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Research Article

# Temperature and salinity sensitivity of the invasive ascidian *Microcosmus exasperatus* Heller, 1878

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## Abstract

Environmental factors, such as temperature and salinity, are known to influence distribution patterns and invasion success in ascidians. The solitary ascidian *Microcosmus exasperatus* Heller, 1878 has a wide global distribution and can be found in both tropical and sub-tropical waters. In the Mediterranean Sea, it is considered to be an invasive species introduced through the Suez Canal, with a restricted distribution in the eastern Mediterranean. Despite its global distribution, the environmental tolerances of this species are poorly known. We examined the effect of varying temperature and salinity on the survival of adult individuals of *M. exasperatus* in a laboratory setting to partially determine its environmental tolerance range. In addition, it's global and local distribution as well as the seasonal abundance in 'Akko Bay (northern Mediterranean coast of Israel) were examined. Field observations and laboratory experiments show that *M. exasperatus* is able to tolerate a temperature range of 12–30 ºC, and salinity of 37–45, but it survived poorly in salinity of 33–35 and temperatures > 32 ºC. Considering this relatively wide tolerance range of *M. exasperatus* to temperature and salinity together with the anticipated rise in anthropogenic disturbances, we expect this species to further spread into new locations along the Mediterranean coast.

**Key words:** invasive ascidians, tunicates, environmental tolerance, survival, global warming, Mediterranean Sea, Suez Canal

## Introduction

Invasive species can have severe impacts on marine communities as well as on human health and economy (Musselman 1994; Galil and Zenetos 2002; Pimentel et al. 2005; Streftaris and Zenetos 2006). Ecologically, they affect the structure and function of native communities, which can eventually lead to alteration of the physical and biotic features in the invaded ecosystem (Carlton 1996; Stachowicz et al. 1999; Elton 2000; Grosholz et al. 2000; Castilla et al. 2004a,b). To survive and reproduce in the new environments, invasive species often possess a wide physiological tolerance towards environmental stressors such as temperature, salinity, light intensity, oxygen availability, and pollution (Kinne 1963; Guisan and Thuiller 2005; Piola and Johnston 2006; Epelbaum et al. 2009; Lenz et al. 2011). It has been estimated that only one out of ten introduction events will manage to establish a thriving population in its new environment (Williamson 1996). Many of the introduced species are opportunistic, often exploiting temporal windows of tolerable conditions to settle and establish in a new habitat (McKinney 2002).

The Mediterranean Sea is considered a "hot spot" for marine invasive species (Coll et al. 2010; Seebens et al. 2013; Galil and Goren 2014), with check-lists including between 680 to nearly 1000 non-indigenous species (Zenetos et al. 2012; Galil et al. 2014a). The Suez Canal, which connects the tropical Red Sea to the sub-tropical and temperate Mediterranean Sea, plays a major role as a passage for non-indigenous species into the Mediterranean (Galil 2006; Galil et al. 2014b). This phenomenon is known as "Lessepsian migration" (Por 1978) or "Eritrean invasion" (Galil 2006). Hundreds of species have crossed through the Canal into the Mediterranean since its opening in 1869 (Galil and Goren 2014), and this number is expected to significantly increase in the future due to the recent expansion of the Canal (Galil et al. 2014b).

Ascidians (Phylum: Chordata, Class: Ascidiacea) are conspicuous invasive species in both tropical and temperate waters (Lambert and Lambert 1998, 2003; Izquierdo-Muñoz et al. 2009; Shenkar and Loya 2009; Marins et al. 2010; Tamilselvi et al. 2011; Rocha et al. 2012). They are known to be strong competitors (Bullard et al. 2007; Bullard and Carman 2009; Locke and Carman 2009; Lindeyer and Gittenberger 2011), with a wide range of tolerance to physical conditions (Sims 1984; Naranjo et al. 1997; Lowe 2002; Pineda et al. 2012a,b). These traits make ascidians highly successful invaders that can create major economic expenses by heavily fouling marine vessels and man-made structures, as well as by overgrowing aquaculture equipment and organisms (Bullard and Carman 2009; Daigle 2009; Davis and Davis 2010; Aldred and Clare 2014). In addition, invasive ascidians can severely alter the structure and function of the native communities (Lambert and Lambert 1998; Lambert 2002; Castilla et al. 2004b; Bullard et al. 2007; Mercer et al. 2009).

In the Mediterranean Sea, the solitary ascidian *Microcosmus exasperatus* Heller, 1878 is considered a Lessepsian invader that has established thriving populations along the eastern basin (Turon et al. 2007; Izquierdo-Muñoz et al. 2009; Ramos-Esplá et al. 2013) and more recently has expanded its distribution in the Levant basin into Turkey (Ramos-Esplá et al. 2013) and Cyprus (Gewing et al. 2016). In the eastern Mediterranean, *M. exasperatus* is capable of reproducing almost year round, overcoming wide temperature and food availability fluctuations (Nagar-Raijman 2014). It was reported along the Mediterranean coast of Israel from both artificial and natural substrates (Shenkar and Loya 2009) and is capable of forming large aggregations (Nagar-Raijman 2014). The dense populations and typical heavy epibiont coverage of *M. exasperatus* may contribute to its establishment as an ecosystem engineer species, as has been reported for another tunicate *Pyura praeputialis* (Heller, 1878) in Chile (Castilla et al. 2004b). To date, there are no reports of *M. exasperatus* from the western basin, indicating that its distribution is restricted to the eastern Mediterranean (Turon et al. 2007).

Numerous factors influence invasive ascidian recruitment, reproduction, and abundance; the two most significant being temperature and salinity (Kinne 1963; Auker and Oviatt 2008; Epelbaum et al. 2009). Determining the role of tolerance ranges of ascidians and their interaction with environmental conditions is thus critical to an understanding of the patterns of invasive ascidian recruitment, dispersal, and global distribution. Changes in world climate such as elevated sea water temperature and salinity (Somot et al. 2006) are expected to influence the dispersal patterns of ascidian populations, allowing invasive ascidians to become strong competitors that may displace native fouling communities (Stachowicz et al. 2002; Lambert and Lambert 2003; Agius 2007). In view of the ongoing tropicalization of the Mediterranean (Bianchi and Morri 2003), it is crucial to investigate the physiological sensitivity of invasive organisms of tropical origin (such as *M. exasperates*) to achieve a better understanding of its present distribution, persistence, and potential to spread into new environments under the changing environmental conditions.

The aim of this study was to determine the temperature and salinity tolerance of the invasive ascidian *M. exasperatus* found along the Mediterranean coast of Israel. In addition, the local population density and current global and local distributions were examined, and their relation to the environmental tolerance of *M. exasperatus* discussed. We hypothesized that *M. exasperatus* would exhibit a wide range of tolerance to temperature and salinity conditions; thereby greatly contributing to its potential to spread to additional sites in the Mediterranean Sea, particularly in view of the anticipated rise in sea water temperature and salinity (Somot et al. 2006).

## Materials and methods

### *Local and global distribution*

To determine the local spread of *M. exasperatus* along the Mediterranean coast of Israel, underwater surveys were conducted along the coast between October 2012 and August 2014. We surveyed 21 sites, six of which were marine harbors or vessel docks (Figure 1, Supplementary material Table S1). At each site, where possible, specimens of *M. exasperatus* were identified, and three to four specimens were taken to the laboratory for taxonomic verification using the taxonomic key of Kott (1985).

The global distribution of *M. exasperatus* was reviewed using the available literature, on-line taxonomic databases such as Ascidiacea World Database (Shenkar et al. 2015), and examination of open-access on-line libraries such as the Biodiversity Heritage Library (BHL). A distribution map was created using ArcGIS version 10.0 software (Geographical Information System by ESRI, Redlands, CA, provided by Systematics Technologies R. G. Ltd., Tel Aviv).

The seasonal changes in abundance of *M. exasperatus* were monitored from July 2013 until August 2014 in ‘Akko Bay (32°55'17.23"N, 35°04'22.08"E) (Location 2; Figure 1), on the northern Mediterranean coast of Israel. The number of specimens was counted on either side of an 11 $m^2$ submerged shipwreck with two sides, one facing open water (the southern side) and one facing the shore (northern side) at a depth of 0.5–1.5 m. The surveys were conducted on a monthly basis from January 2014 to August 2014 (except of May 2014), and once or twice per season depending on sea conditions, from July 2013 to December 2013.

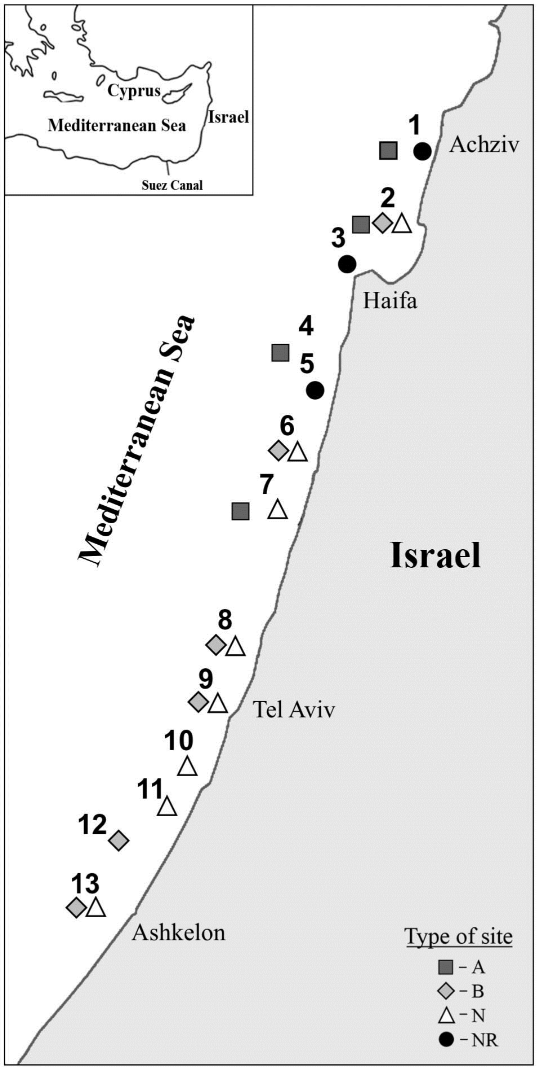


**Figure 1.** Locations surveyed for *Microcosmus exasperatus* and location of study area in the Mediterranean Sea (inset). A. Artificial substrate B. Marina N. Natural substrate NR. Nature reserve. Details of locations corresponding to station numbers are provided in Table S1. ‘Akko Bay is at location 2.

*Temperature and salinity experiments*

Temperature and salinity tolerance experiments were conducted in the laboratory from February-March and May-June of 2014, respectively. Mature individuals of *M. exasperatus* were collected for each experiment from the Ashkelon National Park (31º39'54.55"N 34º32'39.69"E) (Location 13; Figure 1) at a depth of 4–10 m, using SCUBA. The specimens were transported to the laboratory within two hours of collection. To minimize stress, the specimens were separated into numerous natural-seawater filled tanks. In the laboratory, the specimens were placed in 3-liter aquaria (two individuals per aquarium) containing aerated artificial sea water and under artificial lighting matching the natural photoperiod. Specimens were fed daily with a unicellular alga mixture (*Haematococcus* sp., *Pavlova* sp. *Nanno-chloroposis* sp., *Tetraselmis* sp., *Isochrysis* sp., *Thalassiosira* sp.; Brightwell Aquatics, PA) at 2 $\times 10^7$ cells/ml. A quarter of the volume for each aquarium was replaced daily to prevent accumulation of ammonia waste.

Specimen survival was assessed daily based on three criteria: siphon state (open or closed); contraction following gentle contact; and color of the tunic. In the event that the siphons were closed, there was no reaction to contact, and the tunic color had changed to white, the specimen was concluded to be dead and immediately removed from the experiment to prevent a negative effect on the remaining specimen in the aquarium. In both the temperature and the salinity experiments, the artificial seawater was prepared by dilution of artificial sea salt (Aquarium systems, France) with distilled water until reaching the desired salinity. Water salinity was measured on daily basis with a temperature compensated refractometer (REF-211 model, MRC ltd. China) with ± 0.2% accuracy.

Three treatments of temperature and salinity were examined in each experiment, with each of them either representing conditions currently found within the distribution of *M. exasperatus,* or that may occur in the near future as a result of the anticipated changes in world climate (Bianchi and Morri 2003; Somot et al. 2006).

Temperature experiment

Specimens were acclimated at 17–18ºC for three days prior to onset of the experiment. Three treatments were established: 12–13ºC (low), 23–24ºC (control), and 32–33ºC (high). Water temperatures were adjusted gradually at a rate of 1ºC/24h (cooling) and 2ºC/24 h (heating). Day zero of the experiment was considered as that when the water had reached the required temperature in each experimental group. Forty-five individuals (13–16 per treatment group) were used in the experiment, which lasted five weeks. The specimens were held throughout the experiment in artificial sea water that was adjusted to the same salinity (39) as that measured at the collection site. The aquaria in the experiments were kept in bath systems to minimize temperature fluctuations. In the high temperature treatment, the water in the baths was heated using heating coils and monitored using a thermometer. The cooling and monitoring of the water in the low temperature treatment were performed using a chiller (HC-300A, Heilea, China).

Salinity experiment

Specimens were acclimated to salinity of 37–40 for three days prior to onset of the experiment. The specimens were then separated into the three salinity treatments: 33–35 (low); 37–40 (ambient, control); and 43–45 (high). Forty-two individuals (14 animals per treatment group) were used in the experiment, which lasted two weeks. The specimens were held at a constant temperature of 23 ºC.

*Statistics*

Before applying parametric statistics, Shapiro-Wilk's and Levene's tests were carried out to determine whether the data violated the assumptions of normality and homoscedasticity. The comparison of the temperature and salinity survival curve experiments were performed using the Kaplan and Meier (1958) method, followed by the Breslow Day test (Bland and Altman 1998; Liu 2005), which revealed the different variance groups among the survival curves. Analyses of the survival experiments were conducted using SPSS V.19 software (SPSS 2010). To detect possible differences in mean abundance between the two sides of the submerged ship wreck (northern *vs*. southern) throughout the year, a paired t-test was applied using statistical package R Software for Statistical Computing (R Core Team 2013).

## Results

*Local and global geographic distribution of Microcosmus exasperates*

*Microcosmus exasperatus* was widely distributed along the Mediterranean coast of Israel; it was very common on both artificial (piers, artificial reefs, etc.) and natural substrates (Figure 1, Table S1). Moreover, individuals were also present in disturbed and polluted sites such as marinas and ports, as well as in protected sites such as nature reserves (Akhziv Nature Reserve, HaBonim Beach, Figure 1). *M. exasperatus* individuals were found at all depths surveyed (1–30 m, Table S1), usually forming massive and dense aggregations. *M. exasperatus* was abundant in all the surveyed sites, except for Akhziv Nature Reserve, where it was relatively rare.

*Microcosmus exasperatus* has a broad global distribution and is commonly found along all the continental shelves except for that of Antarctica. It also is present in such remote locations as Hawaii and the Mariana Islands (Supplementary material Table S2).

*M. exasperatus* is typically found in tropical to temperate seas (Figure 2). The northern-most report of the species is from Izmir Bay, Turkey (Ramos- Esplá et al. 2013), while the southern-most report is from south-west Australia (Tokioka 1967; Kott 1985).

The abundance of tunicates at ‘Akko Bay varied seasonally, with the highest densities (18.5 and 21.5 individuals/m$^2$) recorded during April and June 2014 followed by a large decline in July and August (Figure 3). During autumn, abundance once again increased followed by a drop to a winter minimum (4.09 individuals/m$^2$) in January. Moreover, the density of individuals on the southern side (facing the open sea) of the submerged shipwreck were significantly higher than on the northern side (facing the shore, paired two sample t-test, t = -3.436, $df$ = 13, $p$ < 0.005) throughout the entire survey period. The greatest differences were observed in April 2014, when abundance on the southern side reached 15.1 specimens/m$^2$ compared to 3.5 specimens/m$^2$ on the northern side.

*Survival of Microcosmus exasperatus under various temperature and salinity conditions*

Temperature experiment

Over the five weeks of the experiment, there was a distinct difference in survival of the specimens

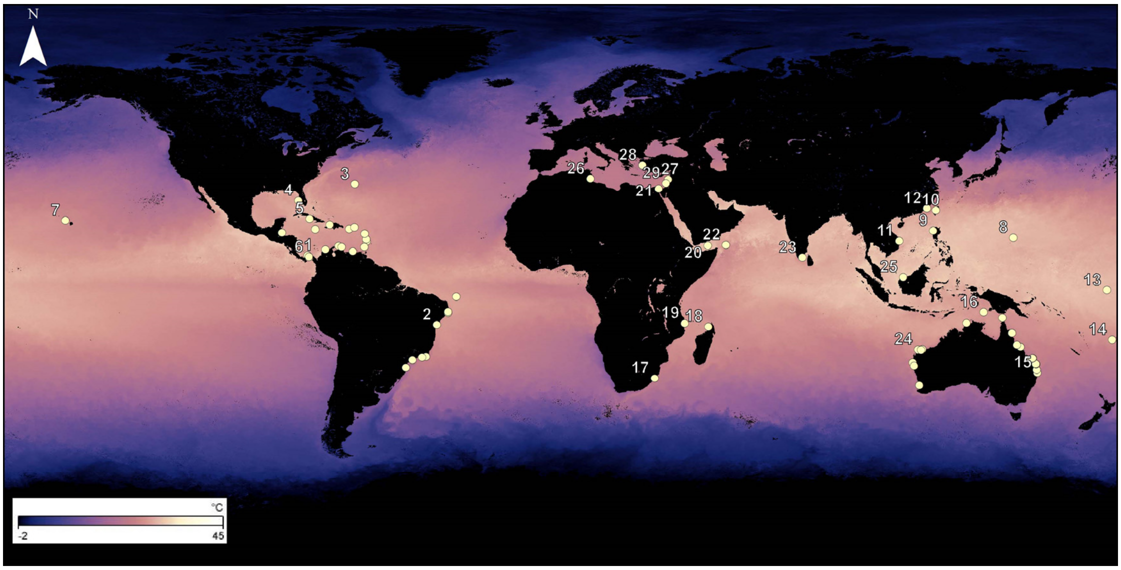


**Figure 2.** *Microcosmus exasperatus* global distribution. Sea-surface temperature range (Cº) is indicated on the map; specific locations and supporting references are listed in Table S2.

between temperature treatments (Kaplan-Meier followed by Breslow $\chi^2 = 44.9$, $p < 0.05$, Figure 4). The survival differed between the low and high temperature treatments (Kaplan-Meier $\chi^2 = 28.03$, $p < 0.001$) and between the control and the high temperature treatments (Kaplan-Meier $\chi^2 = 16.66$, $p < 0.001$). Survival between the low and control treatments did not differ (Kaplan-Meier $\chi^2 = 2.384$, $p > 0.05$).

While the tunicates survived relatively well in the low temperature and ambient control treatments, high mortality (10% survival) occurred within 5 days in the high temperature treatment and none survived beyond day 12. In contrast, survival was 100% on day 5 in the low temperature and 90% in the ambient control treatment. Moderate mortality occurred in the two remaining groups such that 56% survived to day 32 in the ambient control group and 81% survived in the low temperature treatment.

Salinity experiment

A significant difference in the survival of individuals was found between salinity treatments (Kaplan-Meier followed by Breslow test $\chi^2 = 9.4$, $p < 0.05$, Figure 5). Survival differed between the control and the low salinity treatments (Kaplan-Meier followed by Breslow test $\chi^2 = 7.77$, $p < 0.01$) but not between the control and high salinity treatment or the low and high salinity treatment ($p = 0.132$ and $p = 0.121$, respectively).

In contrast to the temperature experiment, the salinity experiment only lasted two weeks and the reduction in survival was almost immediate. In the low salinity treatment, only 64% remained alive on day 2 and only 14% were alive by day 14 when the experiment was terminated due to the low numbers surviving. Survival in the high and ambient salinity treatments were equivalent (about 80%) on day 4 and then diverged. There was little mortality in the ambient salinity treatment until the end of the experiment – 71 % survived to day 14. In contrast, there was steady mortality in the high salinity treatment; similarly to the low salinity curve and 36% survived until day 14.

## Discussion

This study indicates that the invasive populations of *Microcosmus exasperatus* in the Mediterranean has high tolerance to low temperatures and a limited tolerance (short term) to high salinities. These results also correspond with *M. exasperatus*'s broad global distribution where it inhabits tropical as well as temperate waters (Table S2). The limited tolerance of *M. exasperatus* to high salinities is also compatible with the assumption that it is a Lessepsian invader that likely passed through the high salinity waters of the Suez Canal. In addition, the high abundance of *M. exasperatus* throughout the year on both natural and artificial substrates indicates that it has become

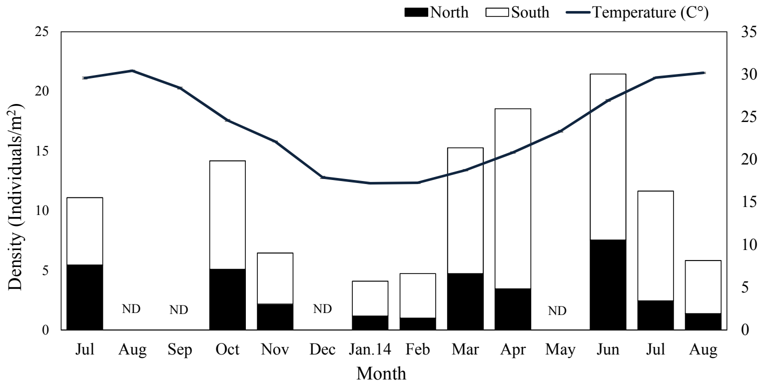


**Figure 3.** Seasonal abundance of *Microcosmus exasperatus* and average sea-surface temperature (right Y-axis in ºC) on the northern (shore facing) and southern (sea facing) sides of the submerged shipwreck at ‘Akko Bay.

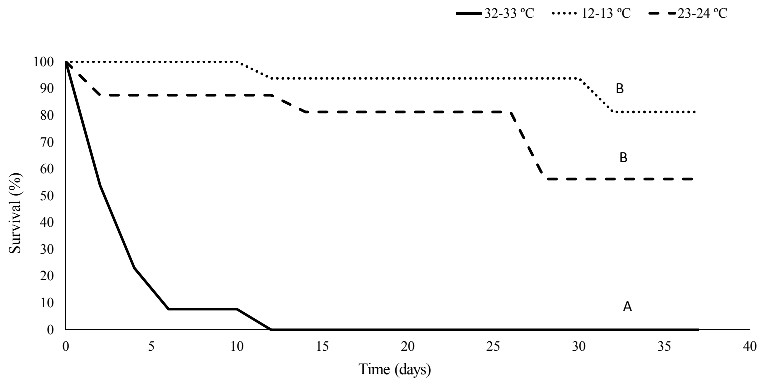


**Figure 4.** Survival curves of *Microcosmus exasperatus* exposed to three treatments of temperature; low (12–13 ºC); high (37–40 ºC); and control (23–24 ºC). Curves sharing the same letter did not differ significantly ($p > 0.05$).

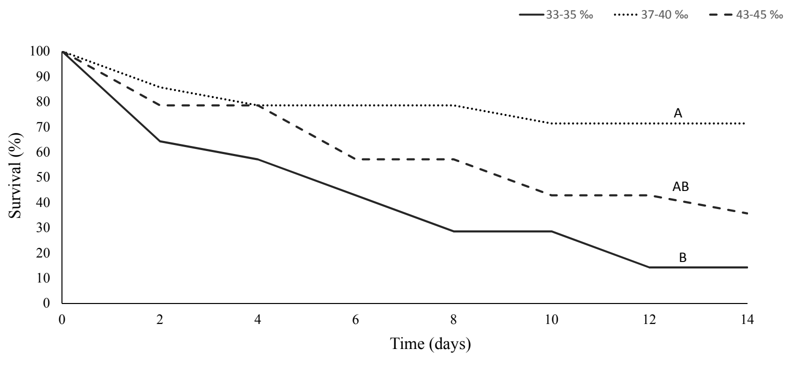


**Figure 5.** Survival curves of *Microcosmus exasperatus* exposed to three treatments of salinity; low (33–35); high (43–45); and control (37–40). Curves sharing the same letter did not differ significantly ($p > 0.05$).

well-established along the Mediterranean coast of Israel.

The abundance of *M. exasperatus* on the side of the shipwreck that was exposed to wave action and sea currents was higher than on the more protected side, which suggests a high tolerance to dynamic sea conditions. The presence of *M. exasperatus* in the marinas and ports surveyed is consistent with the mode of introduction being primarily through shipping vectors (Lambert and Lambert 1998; Lambert 2002; Godwin 2003; Davis et al. 2007; Farrapeira et al. 2007). The presence of *M. exasperatus* in such remote locations as Hawaii (Coles et al. 1997) and the Mariana Islands (Tokioka 1967; Lambert 2003) also is consistent with this vector.

The three treatments of temperature and salinity used in our experiments were meant to represent prevailing conditions within the distribution of *M. exasperatus,* or those that may occur in the near future as a result changes in world climate (Bianchi and Morri 2003; Somot et al. 2006). Thus the high temperature treatment (32–33ºC) represented a predicted 3.1ºC temperature increase for the eastern Mediterranean (Somot et al. 2006). In contrast, the low temperature treatment (12–13ºC) simulated temperatures that can be found in the winter months along the western Mediterranean (Manca et al. 2004), where *M. exasperatus* has not been detected yet. While the high temperature treatment had a major and significant effect on the survival, the low temperature treatment had the least effect on their survival. These results are consistent with the field observations of a decline in *M. exasperatus* abundance during the summer months and suggest the species has an upper temperature threshold of 31–32ºC. Accordingly, a rise of 3.1ºC in sea-surface temperature (Somot et al. 2006) may restrict the distribution of *M. exasperatus* in the eastern Mediterranean, unless the invasive population can adapt to warming sea water conditions.

Surprisingly, the highest survival was observed under the low temperature treatment although the population density at the study site was lowest during winter months. However, during the winter months high mortality can occur due to storms events where there is both an influx of fresh water (heavy rainfall) and high turbidity; factors that can lead to death and alteration of the population dynamics (Goodbody 1962; Monniot 1965, cited in Millar 1971). In addition, *M. exasperatus* does not reproduce during these months (Nagar-Raijman 2014), therefore, it is possible that together with the mortality following the winter storms, a synergistic effect is created leading to a decrease in the population size of *M. exasperatus* during those months. Hence, the high survival of *M. exasperatus* under the low temperature treatment indicates that low temperatures do not constitute an abiotic barrier for further spread of this species. Indeed, individuals of *M. exasperatus* were recently reported from Izmir Bay, Turkey (Çinar et al. 2011), where the temperature in the winter months can be 12–13ºC or lower (Sayın 2003). The reproduction process and early life stages of *M. exasperatus* need to be examined to better predict its ability to reproduce and establish populations in colder environments (Thiyagarajan and Qian 2003; Pineda et al. 2012a).

Salinity, particularly at low levels, is a major factor in determining the abundance and distribution of ascidians (Dybern 1967; Sims 1984). Salinity stress has dramatic consequences on ascidian survival due to direct osmotic effects or due to its interruption of the normal function of enzymes and metabolic process (Gilles 1975; Hochachka and Somero 2002). Sudden changes in salinity, such as flood runoff, can lead to a mass mortalities and eventually to changes in population dynamics (Goodbody 1962). Similar to the temperature experiment, the salinity treatments were chosen to mimic the prevailing natural conditions, or those that are predicted to exist, within the distribution of *M. exasperatus*. Thus, the low salinity treatment represented the lower range of salinity found in the western Mediterranean Sea (Manca et al. 2004; Locarnini et al. 2006) while the high salinity treatment simulated the salinity along the Suez Canal (Por 1978; Fouda and Gerges 1994; El-Serehy et al. 2013). The low salinity treatment had a major effect on the survival of *M. exasperatus* individuals which is consistent with effects of low salinity on the survival, growth, and reproduction in ascidians elsewhere with "low" being relative to species-specific tolerances (Dybern 1967; Vercaemer et al. 2011). Vázquez and Young (2000) found that low salinity had a dramatic effect on settlement and metamorphosis of early life stages of colonial ascidians as well as on the survival of adult individuals. The high sensitivity of *M. exasperatus* to low salinity conditions observed in the current study is also supported by C. Monniot (1965, cited in Millar 1971), who stated that the dispersal of ascidians belonging to the Pyuridae family (which include *M. exasperatus*) is restricted in areas where salinity is < 20. *M. exasperatus* does occur in salinity environments lower than those tested in this study, such as the waters adjacent to the Panama Canal (Carman et al. 2011) and in various Brazilian bays (Marins et al. 2010), with salinities of 30–33 and 24–32, respectively. It is not immediately obvious why the low-salinity tolerance reported in the current study is higher than those reported elsewhere.

Numerous studies on ascidian populations have found that life history traits such as survival, growth rates, and reproduction revealed under laboratory conditions differ from those assessed in the natural environment (Brunetti and Copello 1978; Boyd et al. 1986). In addition, tolerance ranges among different populations can vary, with the sensitivity of one population not directly implying that of others. Vercaemer et al. (2011) found the tolerance range of the invasive ascidian *Ciona intestinalis* (Linnaeus, 1767) population in Nova Scotia to be narrower than that of the natural population in northern Europe. Thus, it is possible that the *M. exasperatus* population from the eastern Mediterranean exhibits a different tolerance range to those of populations from other locations in the world, possibly due to a founder effect.

The limited tolerance of *M. exasperatus* in the high salinity treatment supports the assumption that it is a Lessepsian invader introduced by shipping through the Suez Canal (Turon et al. 2007; Shenkar and Loya 2009). While it seems able to tolerate the high salinity found along the canal for a short term, with the exception of a single report (Monniot 2002), this species had never been recorded in the canal.

In conclusion, this study indicates that *M. exasperatus* populations along the Mediterranean coast of Israel exhibit a relatively high tolerance to low temperature, and a limited tolerance (short term) to high salinities. This, combined with possible environmental and anthropogenic changes such as human-mediated transport mechanisms, extensive development of coastal structures, and anticipated climate change, may greatly enhance the invasion success and potential to spread of this species into new locations in the Mediterranean Sea. The presence of *M. exasperatus* along the Israeli coast (Shenkar and Loya 2009; this study), its high resistance to wave action, together with high reproductive abilities (Nagar-Raijman 2014), suggest that it may have a significant effect on the local fauna. Moreover, the large aggregations that *M. exasperatus* forms on both artificial and natural substrates suggest a potential role as an ecosystem engineer; i.e., a species that can increase habitat complexity and provide new habitat (Reichman and Seabloom 2002; Wright et al. 2002; Voultsiadou et al. 2007). This emphasizes, among other things, the need to acquire additional knowledge on the physiological and ecological traits of *M. exasperatus* to aid in assessing its invasion potential.

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The following supplementary material is available for this article:

**Table S1.** Locations and depth where *Microcosmus exasperates* was detected along the Mediterranean coast of Israel.

**Table S2.** *Microcosmus exasperatus* global distribution and the supporting references.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2016/Supplements/AI_2016_Nagar_Shenkar_Supplement.xls