versus ~25 mm/sec A. Abelson unpublished data). Sets of experimental ceramic plates, 20 x 20 cm each, were attached both to the seabed and floating AMS as depicted in figure 4.1. At each sampling three settlement plates of each combination were removed from each of the four AMS and replaced with new plates at the exact same position. The investigated plates were sampled after three months of submersion, on March, June, September and December 2005, representing four seasons: winter (December 2004-March 2005), spring (March-June 2005), summer (June-September 2005) and autumn (September-December 2005). In the current study, 192 plates were used (4 seasons x 4 AMS devices x 2 orientations x 2 depths x 3 plates).

4.2.2 Assessment of epifaunal assemblages and data processing. Upon sampling, the plates were immediately transported in seawater to the lab and examined under a dissecting microscope for ascidian coverage and number of individuals. This method is recommended for recording the occurrence of small species that can be easily overlooked in situ (Beaumont et al. 2007). Live cover percentage was calculated on the ceramic plates using a 4 x 4 cm grid to estimate the total live cover of each tile surface, on both sides: front, represents side facing the open water; back, represents side facing the Artificial Marine Structure (AMS) surface, with a 10 cm distance from the plates to the AMS. Since very low ascidian coverage was observed throughout the study period on the plate front, only the plate back was taken into consideration for data analysis.

In order to identify the ascidians to species level, representatives of each species were narcotized with menthol crystals for up to three hours and fixed in 4% formalin in filtered
sea water (deposited at the National Collections of Natural History at Tel Aviv University). Taxonomic identification was carried out using authoritative keys and texts (Monniot et al. 1991). The data obtained for other taxa will be published elsewhere.

### 4.2.3 Statistical analysis.

Data analysis was performed on the pooled data of every three settlement plates representing each treatment (orientation x depth on each AMS). Differences in the cover percentage of each species, the total cover percentage of colonial and solitary species and the number of solitary individuals on the settlement plates were tested by factorial ANOVA. The factors used were: season, depth (12 m, 20 m) and orientation (vertical, horizontal). Data analyses also allowed testing for interactions between these factors. Data were tested following an arcsin√p transformation to the percent coverage and square-root transformation to the number of individuals, and checked for normality with Shapiro-Wilk’s test, and for homogeneity of variance with Cochran’s test (Zar 1999). The AMS identity factor was removed after a nested design ANOVA for each of the measurable variables (with orientation and depth nested within AMS identity) had shown a non-significant effect (p > 0.05) of this factor. Fisher’s LSD (least significant differences) tests were used as post-hoc comparisons when significant differences were detected. Differences between total colonial and solitary species cover percentage on horizontal and vertical surfaces and on the seabed and floating unit were tested with student’s t-test on transformed data. Statistical analyses were carried out using Statistica (Version 7.1, StatSoft, Inc).

Associations between ascidian species composition, season, orientation and depth were examined using multivariate statistical methods within the software package PRIMER (PRIMER-E Ltd., 2000, Clarke & Warwick 2001). The analysis was carried out
separately for the colonial species’ percent coverage after log (x + 1) transformation and for the solitary species’ counts after fourth-root transformation. Analysis of similarities (ANOSIM, Clarke 1993) was performed to test for significant differences in ascidian species composition for each parameter. In this non-parametric test an $R$- statistic is calculated in the range of -1 and +1. $R$ approximates zero if the null hypothesis is true, the average similarity between and within the factor groups (i.e. season, orientation and depth) is the same, indicating no difference in species composition. In order to determine the contribution of each species to the average similarity within the replicate panels, we applied the similarity percentage program (SIMPER) within the PRIMER software. This program was also used to calculate the dissimilarity percentage in ascidian species composition between the different sampling periods, orientation and depth. Results are presented as averages ± standard errors throughout the text.
Figure 4.1 Schematic illustration of one of the four AMS used in the experiment. At each sampling 48 settlement plates were removed (4 AMS x 2 orientations x 2 depths x 3 plates).

4.3 Results

4.3.1 Ascidian assemblage structure

 Throughout the study a total of 14 different ascidian species belonging to six families were identified attached to the settlement panels. The rare species included: *Rhodosoma turcicum* (Savigny 1816), *Phallusia arabica* (Savigny 1816), *Polycarpa mytilegra* (Savigny 1816), *Pyura gangelion* (Savigny 1816) and *Rhopalaea* sp., and *Boltenia* sp. both suspected as new species (F. Monniot pers. comm.). The dominant species which appeared in sufficient numbers to allow statistical analysis were: the solitary ascidians
Herdmania momus (Savigny 1816), Halocynthia spinosa (Sloiter 1905), Phallusia nigra (Savigny 1816), Ascidia cannelata (Oken 1820), and the colonial ascidians Didemnum candidum (Savigny 1816), Didemnum granulatum (Tokioka 1954), Eusynstyela latericius (Sluiter 1904), Botryllus eilatensis (Shenkar & Monniot 2006).

4.3.2 Seasonal changes in ascidian cover

Total ascidian percent coverage did not change significantly throughout the study period (Figure 4.2). The highest percent coverage was observed on plates immersed during spring and autumn 2005, with an average of 3.7 ± 0.7 (n = 32) percent coverage, in comparison to plates immersed during winter with 1.4 ± 0.4 (n = 16) percent coverage (Figure 4.2). In addition, multivariate analysis revealed that the ascidian assemblages that developed on the settlement plates during each of the seasonal exposure periods did not differ significantly from each other (ANOSIM, \( P > 0.05 \), table 4.1). The highest dissimilarity in the solitary species composition was found between winter and autumn (49%, Table 4.2) and is a result of the appearance of \( H. \) spinosa during winter and \( P. \) nigra during autumn (Figure 4.3a). The highest dissimilarity in the colonial species composition was found between winter and spring (61%, Table 4.1) and is a result of a higher cover percentage of \( E. \) latericius in spring (Figure 4.3b). The highest contribution to the similarity degree between the plates for each season is from the presence of \( H. \) momus with an average contribution of 88.43 ± 5.2 % and \( D. \) granulatum with an average contribution of 51.8 ± 7.4 % during the four seasons (Table 4.2).
Table 4.1 Dissimilarities (%) and pairwise $R$ values (in brackets) between colonial and solitary assemblage structures for each seasonal period and according to orientation and depth.

<table>
<thead>
<tr>
<th>Factor group</th>
<th>Spring</th>
<th>Summer</th>
<th>Autumn</th>
</tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Winter</td>
<td>Colonial</td>
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<td>56 (-0.048)</td>
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<td></td>
<td>Solitary</td>
<td>39 (0.136)</td>
<td>44 (-0.048)</td>
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<tr>
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<td>Colonial</td>
<td>60 (-0.035)</td>
<td>53 (0.115)</td>
</tr>
<tr>
<td></td>
<td>Solitary</td>
<td>27 (0.18)</td>
<td>26 (0.442)</td>
</tr>
<tr>
<td>Summer</td>
<td>Colonial</td>
<td></td>
<td>48 (-0.016)</td>
</tr>
<tr>
<td></td>
<td>Solitary</td>
<td></td>
<td>38 (0.103)</td>
</tr>
<tr>
<td>Orientation (Vertical vs. Horizontal)</td>
<td>Colonial</td>
<td>52 (-0.053)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solitary</td>
<td>38 (-0.061)</td>
<td></td>
</tr>
<tr>
<td>Depth (Floating vs. Seabed unit)</td>
<td>Colonial</td>
<td>56 (0.117)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solitary</td>
<td>37 (0.086)</td>
<td></td>
</tr>
</tbody>
</table>

Data were log ($x + 1$) transformed for colonial species and fourth-root transformed for solitary species. ANOSIM results: $R = 1$ all similarities within groups are less than any similarity between groups; $R > 0.75$ groups are well separated; $R > 0.5$ overlapping groups but clearly different; $R < 0.25$ barely separable; $R = 0$ indistinguishable.

Table 4.2. Results from SIMPER analysis of colonial and solitary species assemblage data from settlement plates exposed during each of the four seasonal exposure periods and according to orientation and depth (colonial species cover percentages after log ($x + 1$) transformation, solitary species counts after fourth-root transformation). Average abundance and contribution percentage for each species, and the overall average similarity between treatments. Note: the analysis was performed separately for the solitary and the colonial species.
<table>
<thead>
<tr>
<th>Factor group</th>
<th>Season</th>
<th>Solitary</th>
<th>Species</th>
<th>Average abundance</th>
<th>Contribution (%)</th>
<th>Average similarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Winter</td>
<td></td>
<td><em>Herdmania momus</em></td>
<td>5.33</td>
<td>75</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Halocynthia spinosa</em></td>
<td>0.8</td>
<td>22</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Eusynstyela latericius</em></td>
<td>0.57</td>
<td>50</td>
<td>41.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum granulatum</em></td>
<td>0.43</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>0.36</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spring</td>
<td>Solitary</td>
<td><em>Herdmania momus</em></td>
<td>7.79</td>
<td>100</td>
<td>95</td>
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<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Didemnum granulatum</em></td>
<td>1.33</td>
<td>52.5</td>
<td>35.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>1.89</td>
<td>40</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Solitary</td>
<td><em>Herdmania momus</em></td>
<td>4.23</td>
<td>87.1</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Halocynthia spinosa</em></td>
<td>0.46</td>
<td>8.48</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Didemnum granulatum</em></td>
<td>0.75</td>
<td>65.4</td>
<td>41</td>
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<td></td>
<td></td>
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<td>0.5</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>1.05</td>
<td>9</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Autumn</td>
<td>Solitary</td>
<td><em>Herdmania momus</em></td>
<td>4.64</td>
<td>91.63</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Didemnum granulatum</em></td>
<td>4.18</td>
<td>58.4</td>
<td>56</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>Eusynstyela latericius</em></td>
<td>1.09</td>
<td>16.07</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>3.32</td>
<td>13.08</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>Botryllus eilatensis</em></td>
<td>1.05</td>
<td>8.39</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td>Floating unit</td>
<td><em>Herdmania momus</em></td>
<td>5.65</td>
<td>87.38</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Phallusia nigra</em></td>
<td>0.81</td>
<td>4.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Didemnum granulatum</em></td>
<td>1.38</td>
<td>50</td>
<td>43.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>4.38</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Botryllus eilatensis</em></td>
<td>1.23</td>
<td>13.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seabed unit</td>
<td>Solitary</td>
<td><em>Herdmania momus</em></td>
<td>5.48</td>
<td>95.03</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Didemnum granulatum</em></td>
<td>2.1</td>
<td>53</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Eusynstyela latericius</em></td>
<td>2.06</td>
<td>33.83</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>0.4</td>
<td>11.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Orientation</td>
<td>Horizontal</td>
<td><em>Herdmania momus</em></td>
<td>7.36</td>
<td>97.11</td>
<td>78.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Halocynthia spinosa</em></td>
<td>0.56</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colonial</td>
<td><em>Didemnum granulatum</em></td>
<td>2.56</td>
<td>33.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Eusynstyela latericius</em></td>
<td>2.47</td>
<td>18.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>Didemnum candidum</em></td>
<td>2.34</td>
<td>18.89</td>
<td></td>
</tr>
</tbody>
</table>

64
Factorial ANOVA performed separately for each of the ascidian species showed significant differences in the numbers of *H. spinosa* \( F_{(df=3,48)} = 4.7, P < 0.01 \) between the seasons (Figure 4.3a) with significantly higher numbers observed in winter (Fisher’s LSD, \( P < 0.01 \)). The numbers of *A. cannelata*, *P. nigra* and *H. momus* did not change significantly between the seasons, although higher numbers of *H. momus* were observed on plates immersed in spring. The number of *H. momus* at each sampling was significantly associated with water visibility \( r^2 = 0.91, y = -8.93X + 280, p < 0.01 \). The total colonial percent coverage changed significantly between the seasons \( F_{(df=3,48)} = 3.2, P < 0.05 \) due to a significant change in the percent coverage of *D. granulatum* \( F_{(df=3,48)} = 6.5, P < 0.001 \), which showed a significantly higher percent coverage during autumn (Fisher’s LSD, \( P < 0.01 \), Figure 4.3b).

![Figure 4.2](image)

**Figure 4.2** Ascidian percent coverage (means ± SEs) during the study period (n = 16).
Figure 4.3 (A) Average number (± SEs) of solitary species and (B) average percent coverage (± SEs) of colonial species per treatment (n = 16).

4.3.3 Vertical versus horizontal surfaces

No significant differences were found in total ascidian percent coverage between the vertical and the horizontal surfaces (factorial ANOVA, $P > 0.05$). In addition, the close to zero $R$ values (ANOSIM, Table 4.1) indicate that the colonial and solitary species assemblages on vertical versus horizontal surfaces are almost indistinguishable. However, in comparison to colonial species, a significantly higher percent coverage of solitary species was measured on the horizontal surfaces (t-test, transformed data, $P < 0.05$, 
Figure 4.4). Out of the solitary species only *H. momus* had significantly higher numbers ($F_{(df=1,48)} = 8.1, P < 0.01$) and percent coverage ($F_{(df=1,48)} = 10.8, P < 0.01$) on horizontal surfaces (Figure 4.5a). The colonial species examined (excluding *D. granulatum*) showed a preference for vertical surfaces, although this was not statistically significant (Figure 4.5b). The highest contribution to the similarity percentages of the ascidian assemblage on horizontal and vertical surfaces was again by *H. momus* and *D. granulatum* (Table 4.2).

**Figure 4.4** Total percent coverage (means ± SEs) of the solitary (hatched bars) and colonial (blank bars) species on vertical versus horizontal orientations and on the seabed versus floating units (n = 32).
Figure 4.5 (A) Average number (± SEs) of solitary species and (B) average percent coverage (± SEs) of colonial species on horizontal (hatched bars) and vertical (white) oriented treatments (n = 32).

4.3.4 Seabed unit versus floating unit

Similar to the vertical vs. horizontal analysis, no significant difference was found in total ascidian percent coverage (factorial ANOVA, $P > 0.05$) and species assemblages between the floating units and the seabed units (Table 4.1). However, a significantly higher percent coverage of solitary species in comparison to colonial species was measured on the floating units (t-test, transformed data, p > 0.05, Figure 4.4). Separate analysis for each species revealed significantly higher numbers of *P. nigra* ($F_{(df=1,48)} = 7.3, P < 0.01$) and *A. cannelata* ($F_{(df=1,48)} = 4.9, P < 0.05$) on the floating units. No
significant difference was found in the number of individuals of *H. spinosa* and *H. momus* on the floating versus the seabed units (Figure 4.6a). Out of the colonial species only *D. granulatum* and *E. latericius* had significantly higher percent coverage on the seabed unit ($F_{(df=1,48)} = 5.6, P < 0.05$, $F_{(df=1,48)} = 4.7, P < 0.05$ respectively, Figure 4.6b). For *D. granulatum* a significant interaction existed between season and depth ($F_{(df=3,48)} = 6.1, P < 0.01$) as a result of significantly higher percent coverage on the seabed units in autumn (Fisher’s LSD, $P < 0.01$). Again, *H. momus* and *D. granulatum* had the highest contribution to the similarity percentages of the ascidian assemblage on the seabed and floating units (Table 4.2).

![Graphs](image)

**Figure 4.6** (A) Average number (± SEs) of solitary species and (B) average percent coverage (± SEs) of colonial species on seabed unit (hatched bars) and floating unit (white) treatments ($n = 32$).
4.4 Discussion

The data that can be obtained from fouling community development studies are often limited by the ability to identify the benthic assemblage correctly and reliably. By providing an accurate identification of the fouling organisms to species level it is possible to distinguish among the specific recruitment patterns of a certain species, rather than generalizing patterns among phyla as shown in most studies (Fitzhardingh & Bailey-Brock 1989, Perkol-Finkel & Benayahu 2007). Hence, it is possible to indicate which species are the dominant ones that establish the pattern for the entire group, and also to locate differential recruitment patterns of less dominant species that would have been overlooked when working at group level identification.

In the current study no significant difference in total ascidian percent coverage between the seasons was found (Fig. 4.2). However, a separate analysis performed on each of the species revealed a seasonal appearance of several ascidian species (Table 4.1, Fig. 4.3a,b). The colonial ascidian *Didemnum granulatum* appeared with significantly higher percent coverage during autumn, while the solitary species *Halocynthia spinosa* appeared in significantly higher numbers during winter, and the solitary species *Phallusia nigra* was absent from settlement plates submerged during spring and winter.

Seasonal periodicity in recruitment is highly dependent on reproduction; hence, on larval availability from the plankton (Hurlbut 1992, Brown & Swearingen 1998). The main factor regulating the reproduction of ascidians is temperature (Millar 1971) and most coral reef ascidians reproduce year round (Goodbody 1961, van Duyl et al. 1981, Stoner 1990). Nevertheless, whereas in the coral reefs of Jamaica *P. nigra* shows continuous breeding throughout the year (Goodbody 1961), in Brazil, in a subtropical environment it
shows a depressed gonadal activity during winter (Rocha et al. 1999). The coral reefs of Eilat are situated at the most northern boundary of coral reef distribution (i.e., 30°N); therefore it is intriguing that in this region this cosmopolitan species has a seasonal appearance similar to that in subtropical environments.

Another factor that may contribute to the seasonal fluctuations in ascidian appearance is that of food availability (Becerro & Turon 1992, Sahade et al. 2004, López-Legentil et al. 2005). The higher percent coverage of ascidians in spring and autumn (Fig. 4.2) may be a result of higher nutrient levels in the water column during the transitional seasons (Shaked & Genin 2007). Indeed, the solitary ascidian *Herdmania momus*, which exhibits year-round reproduction in Eilat (Shenkar & Loya, personal observations), appeared in significantly higher numbers as water visibility decreased (indicative of particulate matter in the water, $r^2 = 0.91$) possibly due to additional food supply.

Multivariate analysis revealed that the solitary species *H. momus* and the colonial species *D. granulatum* both had a very high contribution (> 50 %) to the similarity degree between the sampled plates (Table 4.2). Therefore, data analysis should be treated with caution in order to avoid imposing the recruitment patterns of one dominant species upon the entire ascidian group. For instance, the significant change in the colonial percent coverage between the seasons was a result of the significantly higher percent coverage of *D. granulatum* in autumn; and the high percent coverage of solitary species on horizontal surfaces was due to the high abundance of *H. momus*. Knott et al. (2004) describe similar results in which the pattern of cover of ascidians on artificial substrates in Sydney’s south coast was due primarily to *H. momus*. 
A major physical difference between horizontal and vertical surfaces is that of shading (Connell 1999), which has been known to attract settlement of ascidian larvae (Young & Chia 1984, Gotelli 1987, Hurlbut 1992, Oren & Benahyau 1998, Glasby 1999). However, while some studies describe higher ascidian coverage on horizontal surfaces (Logan et al. 1984, Oren & Benayahu 1998, Glasby 2000), others report an opposite trend (Gotelli 1987, Knott et al. 2004). In the current study only *H. momus* showed a significant preference to the horizontal versus vertical surfaces while the other species did not show a specific trend. Thus, it is possible that either these particular species recruitment patterns are not affected by orientation or that in the Red Sea, the orientation factor in such study designs is negligible as also been found in other studies (Perkol-Finkel & Benayahu 2007).

The current study design does not allow to separate between the effect of depth (12 m versus 20 m) and position in the water-column (floating versus seabed unit). Due to different hydrodynamic features, primarily stronger water flow which has an important role in larval settlement into fouling communities (reviewed in Koehl 2007), floating habitats (such as pontoons and other artificial structures) develop unique communities in comparison to adjacent reefs and identical artificial fixed structures (Connell 2000, Glasby 2001, Perkol-Finkel et al. 2006). The high mass-transfer rates and current velocities encourage massive settlement of benthic filter feeders such as ascidians, sponges and bryozoans (Baynes & Szmant 1989, Holloway & Connell 2002, Perkol-Finkel et al. 2006). Another factor affecting the seabed units that may influence recruitment is that of sedimentation. Sedimentation can clog the pores of filter-feeding organisms, inhibit the exchange of dissolved nutrients and gases, lead to increased energy
expenditure due to sediment rejection activities, inhibit larval settlement and physically abrade and bury encrusting organisms (Baynes & Szmant 1989). The present results show a higher total ascidian cover on the floating units (Fig. 4.4) as a result of higher numbers of the solitary species *P. nigra* and *Ascidia candela* and higher cover of the colonial species *Didemnum candidum* (Fig. 4.6a,b). Similar findings are described by Glasby (1999), with higher abundance of solitary ascidians on shaded plates far from the seabed in Sydney harbor, Australia. In addition, Hurlbut (1991) describes the same settlement pattern for *D. candidum* in Hawaii, with higher abundance at shallow depths on floating docks than at greater depths along pilings, as a result of greater larval supply and settlement intensity at shallow depths. By contrast, in the present study, both *D. granulatum* and *E. latericius* had a significantly higher percent coverage on the seabed units (Fig. 4.6b). Their ability to settle and grow on these units may be a result of efficient sediment rejection and/or preference for deeper zones. Moreover their short larval swimming stage may limit the distance the larvae can disperse from the parent reef resulting in their settlement on the seabed units.

The pattern and process of recruitment by organisms has long been known to be extremely complex (Watson & Barnes 2004) and much work is required in the future in order to ascertain the role of the biotic and a-biotic factors in determining the patterns observed in the current study. We conclude that the ascidian recruitment patterns are species-specific and vary between seasons, orientation and position in the water column. Most importantly, the epifaunal assemblage developing on an artificial substrate in a tropical environment is influenced by the season of deployment and this therefore should be taken into consideration when investigating community development on such
structures, especially when analyzing fouling development on anti-fouling coated panels. The data acquired from the current study will greatly contribute to the accumulating knowledge regarding ascidian ecology in coral reef regions and differential recruitment patterns of ascidians to artificial marine structures.
SECTION IV

Population dynamics of a coral reef ascidian in a deteriorating environment

5.1 Introduction

In the past few decades coral reefs have suffered a massive, long-term decline in abundance, diversity and habitat structure due to both natural (e.g., coral bleaching, predation by crown of thorns starfish) and anthropogenic (e.g., overfishing, sedimentation, pollution) disturbances (Hughes 1994, Hughes et al. 2003, Pandolfi et al. 2003). Live coral cover is declining rapidly, with reports of up to 80% decrease in the Caribbean and East Africa (Gardner et al. 2003, Wilkinson 2002) and ~50% decrease in the Red Sea (Loya 2007). The decline in live coral cover has a negative effect on other reef inhabitants (Pandolfi et al. 2003, Attrill et al. 2004, Feary et al. 2007). However, the conditions that are deleterious for corals (e.g., eutrophication) may create a favorable environment for filter-feeding organisms, accelerating their growth and spread (Bak et al. 1996, Aerts & van Soest 1997, Aronson et al. 2002, Ward-Paige et al. 2005). Sponges and ascidians control the removal of particulate organic nitrogen from coral reef waters (Ribes et al. 2005). Their ability to effectively filter small plankton, less than 10 µm in size (Yahel et al. 2005), plays an important role in the carbon and nutrient pathway in coral reef ecosystems (Ribes et al. 2005). It has been suggested that changes in water quality in tropical coasts, particularly pertaining to the availability of nutrients and organic matter, may result in vast changes in these benthic communities (Bak et al. 1998, Ward-Paige et al. 2005).
In general, ascidians constitute a minor benthic component on exposed surfaces on natural coral reefs. They are often found in cryptic environments such as grottos, crevices and the sides or undersides of rocks and corals (Monniot et al. 1991). However, there have been recent reports of a rapid spread of several ascidian species in various tropical regions of the world (Bak et al. 1996, Lambert 2002). Bak et al. (1996) reported a nine-fold increase in the density of *Trididemnum solidum*, a colonial ascidian, along the 84 km of fringing reef in Curacao, over a period of 15 years. Witman & Smith (2003) reported a three-fold increase in ascidian biomass over one year in an upwelling site in the Galápagos. Whereas numerous studies have investigated the competitive relationship between different coral species (Lang 1973, Sheppard 1979, Connell 1983, Abelson & Loya 1999, Connell et al. 2004), only a few studies have investigated the interactions of corals with other bottom occupying organisms (Aerts & van Soest 1997, Aerts 1998, López-Victoria et al. 2006) and none of them have investigated coral-ascidian interactions.

Acsidians have a rapid growth rate (Bak et al. 1981), reach sexual maturity when only a few weeks old and produce large numbers of short-lived, non-feeding larvae (Berrill 1950, Millar 1971). These characteristics, combined with a lack of significant predators makes ascidians very successful competitors (Lambert 2002). The rapid spread of several ascidian species in both tropical and temperate regions of the world is of growing concern (Lambert 2002, Paulay et al. 2002, Blum et al. 2007, Valentine et al. 2007).

In the coral reefs of Eilat (Red Sea) a newly-described colonial ascidian, *Botryllus eilatensis* (Shenkar & Monniot 2006), has been observed overgrowing dead coral skeletons and rapidly colonizing artificial substrates. This genus has a wide distribution.
and is a common fouling organism in estuaries and shallow water environments (Kott, 1985). It is difficult to determine whether *Botryllus eilatensis* is native or non-indigenous, as it has colonized harbor structures, as well as natural substrates (Shenkar & Monniot 2006). Due to a rise in anthropogenic activity the coral reefs in the northern Red Sea are experiencing increased eutrophication and the corals are severely stressed (Ben-Tzvi et al. 2004, Abelson et al. 2005, Loya 2004, Loya 2007). Although much effort is being invested in monitoring the status of the corals and fish in this area, the possible role of other invertebrate taxa in affecting the community structure has unfortunately been overlooked. The aim of the current study is to investigate the ecology and life history of the ascidian *B. eilatensis*, focusing on its interaction with stony corals in a deteriorating coral reef environment.

### 5.2 Materials and Methods

The study was performed from 2004 to 2006 at Eilat (Red Sea coast of Israel). Observations were carried out using SCUBA diving. Ambient seawater temperature, chlorophyll-α levels and visibility data were obtained from The Israel National Monitoring Program (NMP, http://www.iui-eilat.ac.il).

#### 5.2.1 Geographic and depth distribution of *Botryllus eilatensis* in the coral reefs of Eilat.

Preliminary observations showed that *Botryllus eilatensis* appears in the natural reef during April and May. In 2006, during these months, ascidian surveys were conducted on both natural and artificial surfaces in the Gulf of Eilat: the natural coral reefs at Taba (N 29° 29.65' E 034° 54.345') and at the Inter-University Institute marine laboratory (IUI, N 29° 30.131' E 034° 55.045'), and on artificial surface (composed of wood, metal and Polyethylene pontoons) at the marine sport jetty at the eastern part of the
North Beach (NB, N 29° 32.856′ E 034° 57.265′). The NB site is considered as a highly disturbed site due to its proximity to the naval port area and other anthropogenic disturbances, in comparison to the Taba and IUI sites from which pollution sources are relatively distant and where recreational activities are limited (Ben-Tzvi et al. 2004). The different nature of the surveyed sites in terms of substratum availability and topography dictated the use of different survey methods. At the Taba site, due to very low densities of *B. eilatensis*, each colony observed within a defined area of 500 m² was measured and photographed. At the IUI site four belt-transsects (50 m long, 2 m wide) were performed perpendicular to the shore at 5-10 m depth. At the NB sport-jetty *B. eilatensis* density was estimated by counting colonies on 20 pilings (40 x 80 cm, data corrected to 1 m² density).

5.2.2 Seasonal appearance of *Botryllus eilatensis*. In order to study ascidian overgrowth dynamics and seasonal appearance, a total of 70 interactions between tagged coral colonies and the colonial ascidian *Botryllus eilatensis* were photographed monthly from April 2005 to June 2006 (Fig. 5.1a). The monitored corals had been previously attached in May 2001 by the staff of the Underwater Observatory Marine Park to a dome-shaped steel construction with a base diameter of 10m and a height of 3 m, at a depth of 10-14 m, between the IUI site and the underwater observatory (Fig. 5.1b). Colonies of *B. eilatensis* recruited onto the artificial structure and overgrew the corals that had been previously transplanted onto the dome structure. During the study all monitored interactions (approximately 50-70 interactions per month, except for November 2004 and February 2005 during the preliminary study, when n = 14 interactions) were photographed using a digital Sony T-11 and a Nikon CoolPix7900 cameras, keeping a fixed angle and distance from the object. Total surface area of the coral colony and the area covered by *B.*
eilatensis was measured using the CPCe (Kohler & Gill 2006) photo analysis program. Ascidian cover percentage was calculated as the percentage of available coral area (dead and live coral tissue) covered by the ascidian. The following film clip demonstrates the ascidian overgrowth at the study site


5.2.3 Botryllus eilatensis reproduction. During 2006, monthly samples (ca. 2 x 2 cm) from five different Botryllus eilatensis colonies (separated from each other by at least one meter) were taken from the NB site. The samples were narcotized with menthol crystals for up to three hours and fixed in 4% formalin buffer in filtered sea water. Twenty zooids from each sample were dissected under a stereomicroscope, and the presence or absence of testes and oocytes were recorded. The gonad index (in percentage, GI) was calculated as the ratio of zooids containing gonads (testis/oocytes) to the total number of zooids examined.

5.2.4 Botryllus eilatensis growth and survivorship.

In order to quantify differences in the growth and survivorship of Botryllus eilatensis in a high nutrient versus low nutrient sites a transplantation experiment was conducted during June 2006 at the NB (high nutrient levels) and IUI (low nutrient levels) sites. Five large colonies (> 20 cm in diameter) of B. eilatensis were sampled at the IUI site, separated into similar sizes and attached using sewing thread to a PVC plate (7 x 7 cm). The plates were placed in unfiltered, running seawater containers at the Inter University Institute (IUI) Marine Laboratory. Two days following successful attachment, 28 fragments were placed at the IUI jetty and 37 fragments were attached to the marine sport jetty (NB),
both at 1 m depth. Each plate was photographed weekly and the ascidian surface area was
measured using the CPCe (Kohler & Gill 2006) photo analysis program.

5.2.5 Statistical analysis. All statistical analyses were carried out using Statistica 7.
Analysis of ascidian percentage cover and gonad index data were performed after
arcsine\sqrt{p} transformation and tested for normality with Shapiro-Wilk’s test and
homogeneity of variance with Cochran’s test (Zar, 1999). Analysis of a possible
relationship between coral morph (branching corals versus massive corals), orientation
(north-facing corals versus south-facing corals) and size (coral surface smaller or larger
than 100 cm²) to ascidian overgrowth was investigated during the peak of the ascidian
overgrowth (Apr- Jun 2005) for each category using student’s t-test. Only the maximal
ascidian cover percentage scored per coral during this period was included. We were not
able to perform a factorial design ANOVA since there were not enough interactions for
the south-facing-small-branching corals group. Linear regression analysis was used to
determine whether the monthly average of the ascidian cover percentage is associated
with ambient seawater temperature, chlorophyll-a levels and visibility data. One-way
ANOVA was performed in order to investigate possible differences in gonad index
throughout the study. Fisher’s LSD (least significant differences) tests were used as post-
hoc comparisons when significant differences were detected. Separate t-tests were
performed in order to test differences in fragment size measurements between the sites
for each week and a comparison between the survivorship curves at each site was made
using the log-rank test. Results are presented as averages ± standard errors throughout the
text unless denoted otherwise.
Figure 5.1 (A) a monitored interaction between *Botryllus eilatensis* and the massive coral *Platygyra* sp. during March 2005. (B) The dome construction serving as substrate for the monitored corals.

5.3 Results

5.3.1 Geographic and depth distribution of *Botryllus eilatensis* in the coral reefs of Eilat.

Field surveys revealed that during April and May 2006 *Botryllus eilatensis* was common along the entire coast on artificial substrates (piers, artificial reefs) and in the natural environment, overgrowing coral skeletons and rock pebbles from shallow depth (1 m at
the NB site) to 10-15 m depth at the IUI and Taba sites. The number of *B. eilatensis* colonies at the IUI site was 5.5 ± 0.8 per 100 m² transect (n = 4) during April and 3.8 ± 1.1 during May (n = 4). At the Taba site the number of colonies observed increased from 7 to 15 per 500 m² from April to May. At the NB site the number of colonies increased from 5 ± 1.8 per 1 m² during April to 12 ± 5 per 1m² during May (n = 20).

### 5.3.2 Seasonal appearance of *Botryllus eilatensis*

Monthly photography revealed that the percentage of *B. eilatensis* cover over the monitored corals fluctuated throughout the year (Fig. 5.2b). The highest cover was measured during April and May 2005 (mean 27.0 ± 4.6 %), decreasing rapidly in the following months to a minimum of 0.18 ± 0.11 % in September. By April 2006 *B. eilatensis* had disappeared from the monitored area and reappeared the following month with an average cover percentage of 2.8 ± 1.3 %. No significant association was found between monthly average of ascidian cover percentage and sea water temperature ($r^2 < 0.01$, $p = 0.75$) or chlorophyll- $a$ measurements ($r^2 = 0.01$, $p = 0.7$). A weak but significant association was found between visibility (measured as secchi disk depth) and ascidian cover percentage ($r^2 = 0.42$, $y = -2.43x + 69$, $p < 0.01$) from November 2004 to June 2006. During the peak of the ascidian overgrowth on corals (April-June 2005) a significantly stronger association was found between visibility and ascidian cover percentage ($r^2 = 0.89$, $y = -1.53x + 65$, $p < 0.01$). During this period higher cover percentage was observed over massive corals (*Platgyra* sp., *Porites* sp., *Favia* sp., *Favites* sp.) in comparison to branching corals (*Stylophora* sp., *Acropora* sp., t-test, $t = 2.38$, df = 81, $p < 0.05$); north-facing corals in comparison to south-facing corals (t-test, $t$
= 2.37, df = 81, \( p < 0.05 \)); and small corals (< 100 cm²) in comparison to large corals (t-test, \( t = 2.56, \text{df} = 81, p < 0.01 \), Fig. 5.3).

**Figure 5.2** *Botryllus eilatensis*. Seasonal variation of mean percentages of ascidian cover (+ SE) relative to available coral area.

![Figure 5.2](image)

**Figure 5.3** *Botryllus eilatensis*. Comparison of mean ascidian cover percentage (+ SE) measured from April to June 2005 overgrowing north-facing corals (\( n = 41 \)) versus south-facing corals (\( n = 42 \)); massive corals (\( n = 47 \)) versus branching corals (\( n = 36 \)); and small (< 100 cm², \( n = 24 \)) versus large corals (\( n = 59 \)).

5.3.3 *Botryllus eilatensis* reproduction

Monthly samplings show that *Botryllus eilatensis* in the coral reefs of Eilat demonstrates seasonal reproduction (Fig. 5.4). Zooids with gonads were present throughout the year,
but the sequence of reproductive stages exhibited a clear temporal trend. While zooids with testes were found throughout the entire research period, zooids containing testes and ovaries (with one or two oocytes) appeared only from May to October when sea water temperature fluctuated between 23ºC to 27 ºC. During this time a significantly higher gonad index was measured, excluding August (one way ANOVA, followed by Fisher’s LSD, $F(10,43) = 7.48, p < 0.01$), reaching a maximum of $82 \pm 15 \%$ during June and July ($n = 10$). No embryos and no larvae were found in any of the samples.

![Figure 5.4 Botryllus eilatensis. The relationship between gonad development (average gonad index + SE) and sea surface temperature (SST± SDs). Gonad state is denoted by black bars for zooids containing testes and blank bars for zooids with testes and ovaries ($n = 5$ colonies).](image)

**Figure 5.4 Botryllus eilatensis.** The relationship between gonad development (average gonad index + SE) and sea surface temperature (SST± SDs). Gonad state is denoted by black bars for zooids containing testes and blank bars for zooids with testes and ovaries ($n = 5$ colonies).

### 5.3.4 Botryllus eilatensis growth and survivorship

Weekly monitoring of the transplanted fragments revealed a significant difference in fragment size between the sites after two weeks of transplantation ($t$-test, $t = 2.2$, df = 62, $p < 0.01$, Fig. 5.5a). Whereas the fragments transplanted to the NB site increased by 3-
fold in size reaching an average size of $15 \pm 8 \text{ cm}^2$ (SD) after one month, the fragments at the IUI site remained at the same size throughout the experiment. In addition, higher survivorship rates were observed at the NB site of 50% by the end of the experiment ($n = 37$), in comparison to only 30% at the IUI site ($n = 28$) but these differences were not significant (log-rank test, $p = 0.25$, Fig. 5.5b).

![Figure 5.5 Botryllus eilatensis. A) Average size (± SD’s) and (B) survivorship percentage of fragments transplanted to the NB site (black circles, $n = 37$) in comparison to the IUI site (blank circles, $n = 28$).](image)

**5.4 Discussion**

One of the major questions faced by coral reef researchers is that of the ongoing change to reef community assemblages. While phase-shifts from coral to macroalgal dominance have been a major focus in many studies (reviewed in McManus & Polsenberg 2004) only a few studies have discussed the role of filter-feeding organisms in deteriorating reef environments (Birkeland 1987, Bak et al. 1996). In deteriorating coral
reefs hermatypic corals and filter-feeding organism biomass are negatively correlated (Aerts & van Soest 1997), with sponge and ascidian biomass increasing as coral biomass decreases (Bak et al. 1996, Aerts 1998, Ward-Paige et al. 2005). In the present study we found that in the coral reefs of Eilat the newly-described colonial ascidian *B. eilatensis* (Shenkar & Monniot 2006) may rapidly overgrow corals during periods of nutrient enrichment and become a potentially important competitor for space with the reef building corals.

The monitoring of the coral-ascidian interactions revealed that *Botryllus eilatensis* has a seasonal appearance. During spring (April - June) the average percentage coral cover by the ascidian reached approximately 30% and decreased rapidly during the summer months (Fig. 5.2b). This rapid growth may be a result of the vertical mixing phenomenon that occurs in Eilat every winter, which results in higher nutrient levels in the water column (Genin et al. 1995). Ascidians are able to filter even very small particulate matter ranging between 0.5 to 2 µm diameter (Bak et al. 1998, Bone et al. 2003). Indeed, as visibility decreased, indicating an increase in particulate matter in the water column (Udy et al. 2005), ascidian percentage cover increased significantly, most likely due to the additional food supply. Similarly, Ward-Paige et al. (2005) reported an increase in Clionid sponge cover due to increase sewage contamination at the Florida Keys. As the water column gradually becomes depleted of particulate matter, the ascidian percentage cover declines until the following winter. The discrepancies in percentage cover between 2005 and 2006 may be a result of the deep and prolonged vertical mixing event that occurred in 2005 (*ca.* 600 m depth), which produced more
particulate matter, in comparison to the shallower (ca. 500 m depth) and shorter event during 2006 (Shaked & Genin 2007).

*Botryllus eilatensis* is very common on both natural substrates (IUI and Taba sites) and artificial substrates (NB) during April and May. However, while it became scarce in the natural environment during the summer, live colonies were still found in high numbers on artificial substrates at the NB site (N. Shenkar, unpublished data). Saito & Nagasawa (2003) described a similar phenomenon in another botryllid species in Japan, in which during unfavorable conditions (e.g., high SST) the blastozooids degenerate and only the vascular system remains. These colonies can live more than one month and recover by vascular budding if surrounding environmental conditions improve. Indeed, following one month of translocation of *B. eilatensis* fragments from the IUI site to the NB site, the fragments increased by 3-fold in size while the fragments at the IUI site remained at the same size (Fig. 5.5a). The NB fragments also exhibited higher survivorship rates (Fig. 5.5b). Again, this may be a result of higher food availability, since the NB site is subjected to more human-mediated disturbance due to its proximity to a port and a tourist area, resulting in higher nutrient levels (NMP, [http://www.iui-eilat.ac.il](http://www.iui-eilat.ac.il)) and consequently deterioration of the coral reefs at this area (Ben-Tzvi et al. 2004, Abelson et al. 2005).

We found significantly higher cover percentages of *Botryllus eilatensis* over the massive corals in comparison to the branching corals, over small coral colonies in comparison to large ones and over north-facing corals in comparison to the south facing ones (Fig. 5.3). The higher ascidian cover percentage observed over the north-facing corals may be a result of the north to south currents in the monitored area (Abelson et al.
which facilitate transport of food particles to the ascidians. The susceptibility of both the massive and the small coral colonies may be explained by the ascidian larval behavior and by the growth dynamics of the ascidian colony. Ascidian larvae are known to prefer dead coral skeletons for settlement (van Duyl et al. 1981, Stoner 1994) and shaded areas (Young & Chia 1984, Oren & Benayahu 1998). Although not quantified, it is possible that the massive coral growth forms on the dome construction provide more shaded area on their undersides in comparison to branching corals and therefore may be more "attractive" to ascidian larvae for settlement. In addition, field observations reveal that *B. eilatensis* advances over the coral skeleton from the coral base (demonstrated in: [http://www.tau.ac.il/lifesci/departments/zooology/members/loya/shenkar.html](http://www.tau.ac.il/lifesci/departments/zooology/members/loya/shenkar.html)). Branching corals may thus “escape in height” from ascidian overgrowth due to smaller colony surface area at the bottom of the colony (Meesters et al. 1996); while the small coral colonies could “escape in size” from ascidian larvae settlement. However, the latter have greater circumference relative to the total surface area ratio and are therefore more susceptible to overgrowth by benthic organisms (Meesters et al. 1996). During the peak ascidian appearance this overgrowth over the small monitored corals was 100% for a period of two months. Surprisingly, once the ascidian cover had disappeared (end of summer), the coral tissue seemed healthy and unbleached. Pawlik et al. (2007) found that coral reef sponges that interact with corals are able to cause coral bleaching by producing secondary metabolites. Ascidians too are able to produce noxious secondary metabolites (Pisut & Pawlik 2002, McClintock et al. 2004), but their effect on coral tissue has not yet been examined. It seems that the rapid growth rate of colonial ascidians (up to 14 cm
linear growth per month, Bak et al. 1996, ca. 10 cm² per month, current study) is the key to their ability to overgrow reef-building corals and occupy newly available surfaces.

There are a number of factors regulating sexual reproduction of ascidians, for which temperature (Millar 1971) and food availability (Sahade et al. 2004, López-Legentil et al. 2005) have been suggested as the main factors. We observed a significantly higher number of zooids containing gonads (testes and ovaries) from May to October (Fig. 5.4), when water temperature and solar irradiation rise. However, since no embryos or larvae were detected, it was difficult to ascertain the exact timing of larval release. Several studies have showed that the ascidian spawning season occurs after the period of highest water temperature (Goodbody 1961, Becerro & Turon 1992); therefore, it is likely that Botryllus eilatensis reproduces sexually from August to October. Although year-round reproduction is typical to tropical coral reef ascidians (Goodbody 1961, van Duyl et al. 1981), similar seasonality was observed by Hirose et al. (2005) for Diplosoma virens in Ryukus, Japan, which, like Eilat, is situated at the northern limit of coral reefs, and exhibits higher seasonal amplitude in water temperature than regions in low latitudes. In addition, Okuyama & Saito (2001) observed sexual reproduction in a Botryllid ascidian in Japan from July to December with a peak in August. López-Legentil et al. (2005) discussed the possibility of a trade-off between resource allocation to reproduction and asexual growth in a Mediterranean colonial ascidian. During the summer months, when there is "energy shortage" in the Mediterranean littoral system, growth rates of Cystodytes sp. were at their maxima, while reproduction occurred during spring and early summer, before the season of limited food availability. Here we found an opposite trend, in which during the winter months there is investment in asexual growth, following the
vertical mixing event that causes nutrient enrichment, while during the summer, as seawater temperature rises, there is an investment in sexual reproduction.

Birkeland (1987) suggested that along a eutrophication gradient the dominance of algae and filter feeders will increase, with filter feeders alone dominating under the most eutrophic conditions. We suggest that the rate of *Botryllus eilatensis* overgrowth on Eilat’s corals is influenced by food availability in the water column. Nutrient enrichment in this region occurring either due to human activities (i.e., mariculture, tourist area development, Loya 2007) or naturally (deep vertical mixing events, Genin et al. 1995), may result not only in algal blooms (Lindell & Post 1995, Smith et al. 2005) but also in booming populations of filter feeding organisms, as demonstrated in other coral reefs (Bak et al. 1996, Witman & Smith 2003, Ward-Paige et al. 2005). This is of ecological significance, because in contrast to an algal phase shift that may be controlled by herbivore pressure (Hughes et al. 1999, Szmant 2002, McManus & Polsenberg 2004) and is limited by light, in the case of *B. eilatensis* no significant predator is evident in the area nor is there an effect of light on its distribution (N. Shenkar unpublished data). A rise in monopolization of the substrate by ahermatypic organisms, such as ascidians, may reduce coral cover by preventing planulae from settling and decreasing the availability of the substrate for coral growth (Aronson 2002). The increasing evidence of the negative effects of rapid overgrowth by ascidians (Valentine et al. 2007, Bullard et al. 2007, Dijkstra et al. 2007) and their ability to alter marine communities and economically affect commercially important activities, such as fishing and aquaculture (Bourque et al. 2007), highlights the need to include this group in coral-reef monitoring programs worldwide.
GENERAL DISCUSSION

The current study is the first to focus on the ecology of ascidians along the Israeli coasts of the Red Sea and the Mediterranean. Since this group is relatively unknown in these areas, I used a multi-disciplinary approach combining taxonomy, ecology and life history. First, I ascertained the dominant species found in the region, and whether these include any non-indigenous species. I then assessed the annual appearance and spatial distribution of these ascidians and how this appearance may be related to different abiotic factors. Finally, I studied the possible impact of anthropogenic activities on ascidian ecology in both coral reef and Mediterranean shore environments.

Taxonomy constitutes the integrative basis of biology. Without taxonomists it would be impossible to (1) describe and name new species, (2) survey biodiversity and identify biodiversity hotspots or other areas of special conservation concern, and (3) correctly identify the source of natural products (Winston 2007). Therefore, in order to properly study this relatively unknown group of organisms in these environments, the first step I undertook was to correctly identify them. In section I, I review the available data regarding the ascidian fauna along the Israeli coasts and described the most dominant species found along the Red Sea coast of Israel. This part of the study led to the discovery and description of a new species, *Botryllus eilatensis* (Shenkar & Monniot 2006, Appendix I). In addition, two solitary species, *Rhopalaea* sp. and *Boltenia* sp., found in Eilat, are also suspected to be new species (F. Monniot personal communication). These findings emphasize the little attention and poor sampling effort that the ascidian fauna has received in past years in the coral reefs of Eilat. The Red Sea’s marine and coral habitats are unique and are world-renowned for the highly diverse fauna
and high numbers of endemic species (Getahun 1998). However, from a historical perspective, since the major oceanographic expeditions, such as that of the R/V Pola at the end of the 19th century, the Cambridge Expedition to the Suez Canal in 1924 and the Israel South Red Sea Expeditions in 1962 and 1965, the study of the Red Sea fauna has been limited to the dominant groups such as corals, fish and mollusks, with very little information being available on other groups, including sparse comparative data and no good data baseline. My study therefore attempts to provide a classical base-line list of species common in the northern Gulf of Eilat/Aqaba. Such an established data-base is imperative when considering the vast changes that this region is undergoing (Loya 2007). These are already evident when observing the disparity between the current species list and that published by Pérès (1962).

One of the major threats to the integrity of natural communities in the modern era is that of human-mediated biological invasion. In its own natural environment, a potentially invasive organism lives in balance with other organisms and is controlled by the ecosystem interactions (i.e. predation, competition). Once such an organism invades an alien environment, however, it can turn out to be a threat to the indigenous species. Such nuisances threats are demonstrated in the introduction of the comb jellyfish (*Mnemiopsis leidy*) into the Black Sea in the early 1980s (Travis 1993, Kideys 2002), the zebra mussel (*Dreissena polymorpha*) introduced into North American lakes (Martel et al. 2001), and the introduction of the Nile perch (*Lates nilotica*) that has resulted in the disappearance of over a hundred species of cichlid fishes in Lake Victoria (Goldschmidt et al. 1993).

During the current study six non-indigenous species (*Phallusia nigra*, *Herdmania momus*, *Microcosmus exesperatus*, *Rhodosoma turcicum*, *Ascidia canellata*, and
Symplegma brakenhielmi) were recorded from the Mediterranean shores of Israel and described in section I. These species probably reached the Mediterranean via the Suez Canal, since all have an extra-tropical Indo-Pacific distribution and a restricted distribution in the Eastern Mediterranean (Streftaris et al. 2005, Çinar et al. 2006, Izquierdo-Muñoz et al. 2007, Turon et al. 2007). Interestingly, two species, *M. exasperatus* and *H. momus*, were found to be restricted to artificial substrates. In a study conducted in Guam, it was reported that all the 15 known non-indigenous ascidian species in that area were found on artificial surfaces; while only three of them (*Botryllus niger*, *Cnemidocarpa irene*, and *Herdmania pallida*) also occurred on nearby reefs (Paulay et al. 2001). On natural reefs these three species were small, sparse, and formed only a negligible part of the reef fauna, in striking contrast to their larger size and numbers on artificial substrates (Paulay et al. 2001). A similar phenomenon was also found at some sites along the Mediterranean coast (Figure 6.1), where species with Indo-Pacific origin form dense populations on artificial substrates.

Figure 6.1. Non-indigenous ascidians (mainly *Phallusia nigra* and *Rhodosoma turcicum*) covering the Electric Company pier tunnels in Ashdod, Mediterranean coast of Israel.
This demonstrates the opportunistic nature of non-indigenous ascidians in the fouling community (Blum et al. 2007, Osman & Whitlatch 2007). The inability of *H. momus* and *M. exasperatus* to settle in the natural environment along the coasts of Israel may indicate that they are currently in the primary stages of establishment and that, in the future, they may well be found not only on artificial substrates but in the natural environment as well. The increasing evidence of negative effects of non-indigenous ascidians on the natural fauna (Castilla et al. 2004, Cohen et al. 2005, Valentine et al. 2007, Bullard et al. 2007, Dijkstra et al. 2007a) emphasizes the need for comparative studies of the introduced species in their natural versus their introduced site. Such data are currently limited but are of great importance since they may provide a tool for estimating invasion rates and possible effects on the natural fauna at the “invaded” site. The uniqueness of the Israeli coast line, namely the combination of a coral reef environment and a Mediterranean rocky shore only few hours apart, provides a fertile ground for such studies. Thus, in section II, I focus solely on the study of comparative aspects of the ecology of the solitary ascidian *H. momus*, in its native (Red Sea) versus its introduced (Mediterranean) site. The results of both molecular analyses and field surveys strengthen the evidence for *H. momus* being an introduced species that arrived via the Suez Canal (table 3.1, 3.2, figure 3.5).

In addition, clear differences were found in the ecology of *H. momus* populations from the Mediterranean site (Hadera) and the Red Sea site (Eilat). In the Mediterranean *H. momus* exclusively inhabits artificial substrates and is common at greater depths than in Eilat (Red Sea). Individuals from the Mediterranean were significantly larger than individuals collected in Eilat, and were inhabited by different symbionts (figures 3.2,
3.3). These differences are attributed to different temperature ranges, food availability, water currents and wave exposure. One of the most marked differences found was that of the reproductive cycles (figure 3.4). Whereas in Eilat *H. momus* reproduces year round, in the Mediterranean it displays a short reproductive season, occurring only when water temperature conditions resemble those measured in the Red Sea (22 – 25 °C). I suggest that the restricted reproductive period of *H. momus* in the Mediterranean may be the reason why this species has not yet spread to colder regions in the western Mediterranean.

Stachowicz et al. (2002b) showed that, under laboratory conditions, the growth rate of non-indigenous ascidians at high temperature was higher compared to that of native species. Therefore, they contend that global warming may facilitate a shift to dominance by non-native species, accelerating the homogenization of the global biota. Considering the anticipated rise in sea-water temperature due to global warming (Rahmstorf & Ganopolski 1999), the ongoing process of the arrival and spread of non-indigenous ascidians with a tropical origin along the Mediterranean coasts are anticipated. Indeed, it has recently been reported that *H. momus* has dispersed farther north and is now found at one site along the western Levantine coasts of Turkey, overgrowing rocks with algae (Çinar et al. 2006).

As demonstrated earlier, for a broader view of the ecological aspects of the studied group, the ascidiaceae, the effects of both temporal and spatial parameters cannot be ignored. The location and timing of one species’ appearance may be the result of seasonality of reproduction (Benayahu & Loya 1985, Gotelli 1987), larval preferences (Bingham & Young 1991), and effects of different a-biotic factors such as food availability (Sahade et al. 2004), light intensity (Glasby 1999), water temperature (Becerro & Turon 1992) and
more. These issues are discussed extensively in section III, in which I examine the ascidian recruitment patterns on an artificial reef in Eilat (Red Sea). By identifying the ascidians to species level, we were able to distinguish between the specific recruitment patterns of the different species, rather than generalizing patterns among groups as done in most studies (Fitzhardingh & Bailey-Brock 1989, Perkol-Finkel & Benayahu 2007). I show that these ascidian recruitment patterns are species-specific and vary between seasons, orientation and position in the water column (figure 4.2-6 and tables 4.1, 4.2). The major contributions to the data presented in this section are: 1) the finding that two dominant species, *Herdmania momus* and *Didemnum granulatum*, established the pattern for the entire group, and, 2) the finding that the epifaunal assemblage developing on an artificial substrate in a tropical environment is influenced by the season of deployment. These results should be taken into consideration when investigating community development on such structures, especially since in coral reefs, possibly due to the less dramatic changes between seasons, this aspect has not been considered to date (Goren 1979, Angel & Spanier 2002, Perkol-Finkel et al. 2006). Nevertheless, similar studies from temperate and cold regions conclude that the effects of period of submersion are reduced with increasing length of exposure (Qiu et al. 2003, Brown 2005).

One of the most striking seasonal phenomena observed in the coral reefs of Eilat during the study was that of the seasonal appearance of a new colonial ascidian, *Botryllus eilatensis* (Shenkar & Monniot 2006). This species was observed overgrowing dead coral skeletons and rapidly colonizing artificial substrates. In order to study overgrowth dynamics and the relationship between coral morphology, size, and orientation and ascidian overgrowth, we conducted monthly monitoring of 50 - 70 tagged coral colonies
interacting with *B. eilatensis* (section IV). This study is the first to investigate coral-ascidian competitive interactions. It is of major importance since the environmental conditions that are deleterious for corals (e.g. eutrophication) may create a favorable environment for filter-feeding organisms, such as ascidians, accelerating their growth and spread (Bak et al. 1996, Aerts & van Soest 1997, Aronson et al. 2002, Ward-Paige et al. 2005). The rate of *B. eilatensis* overgrowth on corals was influenced by the coral’s morphology and orientation (figure 5.3) combined with the seasonal appearance of the ascidian (figure 5.2). This seasonality was linked to the vertical mixing phenomenon that occurs in Eilat every winter and results in higher nutrient levels in the water column, and thus in higher food availability (Genin et al. 1995, Lindell & Post 1995). Surprisingly, although during the peak of ascidian appearance some of the smaller monitored corals were completely covered for a period of two months, once the ascidian cover had disappeared (end of summer), the coral tissue seemed healthy and unbleached and the corals continued to grow. I further demonstrated that *B. eilatensis* fragments translocated to a nutrient-rich site increased three-fold in size and exhibited higher survivorship rates in comparison even to intact fragments in a nutrient-poor area (figure 5.5). It has been suggested that changes in water quality in tropical coasts, particularly pertaining to the availability of nutrients and organic matter, will result in vast changes in these benthic communities (Bak et al. 1998, Ward-Paige et al. 2005). However, only the studies by Bak et al. (1996) and Witman & Smith (2003) have reported an increase in ascidian biomass due to changes in water quality. Hence, nutrient enrichment in Eilat’s coral reefs, occurring either due to human activities (i.e.: mariculture, tourist area development, Loya 2007) or naturally (deep vertical mixing events, Genin et al. 1995), may result not only in
algal blooms (Lindell & Post 1995, Smith et al. 2005) but also in booming populations of filter-feeding organisms, such as ascidians. This highlights the need to include this group in coral-reef monitoring programs worldwide.

The data collected in the above studies demonstrate the significant impact of human activities on ascidian ecology. Since ascidians are able to filter even minute particulate matter (Bak et al. 1998, Bone et al. 2003), any rise in nutrient levels and organic material in coastal waters will have a direct influence on their appearance. This was evident in the large size of *H. momus* found in the Mediterranean and the seasonal outbreaks of *B. eilatensis* in the coral reefs of Eilat. In addition, being a major component of the fouling community, ascidians play an important role in marine bioinvasion. Adult ascidians on ship or barge hulls may survive transport over thousands of kilometers to harbors with conditions similar to those they had left (Lambert 2002). This was apparent in field surveys conducted along the Mediterranean coast of Israel, in which six non-indigenous ascidian species with an Indo-Pacific origin were found. These species were particularly abundant on artificial marine structures, which are known to facilitate the spread of non-indigenous species (Bulleri & Airoldi 2005). Thus, another human activity that may contribute to the spread of non-indigenous ascidians is the proliferation of artificial structures along the coastline, which may create corridors for the dispersal of non-indigenous species across areas of otherwise unsuitable habitat (Bulleri & Airoldi 2005).

Aiscidians can be strong spatial competitors, and once they become established they often experience population explosions that can develop into dense mats that overgrow and cover available surfaces (Bourque et al. 2003, Carver et al. 2003). However, although there is a growing awareness that ascidians can become of economic and ecological
concern, unlike these taxa with a direct economic value (i.e., fish, bivalves, crustaceans), relatively little is known at present about the ecology of many of these ascidians. It is my hope that the current thesis will create a renewed interest in the field of ascidian ecology and that it will encourage future studies in this field and help in the development of tools for controlling the negative impacts of human activities on coastal regions.
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APPENDIX I


(Asciidae) from the Red Sea
A new species of the genus *Botryllus* (Asciidiacea) from the Red Sea

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Abstract

A new ascidian species was discovered during a study of the ecology of ascidians off the Red Sea coast of Israel. This species has been provisionally assigned to the genus *Botryllus* as complete details of its life cycle are not available to confirm Botrylloides as a junior synonym. The species displays characteristics of Botryllinae, in which growth is associated with active replication of zooids. The species is distinguished by the opaque pale pigmentation of the colonies, the zooid arrangement in regular double rows converging to common cloacal openings at the top of low lobes, the large number of stigmata rows, and ovary lateral to the testes. We name the new species *Botryllus eilatensis*, after the collecting site, the coral reef of Eilat.

Key words: taxonomy, synonym, ascidian, Red Sea, Gulf of Aqaba, Botrylloides

Introduction

The coral reefs of the northern Gulf of Aqaba (Eilat) are noted for their extraordinarily high biodiversity (Loya 1972). Although situated at the northernmost boundaries of coral reef distribution, these reefs are among the most spectacular and diverse in the world and include many endemic species (Goren 1993). However, whereas corals, mollusks and fish, have been studied extensively, the ascidian fauna remains poorly known. The most recent list of ascidian species from Eilat (Pérès 1962) contains only 19 species. This list is certainly incomplete considering the number of ascidian species reported in the past from the Red Sea (Michaelsen 1918, 1920a,b). In the present paper we describe the morphology and distribution of a new species of the genus *Botryllus* from the coral reefs of Eilat. This genus has a wide distribution and is a common fouling organism in estuaries and shallow water (Kott, 1985). The two known genera in the subfamily Botryllinae, *Botryllus*
Gaertner, 1774 and *Botrylloides*, Milne Edwards, 1841 are distinguished by relative positions of their gonads and aspects of their embryogenesis (see Hartmeyer & Michaelsen 1928, Van Name 1945, Kott 1985). *Botrylloides* having ovaries of mature zooids posterior to the testes and incubating embryos in brood pouches, whereas in *Botryllus* ovaries are anterior to the testes and there are no brood pouches. However, species that are not consistent with the above definition (Mukai 1986, Saito et al. 2001) suggest that the forms may be congeneric and raises the need for a revision of the species in the subfamily. At this stage, we have assigned the new species to the senior synonym, *Botryllus*. The species is named for the collecting site, the city of Eilat.

**Material and methods**

During 2003–2005 routine surveys and samplings of ascidians were conducted along the Red Sea coast of Israel (29°30' N, 34°56' E) in order to establish a current list of the ascidian fauna of this region. During these surveys colonies of the ascidian *Botryllus eilatensis* n. sp. were collected by SCUBA from several sites along the coast (Fig. 1) on different substrates. This ascidian was also present on artificial structures (piers, buoys, artificial reef) as well as overgrowing dead corals and rock pebbles in the natural environment, at a shallow depth from one to 20 meters. The specimens were brought to the laboratories of the Inter University Institute (IUI) in Eilat where they were narcotized with menthol crystals for up to three hours and fixed in 4% formalin in filtered sea water. Dissections were stained with hemalum and mounted on permanent slides.

**FIGURE 1.** Map of Gulf of Aqaba with study sites
Description

Botryllus eilatensis n. sp.


External appearance. Living specimens are a striking opaque white or orange color (Fig. 2). Intermediate colors have not been found. Colonies are soft and vary from being small discs of a few millimeters diameter to large sheets, up to 30cm wide and 0.5cm thick. The colony surface is generally free of epibionts and foreign matter and is raised on low lobes. Ridges separate adjacent double rows of zooids. The small colonies are cushion-shaped with a central excurrent aperture; the largest colonies appear as thick encrusting sheets. The edges of the colonies are fringed with a thick band of parallel, elongated terminal ampullae of the tunic vessels (Fig. 2). The large common cloacal apertures are up to 0.5cm in diameter in life. The oral apertures are wide, in regular double rows, separated by wide spaces. After fixation, the colony becomes light brown and the tunic transparent.

FIGURE 2. Both color morph of Botryllus eilatensis n. sp.

The zooids are perpendicular to the colony surface, in a single layer. The largest ones measure three millimeters in length. The body wall contains sparse, round, brown and white pigmented cells in fixed specimens. Musculature is weak. The oral siphon is short and wide (Figs 3A, B; 4A) with a plain edge or six very shallow lobes. Three orange pigment spots can be seen at the base of the siphon, one at the top of the endostyle, and one on each side. The oral sphincter is weak. The atrial aperture is wide and exposes the whole branchial sac in fully developed zooids. The dorsal lip forms a hood over the opening (Figs 3A; 6). Its size varies, sometimes being as long as the zooids. The posterior edge of the atrial opening is also produced out forming concavity in the postero-dorsal part of the atrial cavity. Fine muscles are present around the margin of the atrial aperture when the...
muscles on the oral siphon and the thorax are not conspicuous. The 12 oral tentacles are short and well spaced (Figs 4A, 5A). The peripharyngeal band is bent in a deep V shape. The button-like dorsal tubercle opens in a vertical slit. The branchial sac is particularly large with 15 to 18 rows of stigmata and, halfway down the thorax (Fig. 3; 4A), about 20 stigmata are in each half row. The second row is incomplete, and does not reach the dorsal line (fig. 4A). The three longitudinal vessels are equidistant, running the whole length of the branchial sac (Figs 3; 5A).

The gut is small, mostly located behind the branchial sac (Figs 3A,B; 4A; 5A). The first loop is narrow, the secondary loop is variable, the plain anus opens at the third row of stigmata counted from the base of the thorax; The stomach has nine folds with the proximal part flaring out into protruding ampullae (Figs 4B,C; 5C). A very small button-like caecum is hardly visible without staining. The pyloric gland forms a ring of vesicles around the intestine at stomach level (Fig. 5C). The stomach and a segment of the intestine are orange in life.

**FIGURE 3.** *Botryllus eilatensis* n. sp. Two nourishing zooids stained with hemalum: A, without gonads; B, with testes. Scale bar: 1 mm
FIGURE 4. *Botryllus eilatensis* n. sp. A, young zooid with fully developed branchial sac; B, internal side of the gut and testes; C, external side of the stomach; D, first order bud with gonads and second order bud. Scale bar: A, B, C: 0.5 mm; D, 0.2 mm.

In the few colonies that contained gonads, the male gonads appeared first. In these specimens (except in a single colony), the nourishing zooids have only testes (Figs 3B, 5A), and the ovaries appear only in buds (Figs 4D; 5B). The testis consists of rounded, branched follicles crowded into a spherical mass on each side of the body, just anterior to the gut lop (Figs 3B; 4B; 5A). One bud on each side is rapidly isolated from the parent zooid and develops in the deepest part of the colony. These buds acquire gonads at a small size, no more than 0.6mm. The testes appear first. The ovaries are small with one or two oocytes in the most developed buds which protrude from the body wall above the gut (Fig. 4D; 5B). A secondary bud grows above the gonads in the first-order buds, whose atrial opening is not yet differentiated but whose branchial sac and longitudinal vessels are similar to those of the adults. The stomach is not fully developed at this stage (Fig. 4d). Ovaries were found in only one colony collected in August. An ovary with a single oocyte
(0.25 to 0.3 mm diameter) is on each side of the zooid, lateral to the testis and slightly extruding from the body wall. In this colony almost all zooids had ovaries and testes, with very small buds located deep close to the substrate. No embryos and no larvae were found in any of the various colonies collected in different seasons.

FIGURE 5. Botryllus eilatensis n. sp. stained with hemalum. A, zooid opened along the ventral line; B, first order bud; C, digestive tract. Scale bars: A,1 mm; B,C, 0.2 mm.
The Botryllinae are common worldwide but have not been well described. It is difficult to determine whether the new species is native to the Gulf of Aqaba or an invader as it has colonized harbor structures as well as natural substrates. Further investigations and collections in more southern parts of the Red Sea may answer this question.

*B. eilatensis* appears to be a very successful competitor for space in the northern Gulf of Aqaba (Eilat), overgrowing dead coral skeletons and rapidly colonizing artificial substrates. This may be partly the result of increased anthropogenic activity, particularly eutrophication (Loya 2004) occurring in this area. This environment creates favorable conditions for filter-feeding organisms such as ascidians, providing an advantage in competition for space with reef-building corals. It is therefore imperative to continue monitoring its populations and further study ecological aspects of this species.

**Remarks.** This new species has some characters in common with *Botrylloides lentus* Saito & Watanabe, 1985, from Japan: numerous stigmata rows, large atrial apertures, and similar number of stomach folds. However, *B. eilatensis* has a smaller gastric caecum, the ovary is lateral to the testis rather than beneath it; and; the opacity and color of the tunic are distinctive. *Botrylloides perspicuum* Herdman, 1886 has also numerous rows of stigmata, but the ovary is anterior to the testis. *Botrylloides violaceus* Oka, 1927 has much less stigmata rows.

In *Botrylloides lentus* the larva is incubated in a brood pouch hanging beneath the testis, as is characteristic of the genus *Botrylloides* Milne Edwards, 1841 (see Hartmeyer & Michaelsen 1928 and other authors). Saito and Okuyama (2003) compared Botrylloides
and Botryllus Pallas, 1766 species. They concluded that morphological and life history characteristics are not good reasons for distinguishing these genera. We have adopted this interpretation and assigned the new species to the senior synonym Botryllus (see also Monniot and Monniot 1987).

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היבטים אקולוגיים של היבטים האיצטלבנים
לארד חופי ישראל

הויר ל乄ן קכלת התואר "דוקטור לפילוסופיה"
מאת
נועה שנקר

הוות לסניאט אוניברסיטת תל-אביב
שבט תחט'ה
פברואר 2008
עבורה וنعשתהvasion

פרופ' יוסי ליזה
אצטלים (Phylum: Chordata; Class: Ascidiae) מתחלקים לאצטליםкровנים (Urochordata) או Tunicata (או Acoelomata), האצטלים כמחלקה מונה לנים - 3000 מינים, יחידנים ואימיים שחיים בים."}

阿拉伯語翻譯

提要

腔腸動物 (Phylum: Chordata; Class: Asciidae) 包括門腔腸動物門 (Urochordata) 或 Tunicata (或 Acoelomata)。腔腸動物作為一個類別包括了 3000 種單個的和懸浮在海中各地的腔腸動物。

腔腸動物的種類包括了海綿屬 (Tunicata) 或腔腸動物門 (Urochordata)，其中包含了生活在各地的海綿屬，最好的例子是 "Botryllus eilatensis" (Shenkar & Monniot 2006) 和 "Phallusia nigra", "Herdmania momus", "Microcosmus exasperatus", "Rhodosoma turcicum", "Ascidia canellata" 和 "Symplegma brakenhielmi" (Davies 等人 2006)。

研究了「A. canellata」和 "H. momus" 兩種腔腸動物的生理、繁殖和自然共生者。研究了 "H. momus" 在 "Eilat" 湾的種群的生長情況，並探索了不同的腔腸動物種群的影響。最終，研究了 "H. momus" 的自然變化，並探索了 "H. momus" 的種群在 "Eilat" 湾的三種不同的腔腸動物種的影響。
מלאכותיים ו디יר מזא להב臨ון אורת ברסיבא התсужית. או פק בועמקו של 10-20m ודעור
חלلتימיו מועמקים רדידים מ-2m. לעופות.zh,秕אול גית לולאה מינו הזג ברסיבא התсужית והאו
מazı באיקר בקע 가운데 העמקים 0-10m. ב-50% מכלל ההנטוט שבנאות קטא בזרום סטרניזים
השטייטים לקשלת השטרליגים (Copepoda) ב-10% מהנטוט שבנאות בולגד מ zza סטרן ארך
הבטן היהודים שאקשרים מיイ איצטיליגים שוויים. שמיבוטו הז עניר החלטימיו מז
הדיריה יהודית תיכון. בהל מובח נמaja בין משקל הפרטס בז הצינוק הנדול פי 3 חותים ממושק
הפרטים הסימפליים. חור גוזל הנקה המאיפוריא תפריטים מיイ צינוק, גודק הגדות
החדישים מארח י יפרטים באית גוזל בלשון אחד לכל חותנה, בעד הפרטים בז צינוק
הגדות בלשון בחזיתים מה骀אי וגי יובר מ שלי. חאפר סטרפיטרוריים המיイ צינוק משונתוה
טורפיטורוריים המיイ באית. י仡ואר אל יദיעות על הצינוק בהזנית בי אוכלי נים האצטלג
י הגדות מבולים בז H. momus
זרימה, והשיפה לילה.

בתקהל השיחות של העבורה נ bakım דפוס החיתוניות של מספר מיני איצטיליגים ו-זמני על
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ונת הידת המים בחיתוניות החיתוניות של כל מיני, ושנויו המזון המוביל הקיסין
Herdmania momus חיתוניות מגברת המחלקים. תבתרה זה חתולו čי שיני יתים ודומיוטיסים
וקשישה חיתוניות דפוס החיתוניות של הקובץ בעד מיני פרגים פוחת
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ולימיו. ממציא זה חורו על השבורה רבע ממכרים מסופי זה.columnName,בראורי שינית האלמנוגנים לא
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 Haram מהפושבי
הגד חるべき האלמנוגנים הפרפרים אילת בצל בורמיט
העונת בשונית. על מצאת הענק אחר דינאירקטרים מ הבריאות והאינתואירקטרים של מינו הזג ברסיבא
וגר במשש שוניים מעקב בצמדתculo האלמנוגים וחוזימי אחר 50 מוסבות אלמנוגים מסומנות

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