



Rising seawater temperatures affect the fitness of *Rhopilema nomadica* polyps and podocysts and the expansion of this medusa into the western Mediterranean

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ABSTRACT: Since first reported in the eastern Mediterranean in the 1970s, the swarm-forming scyphomedusa, *Rhopilema nomadica*, has been continuously expanding westward. We examined the effect of temperature on the benthic stages of this jellyfish (polyps, podocysts, and strobilae) to assess whether it can survive and reproduce in the western Mediterranean temperature regime (12.2–26.2°C). In the first experiment, polyps and podocysts were maintained at 4 constant temperatures (12, 18, 24, and 30°C) for 55 d. In the second experiment, temperatures were gradually reduced over 14 d, kept constant for 18 d, and then gradually brought back to 25°C. High temperatures proved beneficial to polyp survival and asexual reproduction, yet in some cases, polyps were able to survive temperatures as low as 12°C. Strobilation occurred at temperatures between 22 and 30°C. Podocyst production, excystment, and strobilation all take place following a temperature increase, and excystment of podocysts occurs mainly during the first 2 wk following podocyst production. We propose that the role of podocysts in *R. nomadica* is mainly to increase the current season's polyp population, contributing to swarm formation rather than as a means to maintain polyp populations during harsh environmental conditions. Since polyps are able to survive winter temperatures in the western basin (12°C) and reproduce by podocysts and strobilation during summer temperatures (24°C), thermal constraints will probably not restrict the expansion of *R. nomadica* into the western Mediterranean. Expected future climate change conditions will increase the performance and range expansion of this species.

KEY WORDS: Jellyfish · Asexual reproduction · Temperature · Podocyst · Excystment · Strobilation

1. INTRODUCTION

Most scyphozoans have a complex life cycle comprising a pelagic sexual stage and a benthic asexual stage (Arai 1997). The adult medusae release planula larvae, and when the planulae find a suitable substrate, they settle and undergo metamorphosis into polyps, also known as scyphistomae. The benthic polyps reproduce asexually and metamorphose into strobilae, which, in polydisc species, may release sev-

eral ephyrae (young medusae) repeatedly every season (Lucas et al. 2012). Therefore, many studies have emphasized the important contribution of asexual reproduction to the abundance of free-swimming adult jellyfish (reviewed in Arai 2009, Lucas et al. 2012).

Podocysts are dormant cysts produced underneath the pedal discs of polyps and consist of a chitin-covered cell mass (Arai 2009). Through a process of excystment, podocysts may develop into polyps capable of normal functioning, including additional

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§Range notation for temperature values in Table 1 was modified after publication.
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podocyst production and strobilation. A polyp may form a trail of several podocysts by repeatedly moving to a new position, leaving the newly produced podocyst behind (Arai 2009). The role of podocysts is thought to contribute to survival during periods of adverse conditions, such as low food availability (Thein et al. 2012), harsh temperatures, extreme salinities, hypoxia (Cargo & Schultz 1966, Arai 2009, Kawahara et al. 2013, Thein et al. 2013), predation by nudibranchs (Cargo & Schultz 1967, Gröndahl 1988b, Takao et al. 2014), and competition with other sessile organisms (Brewer & Feingold 1991). Moreover, synchronized podocyst excystment may increase polyp populations (Cargo & Schultz 1966, Brewer & Feingold 1991, Widmer 2008, Lucas et al. 2012), potentially contributing to the successful proliferation (outbreak or swarm formation) of medusae (Arai 2009). Early studies had assumed that podocyst production was induced by unfavorable environmental factors (Cargo & Schultz 1966, 1967). Subsequent studies have shown that induction of podocyst production in *Lychnorhiza lucerna*, *Nemopilema nomurai*, and *Rhopilema esculentum* is positively correlated with food supply and temperature (Guo 1990, Kawahara et al. 2013, Schiariti et al. 2014). This difference is species-specific and may be related to the mode of asexual reproduction. In 'mono-mode' species (species displaying only one mode of asexual reproduction apart from strobilation, e.g. *Chrysaora lactea* and *N. nomurai*), where podocysts are the only form of asexual reproduction, an increase in temperature and food supply may promote podocyst production, whereas in 'multi-mode' species (species displaying different modes of asexual reproduction, e.g. *Catostylus mosaicus* and *Chrysaora quinquecirrha*), these factors increase budding and stolon production (Schiariti et al. 2014). The synchronization between strobilation and podocyst production and excystment is also species-specific (Ding & Chen 1981, Gröndahl 1988a, Kawahara et al. 2006). Some species, such as *Aurelia aurita*, produce podocysts after strobilation to maintain the population during harsh conditions (Gröndahl 1988a), whereas in others, such as *Chrysaora pacifica*, podocysts excyst before strobilation to increase the polyp population (Thein et al. 2013). Table 1 summarizes the reproduction modes and temperature conditions (as well as other environmental triggers such as salinity, food availability, light, oxygen, etc.) of many podocyst-producing scyphozoan species.

There is an ongoing debate on whether the frequency and intensity of jellyfish outbreaks have increased globally (Mills 2001, Richardson et al. 2009,

Condon et al. 2012, 2013, Brotz et al. 2012, Sanz-Martín et al. 2016) and whether this shift is related to climate change (Purcell 2005, Condon et al. 2013). Increased temperatures have a positive effect on the reproduction of some (mainly temperate) species of jellyfish (Purcell 2005, Purcell et al. 2012, Lucas et al. 2012, Schiariti et al. 2014, Treible & Condon 2019); for example, podocyst production and overall reproduction rate increased with higher temperatures (15, 20, and 25°C) in *L. lucerna*, *Rhizostoma pulmo*, *R. esculentum*, and *Aurelia* sp. (Schiariti et al. 2014). Thermal stimuli affect different species in different ways, such that one set of thermal conditions may cause increased budding, while another set may promote strobilation (Brewer & Feingold 1991, Willcox et al. 2007, Sokołowski et al. 2016). The rate of podocyst production in *C. quinquecirrha* in Chesapeake Bay increased when temperatures decreased to 2–4°C (Cargo & Schultz 1967), whereas excystment and strobilation in this species occurred in the spring as temperatures rose to 15–18°C (Cargo & Rabenold 1980). Certain processes, such as strobilation and podocyst production, may be induced by a change in temperature, though it appears that for some species the magnitude and rate of temperature change are more important than the absolute temperatures themselves (Purcell et al. 1999, Kroiher et al. 2000, Kawahara et al. 2006, Lucas et al. 2012).

Water temperatures in the Mediterranean vary seasonally as well as regionally (Fig. 1). Whereas sea surface temperatures (SSTs) in the Levantine basin range from 17–31°C (Guy-Haim et al. 2016), the temperature range in the western basin is 13–25°C (Lejeusne et al. 2010, Shaltout & Omstedt 2014). During the past 3 decades, a significant warming trend in overall Mediterranean SSTs of +0.024 to +0.05°C yr⁻¹ was reported (Shaltout & Omstedt 2014). This represents a temperature increase of 0.8–1.4°C in the western basin (Lejeusne et al. 2010) and 1.5–3.0°C in the Levant (Guy-Haim et al. 2016) in 30 yr. Climate models predict that this trend will continue in the long term in all sub-basins except for the northern Adriatic, which is expected to become cooler (Coll et al. 2010, Lejeusne et al. 2010). Hundreds of species introduced into the eastern Mediterranean through the Suez Canal (Lessepsian migrants) since its opening (Katsanevakis et al. 2014a, Galil et al. 2015) have long been confined to the Levant (Lejeusne et al. 2010). The warming of the Mediterranean Sea, along with currents, facilitates the introduction and spread of these thermophilic species (e.g. *Etrumeus golanii* and *Pteragogus trispilus*) into the western basin (Coll et al. 2010, Lejeusne et al. 2010, Katsanevakis et al.

Table 1. Asexual reproduction in podocyst-forming jellyfish species, including seasons and/or temperature conditions for podocyst production (prod.), podocyst excystment (exc.), and strobilation. Other stimulating factors for podocyst production and excystment include salinity, light, food availability, and oxygen levels. Podocyst survival is presented in months (mo) or years (yr)

Species	Reference	Podocyst prod. season (temperature)	Stimulating factors for podocyst prod.	Podocyst prod. rate	Podocyst exc. season (temperature)	Stimulating factors for podocyst exc.	Excysting podocysts (%)	Podocyst survival	Strobilation season (temperature)	Discs strobila ⁻¹ (no. strobilation events)	Asexual reproduction modes
<i>Aurelia aurita</i>	Gröndahl (1988a)	Winter	After strobilation		Spring				Autumn		
	Kroiber et al. (2000)					Onset earlier as temperature decreases (20 to 18°C)					
	Thein et al. (2012)	Temperature increase (19 to 28°C)	Starvation	0.63 podocysts polyp ⁻¹ wk ⁻¹	Temperature decrease (28 to 19°C)	Hypoxic to oxic	Young 39, old 3	3.7 yr			Podocysts, budding, stolons
	Thein et al. (2013)	Summer, high temperatures	Starvation	0.11 podocysts polyp ⁻¹ d ⁻¹	Autumn				Winter (<15°C)		Podocysts, budding, stolons
	Straehler-Pohl (2011)									12–22	Podocysts, budding, stolonoidic budding, pedalocysts
<i>Aurelia coerulea</i>	Feng et al. (2018b)								Winter to early spring (6 to 18°C)	1–12 (1)	Podocysts, budding, stolons
<i>Aurelia limbata</i>	Straehler-Pohl (2011)									8–10	Podocysts, budding, stolonoidic budding
<i>Catostylus tagi</i>	Gueroun et al. (2021)	(15 to 25°C)	No effect of temperature or feeding	1.7 ± 1.5 podocysts polyp ⁻¹					Onset earlier at high temperatures (15 to 25°C)	4 (multiple strobilations at >18°C)	Podocysts
<i>Catostylus mosaicus</i>	Pitt (2000)								(21°C)	Monodisc or 2–5 discs	Podocysts, budding, pedalocysts, longitudinal fission
	Straehler-Pohl (2009)									4	Podocysts, budding
<i>Chrysaora fuscescens</i>	Straehler-Pohl (2011)									<60	stolonocysts

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Table 1 (continued)

Species	Reference	Podocyst prod. season (temperature)	Stimulating factors for podocyst prod.	Podocyst prod. rate	Podocyst exc. (temperature)	Stimulating factors for podocyst exc.	Excysting podocysts (%)	Podocyst survival	Strobilation season (temperature)	Discs strobila ⁻¹ (no. strobilation events)	Asexual reproduction modes
<i>Chrysaora lactea</i>	Straehler-Pohl (2011)						<10				Podocysts
<i>Chrysaora pacifica</i>	Thein et al. (2013)	Summer, high constant temperatures	Increased food and salinity	0.29 podocysts polyp ⁻¹ d ⁻¹	Autumn, temperature decrease	Hypoxic tooxic, low excystment at increased or constant temperatures	48	1 yr	Early winter (<18°C)		Podocysts
<i>Chrysaora quinquecirrha</i>	Littleford (1939)	Autumn	Increased food			Within a few months or dead			Summer		Podocysts, stolonocysts, stolonic budding
	Cargo & Schultz (1966)		Low oxygen	52 podocysts and 6 polyps in <3 mo							
	Cargo & Schultz (1967)	Temperature decrease (2 to 4°C)			Spring, temperature increase (15 to 18°C)				Spring (18 to 19°C)		
	Cargo (1975)				(15 to 18°C)						
	Black et al. (1976)						1–12 monthly	25 mo			
	Cargo & Rabenold (1980)	Late summer	After strobilation	Spring, temperature increase			75		Late spring to early summer (>20°C)	3	Podocysts, stolonocysts
	Purcell et al. (1999)								Onset earlier as temperature increases		
<i>Cyanea</i> sp.	Brewer & Feingold (1991)	Spring, earlier onset at high temperatures		6 ± 1.9 podocysts polyp ⁻¹	Late summer to early winter, temperature decrease		54		Autumn to winter, 1–2 mo after excystment		
<i>Cyanea capillata</i>	Cargo & Schultz (1967)	Winter to spring			Autumn (23°C)				Autumn (15°C)		
	Cargo (1975)	Temperature increase			Temperature decrease				Temperature decrease		
	Gröndahl & Hemroth (1987)	Summer to autumn	By 1 yr old polyps but not newly settled polyps						Winter to early spring		

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Table 1 (continued)

Species	Reference	Podocyst prod. season (temperature)	Stimulating factors for podocyst prod.	Podocyst prod. rate	Podocyst exc. (temperature)	Stimulating factors for podocyst exc.	Excysting podocysts (%)	Podocyst survival	Strobilation season (temperature)	Discs strobila ⁻¹ (no. strobilation events)	Asexual reproduction modes
	Gröndahl (1988a)	Summer to autumn	After strobilation		Winter				Spring		
	Straehler-Pohl (2011)									10–17	Podocysts, budding
<i>Cyanea lamarckii</i>	Straehler-Pohl (2011)										Podocysts, budding
<i>Cyanea nozakii</i>	Dong et al. (2008)								(22 to 26°C)	Monodisc	Podocysts, stolonocysts
	Sun et al. (2013)	High temperatures							(21 to 25°C)		
	Thein et al. (2013)	Summer, high constant temperatures (28°C)	Increased food and salinity	0.11 podocysts polyp ⁻¹ d ⁻¹	Autumn, temperature decrease	Hypoxic tooxic, no excystment at increased or constant temperatures	54	>1 yr	Late spring		Podocysts
	Feng et al. (2015b)	(>18°C)	Increased food	0.39 ± 0.05 podocysts polyp ⁻¹					Summer (22 to 25°C)	1–2 (5)	Podocysts
<i>Lychnorhiza lucerna</i>	Schiariti et al. (2008)		During strobilation	0.33 podocysts polyp ⁻¹ d ⁻¹					(22°C)	3 (<5)	Podocysts
	Schiariti et al. (2014)	High temperatures (25°C)	Increased food		(<20°C)						Podocysts
<i>Nemopilema nomurai</i>	Kawahara et al. (2006)								Sudden temperature increase (13 to 23°C)	3–7	Podocysts
	Kawahara et al. (2013)	(>19°C)	Before strobilation and <3 mo from settlement	3.2 podocysts polyp ⁻¹	(>23°C)	8–27 ppt, hypoxic to oxic	55, podocysts <4 mo old	6 yr	Temperature decrease 19 to 15°C for 1 mo (11 to 15°C)		Podocysts
	Dong et al. (2015)		20–27.5 ppt	>4 podocysts polyp ⁻¹		22.5–27.5 ppt	49				
	Feng et al. (2015b)	(>18°C)	Increased food	9.1 ± 0.6 podocysts polyp ⁻¹					Spring (<18°C), autumn (10 to 13°C)	1–4 (1)	
	Feng et al. (2015a)	Summer (>19°C)	After strobilation, prior low temperatures	2.3 ± 1.7 podocysts polyp ⁻¹					(10 to 13°C)		

Table 1 continued on next page

Table 1 (continued)

Species	Reference	Podocyst prod. season (temperature)	Stimulating factors for podocyst prod.	Podocyst prod. rate	Podocyst season (temperature)	Stimulating factors for podocyst exc.	Excysting podocysts (%)	Podocyst survival	Strobilation season (temperature)	Discs strobila ⁻¹ (no. strobilation events)	Asexual reproduction modes
	Feng et al. (2018a)	(19°C)	After strobilation, 15–30 ppt		Summer	23–33 ppt			Spring (6 to 18°C)	1–4 (2)	Podocysts
	Feng et al. (2018b)	Late spring									Podocysts
	Schiariti et al. (2014)	High temperatures (25°C)	Increased food								Podocysts
<i>Rhopilema esculentum</i>	Ding & Chen (1981)		Before strobilation						Temperature increase (10 to 27°C)	6–10	Podocysts
	Chen & Ding (1983)								Sudden temperature increase (18 to 27°C)	7–8, <17 (5)	Podocysts
	Guo (1990)		Increased food	0.2 podocysts polyp ⁻¹ d ⁻¹			28				
	Jiang et al. (1993)		Increased food, dark			Temperature increase (15 to 30°C)		18–22 ppt			
	Lu et al. (1997)	Temperature increase (15 to 30°C)	20–22 ppt	1.0 podocyst polyp ⁻¹		Temperature increase		Dark			
	Schiariti et al. (2014)	High temperatures (25°C)	Increased food								
	Feng et al. (2018b)								Late spring to early summer (9 to 21°C)	1–12 (1)	Podocysts
<i>Rhopilema nomadica</i>	Lotan et al. (1992)		During strobilation	0.2 podocysts polyp ⁻¹ d ⁻¹						5–6	Podocysts, budding (rare)
	Lotan et al. (1994)								Spring (18 to 22°C)	5.6–7.8 (<3)	Podocysts
	Present study	Spring–summer, temperature increase		0.23 podocysts polyp ⁻¹ d ⁻¹	Spring–summer, temperature increase		23	>18 mo	(>24°C)	4–5.5	Podocysts
<i>Rhopilema verrilli</i>	Cargo (1971)								Temperature increase (9 to 20°C)	<3 (<3)	Podocysts, pedalocysts
	Calder (1973)									Monodisc	Podocysts
<i>Stomolophus meleagris</i>	Calder (1982)								25, 27°C	1–3 (2)	Podocysts

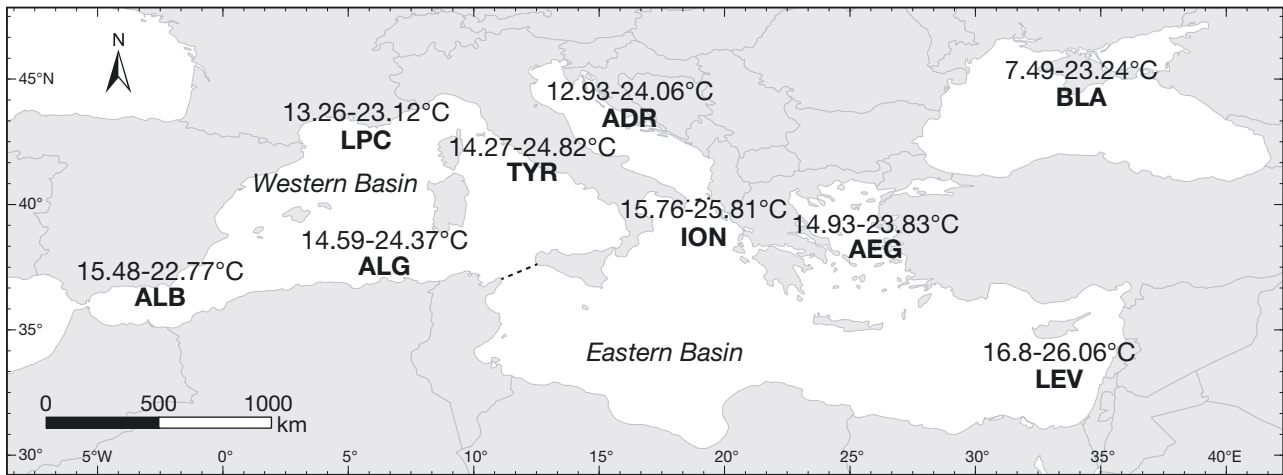


Fig. 1. Winter–summer mean sea surface temperatures between 1982 and 2012 in Mediterranean sub-regions (ALB: Alboran; ALG: Algerian; TYR: Tyrrhenian; LPC: the Liguro-Provençal and Catalan sub-basins; ION: Ionian; LEV: Levantine; AEG: Aegean; ADR: Adriatic). Figure produced using ArcGIS Pro v.2.9 using data provided in Shaltout & Omstedt (2014)

2014a, Zenetos & Galanidi 2020). It is predicted that the Mediterranean Sea will become ‘meridionalized’—inhabited by a combination of species that thrive in the southern and eastern regions along with tropical species, expanding their range (Coll et al. 2010, Lejeune et al. 2010, Boero 2015).

The scyphomedusa *Rhopilema nomadica* was first reported as a Lessepsian species in the eastern Mediterranean ca. 40 yr ago (Galil et al. 1990). The native population of this species is presumably Indo-Pacific, yet no swarming or even large populations have been reported elsewhere. Since the 1980s, *R. nomadica* has been constantly expanding westward (Siokou-Frangou 2006, Öztürk & İşinbilir 2010, Deidun et al. 2011, Daly Yahia et al. 2013, Madkour et al. 2019), recently reaching Sardinia and Sicily (Balistreri et al. 2017). Large swarms of *R. nomadica* disrupt tourism (Ghermandi et al. 2015) and cause damage to the fishing industry (Nakar et al. 2011, Angel et al. 2016) and coastal installations (Rilov & Galil 2009). The detrimental effects of swarms and rapid spread caused this species to be listed as one of the ‘100 worst invasive species’ in the Mediterranean (Streftaris & Zenetos 2006, Zenetos et al. 2010) and one of the most ‘impacting species’ in European seas (Katsanevakis et al. 2014b).

In this study, we conducted 2 experiments to test the effect of different temperatures on asexual reproduction in the early life stages (polyps, podocysts, and strobilae) of *R. nomadica*. We aimed to determine whether all benthic life stages are able to survive and function in the full range of SSTs characteristic of the Mediterranean. In addition, we

wanted to revisit previous findings (Lotan et al. 1994) in light of recent reports (Deidun et al. 2011, Daly Yahia et al. 2013, Balistreri et al. 2017) of the spread of *R. nomadica* medusae to the western Mediterranean. Although our experiments followed all asexual reproduction forms, we focused on podocysts (production and excystment) for 2 reasons: (1) polyps of *R. nomadica* very rarely produce other reproductive forms (buds, stolons, etc.), but they may produce as many as several dozen podocysts each (Lotan et al. 1992), and (2) the factors promoting podocyst production and excystment are not well known, and empirical evidence concerning the role of podocysts in polyp population dynamics is lacking compared to other forms of reproduction (Lucas et al. 2012). In the first experiment, the effect of constant temperature was tested to assess the survival of the various life stages in a wide range of temperatures. The second experiment was a short-term simulation of different intensities of ‘winter’ cooling followed by warming and their effect on asexual reproduction. We hypothesized that a stable jellyfish population, including all reproduction forms, will persist at all tested temperatures. Concerning the role of podocysts in the population dynamics of *R. nomadica*, we hypothesized that podocysts act as ‘seeds’ to maintain populations through adverse conditions and increase the following year’s polyp population. Thus, we expected podocyst production to increase with a temperature decrease, in preparation for the cold season, and podocyst excystment to increase with rising temperatures.

2. MATERIALS AND METHODS

2.1. Experimental system and design

Several sexually mature male and female *Rhopilema nomadica* medusae (20–40 cm bell diameter) were collected ~0.8 km offshore of Mikhmoret (32° 24' 23" N, 34° 52' 24" E) in the eastern Mediterranean Sea using a 60 cm dip net and buckets. In preparation for experimentation, polyps were established on the bottom (down-facing) of glass microscope slides by placing the sexually mature medusae in a 600 l tank filled with natural seawater for 48 h to induce reproduction. Polyps of *R. nomadica* were handled with great care because they do not reattach to a new surface after being detached and their strobilae tend to break easily when moved or during a water change. In a preliminary experiment, changing the water only twice weekly resulted in a rapid increase in salinity (from ~39 to >60) due to evaporation, in the 30°C treatment. These issues, as well as the need to create a more natural environment for the polyps, stimulated us to design a semi-open experimental system that minimizes the disturbance to polyps while maintaining constant water temperature, pH, and salinity. As a result of this design, there were only 4 independent replicates for each treatment. The following is a general description of the experimental system that was used for the 2 experiments (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m14224_supp.pdf). Specifications for each experiment are provided in Sections 2.2 and 2.3. Four 40 l water baths, set to the various experimental temperatures using heaters and chillers, were used to maintain the desired temperatures ($\pm 0.5^\circ\text{C}$) in the experimental containers. Four 250 ml plastic containers, each with a glass microscope slide with polyps, were placed in each water bath to produce 4 independent replicates. The 250 ml containers were covered and aerated to maintain water quality (stable salinity and oxygen levels). The glass slides with the attached polyps were positioned 3 cm above the bottom of the containers to maintain the polyps in an upside-down position. To minimize handling and disruption of the polyps, water in all containers was replaced using an automated water pump that gently pumped water at the correct temperature into each tank so that twice the volume of the tank was displaced daily. Excess water (water entered each container by drip flow) flowed into the surrounding bath with overflow so that there was no mixing of new water with water that was being replaced. To create a map of the locations of polyps and

podocysts on each slide, photographs were taken using a Galaxy Samsung S9+ cell phone camera before and after the experiment, and a number was assigned to each polyp, podocyst, and newly produced podocyst on the slide so that these could be monitored individually (including knowledge of their ancestral lineage). Twice per week, each slide was examined under a stereomicroscope (Motic SMZ-171), and the state of each individual was documented as 'dead', 'polyp', 'podocyst', or 'strobila' + number of discs. A polyp was considered dead when it completely decayed or fell off the slide; a podocyst was marked dead when it was left with just the clear chitin cover and no mass inside; and strobili were noted when the first fission of the polyp into discs was observed. It is possible that there were interactions between adjacent polyps on slides with high numbers of polyps (density effects) (Schiariti et al. 2015), but because *R. nomadica* polyps do not reattach after being moved, we preferred to leave the slides 'as is' after the experiments were initiated. Following each examination, polyps were fed *Artemia salina* nauplii in excess to allow for equal feeding in all treatments, and ca. 2 h later the water was replaced to remove waste. Water temperature, pH, and salinity were measured and recorded daily during the morning hours, and the system was illuminated using fluorescent lighting 12 h d⁻¹.

2.2. Effect of constant temperature on asexual reproduction

Polyps were maintained at temperatures of 12, 18, 24, and 30°C for 55 d (March–June 2020). Temperatures for the experiment were selected to reflect low (12 and 18°C) and high (24 and 30°C) temperatures occurring in the western and eastern Mediterranean, respectively. The duration of the experiment was planned for 60 d but was cut short (55 d) due to a malfunction in the experimental setup. The planned duration was selected based on the rate of response of polyps to thermal change (H. Dror unpubl. data). Moreover, numerous studies on the effect of thermal stimuli on cnidaria were conducted over similar periods, ranging from 6–8 wk. Stock cultures of polyps were produced in the laboratory from fertilization of mature jellyfish collected at Mikhmoret during winter (January) 2020. The polyps were kept in a flowing seawater, 50 µm filtered, open-water aquarium system at a temperature of 17–19°C, fed with *A. salina* nauplii twice a week, and maintained at a 12 h illumination cycle until the beginning of the temperature ad-

justment 45 d later. Temperature adjustment started at SST 18°C, and then the temperature was modified gradually at a rate of 0.5–1°C d⁻¹ until reaching the predetermined temperature for each tank: 12, 18, 24, and 30°C. This temperature adjustment process was conducted during March 2020 and lasted 14 d. Each slide had 1–6 polyps and 0–2 podocysts at the beginning of the adjustment period, and 4 slides were used as replicates so that a similar number of polyps and podocysts (altogether 16–20 polyps and 0–4 podocysts) were placed in each temperature treatment. Many polyps died during the adjustment period, leaving 5, 5, 11, and 9 polyps in the 12, 18, 24, and 30°C temperature treatments, respectively, at the start of the experiment. Newly released ephyrae were documented and transferred to cultivation aquaria, whereas polyps, podocysts, or excysting podocysts (2nd generation polyps) were not removed from the slides during the experiment.

2.3. Effect of changing temperatures on asexual reproduction

Temperatures in the Mediterranean vary seasonally, and low temperatures may be disadvantageous to polyps; therefore, we tested the response of the sessile stages of *R. nomadica* to changes in temperature occurring before, during, and after winter. The target temperatures were selected to reflect warm and cold winter temperatures in the eastern (warm winter: 18°C; cold winter: 15°C) and western (warm winter: 15°C; cold winter: 12°C) Mediterranean. Polyps for this experiment were produced by fertilization from gonads of jellyfish collected at Ga'ash beach (32° 13' 41" N, 34° 49' 08" E), during summer (June) 2020 (SST: 30°C). The polyps were kept in a flowing seawater aquarium system cooled to a temperature of 24–25°C, fed with *A. salina* nauplii twice a week, and maintained at 12 h illumination d⁻¹ until the beginning of the experiment, 70 d later. Four replicate slides in separate containers (see Fig. S1) were introduced into each treatment (bath) and allowed to acclimate to the experimental system for 20 d before the beginning of the experiment. During that time, polyps were not monitored but podocyst production and excystment continued, such that at the start of the experiment the number of polyps and podocysts at each temperature treatment was not the same (36, 285; 57, 334; 54, 286; 29, 268; polyps, podocysts at 12, 15, 18, and 25°C, respectively). In order to minimize disruption, polyps and podocysts were not removed to even out the numbers on the slides. Dur-

ing the experiment (September–November 2020), polyps first experienced a gradual decrease in temperature (0.5–1°C d⁻¹, 14 d) until reaching 12, 15, and 18°C, then a constant temperature for 18 d, and finally a gradual rise in temperature (0.5–1°C d⁻¹, 14 d) until reaching 25°C again; thereafter they were monitored for 14 d (Fig. S2). One treatment was kept at 25°C throughout the experiment as a control. The duration of each phase (decrease, constant, increase) was selected to allow adjustment to the new temperature and minimize the effects of the previous phase on the results of the next. Polyps and podocysts were not removed from the slides during the experiment; numbers of released ephyrae were recorded and they were transferred to aquaria for cultivation.

2.4. Data analysis

Data analysis and graphs were performed/plotted in RStudio v.2021.9.1 (RStudio Team 2021) using packages 'ggplot2', 'lmPerm', 'stats', and 'car'. All tests were conducted at the $\alpha = 0.05$ significance level. Polyp and podocyst survival was calculated as the percentage of the original number at the beginning of the experiment (t_0), and statistical significance was determined using a chi-squared test of dead vs. living polyps on the last day of the experiment (function 'chisq.test'). Podocyst production was calculated as a rate (new podocysts polyp⁻¹ d⁻¹). In the constant temperature experiment, to test the effect of temperature on podocyst production, the production rate was fitted using a linear model through the origin, and a 95% confidence interval was calculated (function 'lm'). Assumptions of the linear model were verified graphically. Podocyst excystment is presented as a percentage, and statistical significance of differences in excystment was tested using a chi-squared test of excysted vs. non-excysted podocysts. To eliminate the effect of age, podocyst excystment in the constant temperature experiment was calculated from the day of podocyst production and not from t_0 . Differences in strobilation indices were calculated using chi-squared tests for percentages, and ANOVA was used to test the change in mean number of discs and time to strobilation onset (function 'aovp'). Assumptions for ANOVA were checked using Levene's test for homogeneity of variance and a Shapiro-Wilk test for normality. Tukey's HSD tests were conducted for multiple pairwise post hoc comparisons of means for significantly different ANOVA results. Results are presented as % or as means \pm SD.

3. RESULTS

3.1. Effect of constant temperature on asexual reproduction

This experiment examined the effect of constant temperature (12, 18, 24, and 30°C) on the asexual reproduction and vitality of polyps. A summary of statistic values is presented in Table 2.

Survival of polyps at the higher temperatures (24 and 30°C) was significantly higher than at the lower temperatures (12 and 18°C) ($\chi^2 = 10.6$, $p = 0.001$), with maximal survival at 24°C. All polyps died at 12°C, and only one polyp was left at 18°C by the end of the experiment, while at the higher temperatures, polyp survival was 82 and 78% (24 and 30°C, respectively) (Fig. 2).

Strobilation occurred only at 24°C, in 63.6% of the polyps. Strobilation began 12 d after the temperature stabilized and continued throughout the experiment, before, during, and after podocyst production. Strobi-

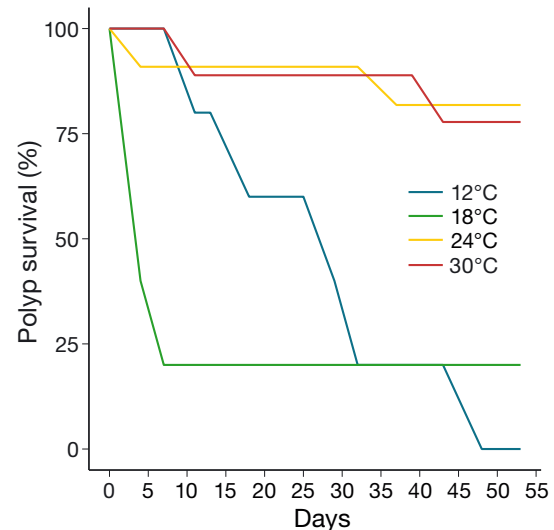


Fig. 2. Mean survival of *Rhopilema nomadica* polyps in 4 different temperature treatments (12, 18, 24, 30°C) over the 55 d experiment. Only polyps present at the beginning of the experiment, not those formed during the experiment, were analyzed

Table 2. Numbers and status of polyps, podocysts, and strobilae during the constant-temperature experiment (55 d) at 4 temperatures (12, 18, 24, 30°C) and statistical values of tests of differences between treatments. CI = confidence interval. Results are presented as % or mean \pm SD; numbers are indicated in brackets. Significant values ($p < 0.05$) are indicated in **bold**

	12°C	18°C	24°C	30°C	Test	p
Polyps						
% survival	0.0	20.0	81.8	77.8	$\chi^2 = 10.6$	<0.005
(No. of polyps at end/polyps at start)	(0/5)	(1/5)	(9/11)	(7/9)		
Mortality d ⁻¹	0.017	0.013	0.003	0.004		
Podocyst production						
% polyps that produced podocysts	20.0	100.0	100.0	77.8	CI = 0.068 \pm 0.015	Adj. R² = 0.98
Production rate podocysts polyp ⁻¹ d ⁻¹	0.01	0.09	0.16	0.21		
(No. of podocysts produced)	(1)	(8)	(88)	(81)		
Podocyst excystment						
% excysting podocysts within 14 d	0	12.5	12.9	23.7		
(No. of excysting podocysts/podocysts)	(0/1)	(1/8)	(14/108)	(23/97)		
Podocysts excysting within 14 d out of excysting podocysts over the entire period (%)		50.0	93.3	100.0		
(No. excysting within 14 d/total excysting)		(1/2)	(14/15)	(23/23)		
Excystment rate first 14 d (polyps podocyst ⁻¹ d ⁻¹) (mean \pm SD)	0	0.009 \pm 0.033	0.009 \pm 0.008	0.017 \pm 0.029		
Strobilation						
% of polyps strobilating 1 st time	0	0	63.6	0		
(No. strobilating/polyps)			(7/11)			
% of polyps strobilating 2 nd time	0	0	42.8	0		
(No. strobilating 2 nd time/strobilating 1 st time)			(3/7)			
Days to 1 st strobilation			27.7 \pm 14.9			
Days from 1 st to 2 nd strobilation			30 \pm 6			
No. of discs 1 st strobilation			4.42 \pm 1.13			
No. of discs 2 nd strobilation			3 \pm 1			
No. of discs per strobilating polyp			5.71 \pm 2.13			

lation occurred between 1 and 3 times for each polyp, with 4–6 wk between each strobilation. The number of discs per strobilation decreased with each strobilation (4.42 ± 0.5 and 3 ± 1 discs for the 1st and 2nd strobilation, respectively). The mean number of discs (all strobilation events) per strobila was 5.71 ± 2.13 .

Podocyst production rate was temperature-dependent and increased significantly with temperature (Fig. 3) ($CI = 0.068 \pm 0.015$, $p = 0.005$, adj. $R^2 = 0.98$). One podocyst was produced during the entire experiment at 12°C, and only 8 were formed at 18°C, while 88 and 81 podocysts were produced at 24 and 30°C, respectively. In all treatments but 12°C, the vast majority of polyps produced podocysts (100, 100, and 77% for 18, 24, and 30°C, respectively); the highest number of podocysts produced by a single polyp was 27, which was observed in one polyp each at 24 and 30°C. Only original polyps (formed by planulae settling on the slide) and 1st generation podocysts (produced by original polyps) that were produced after the adjustment period were included in this analysis.

To focus on the effect of temperature on podocyst excystment and eliminate the effect of podocyst age, podocyst excystment is described as a percentage of excysting podocysts in days from the day of production and not from the beginning of the experiment. Only one podocyst, which did not excyst, was produced at 12°C, and so this treatment was removed from the analysis. Excystment of podocysts was not

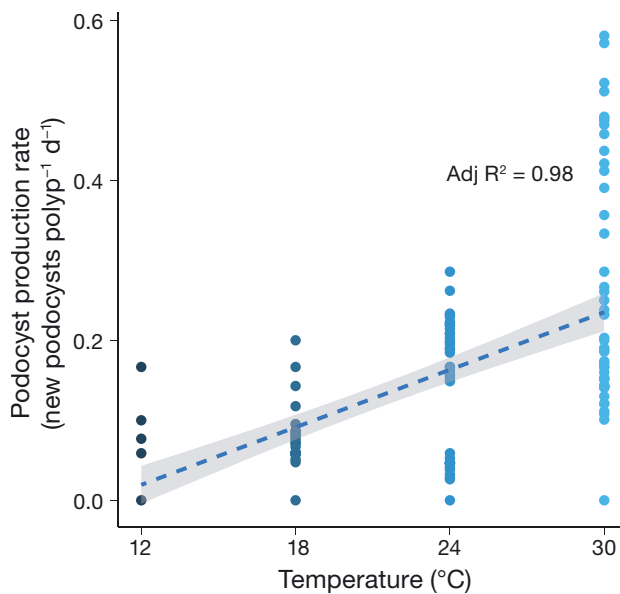


Fig. 3. *Rhopilema nomadica* podocyst production rate at 4 temperatures (12, 18, 24, 30°C) over 55 d ($n = 4$ slides temperature⁻¹). Values are presented as number of new podocysts polyp⁻¹ d⁻¹ and fitted using a linear model with a 95% confidence interval

significantly affected by temperature. The % cumulative excystment was higher in the 30°C treatment (23.7%) than in the 24°C treatment (12.9%), but the low number of podocysts in the 18°C treatment makes statistical analysis unreliable.

Excystment was significantly higher in the first 2 wk following podocyst production than during the following days ($\chi^2 = 8.6$ and 18.1 for 24 and 30°C, respectively; $p < 0.05$ for all tests); 50, 93.3, and 100% of total excystments occurred within 2 wk of podocyst production at 18, 24, and 30°C, respectively; and most excystments occurred within 5 d after podocysts were observed (Fig. 4). All podocysts that were produced after the adjustment period (including those produced during the experiment) were included in the analysis of podocyst excystment. Although many podocysts were monitored on glass slides over lengthy periods of time (months) in the laboratory, most of these podocysts did not excyst (H. Dror unpubl. data).

In some cases, polyps that died during the experiment left podocysts at the bottom of their pedal discs (5 polyps at 18°C and 3 polyps at 24°C). One of these podocysts in the 18°C treatment excysted into a polyp that fully matured but did not produce new podocysts.

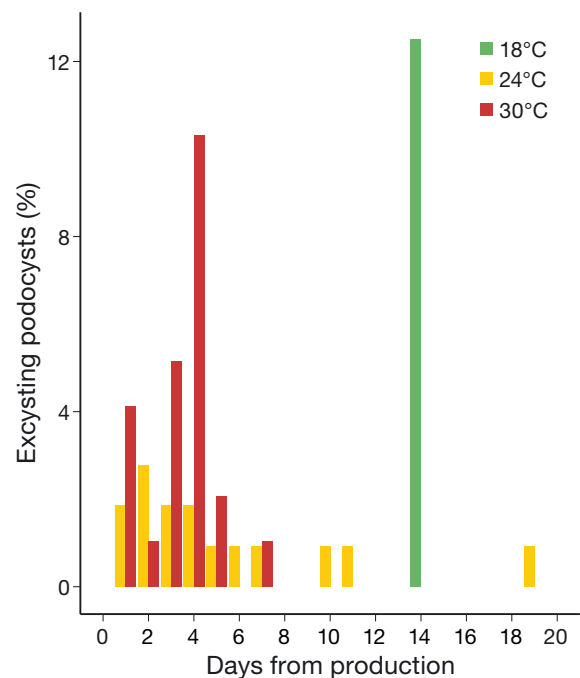


Fig. 4. Percentage of *Rhopilema nomadica* podocysts excysting at 18, 24, and 30°C during the first 20 d from day of podocyst production. The 12°C treatment is not included since only one podocyst, which did not excyst, was produced. In the 18°C treatment, a second podocyst excysted after 48 d (not presented here)

3.2. Effect of changing temperatures on asexual reproduction

This experiment examined the effect of changing temperatures on asexual reproduction. Polyps experienced a temperature decrease (from 25°C to 12, 15, 18°C), then a constant temperature, and finally, a temperature increase (Fig. S2). A summary of statistical values is presented in Table 3.

For the analysis of polyp survival, podocyst survival, and strobilation, only polyps and podocysts

present from the beginning of the experiment were included. For the analysis of podocyst production and excystment, 1st and 2nd generation polyps (or podocysts) were grouped together. Polyp survival was lowest at 15°C, reaching 66.7%, whereas in the control (25°C), 82.8% survived (Fig. 5A). No significant difference in survival was detected between the various treatments ($\chi^2 = 0.3594$, $df = 2$, $p = 0.8$). In addition, polyp survival at all temperatures was much higher in this experiment (lowest survival: 66.7% at 15°C) compared to the constant temperature experi-

Table 3. Numbers and status of polyps, podocysts, and strobilae during different stages of the changing-temperature experiment and statistical values of tests of difference between treatments. Control was maintained at a constant temperature (25°C); temperature in treatments was gradually decreased (0.5–1°C d⁻¹, 14 d) until reaching 12, 15, and 18°C, held constant for 18 d, and then increased (0.5–1°C d⁻¹, 14 d) until reaching 25°C again. Results are presented as % or means \pm SD; numbers are indicated in brackets. Significant values ($p < 0.05$) are indicated in **bold**

	12°C	15°C	18°C	25°C	Test	p
Polyps						
% survival	72.3	66.7	70.4	82.8	$\chi^2 = 0.359$	>0.05
(No. of polyps at end/polyps at start)	(26/36)	(38/57)	(38/54)	(24/29)		
Mortality d ⁻¹	0.006	0.007	0.006	0.004		
Podocyst survival						
% survival	98.25	97.60	98.95	98.88	$\chi^2 = 2.298$	>0.5
(No. of polyps at end/polyps at start)	(280/285)	(326/334)	(283/286)	(265/268)		
Podocyst production						
Podocyst production rate during decrease (new podocysts polyp ⁻¹ d ⁻¹)	0.034 \pm 0.026	0.033 \pm 0.005	0.048 \pm 0.007	0.080 \pm 0.023		
(No. of podocysts produced)	(16)	(25)	(33)	(33)		
Podocyst production rate during increase (new podocysts polyp ⁻¹ d ⁻¹)	0.13 \pm 0.016	0.19 \pm 0.099	0.17 \pm 0.048	0.16 \pm 0.085		
(No. of podocysts produced)	(49)	(83)	(87)	(70)		
Normalized podocyst formation rate during decrease (see Section 3.2)	0.068 \pm 0.052	0.065 \pm 0.011	0.095 \pm 0.014	0.16 \pm 0.047		
Podocyst excystment						
% excystment during temperature increase (No. excysted / total excysted)	98.2	98.0	87.8	69.5		
	(54/55)	(50/51)	(43/49)	(16/23)		
% excystment during temp increase of winter podocysts	3.6	4.6	2.8	0		
(No. excysted / total podocysts)	(11/305)	(19/407)	(11/388)	(0/363)		
% excystment during temp increase of spring podocysts	39.6	26.8	20.9	9.6		
(No. excysted / total podocysts)	(23/58)	(26/97)	(26/124)	(9/84)		
	$\chi^2 = 70.4$	$\chi^2 = 44.5$	$\chi^2 = 43.4$			<0.0005
Strobilation						
% of polyps strobilating 1st time (No. strobilating / polyps)	66.8	64.9	70.3	82.7	$\chi^2 = 0.359$	>0.05
	(24/36)	(37/57)	(38/54)	(24/29)		
% of polyps strobilating 2nd time (No. strobilating 2nd time / strobilating 1st time)	50.0	43.2	65.7	50.0	$\chi^2 = 2.999$	>0.05
	(12/24)	(16/37)	(25/38)	(12/24)		
% of polyps strobilating 3rd time (No. strobilating 3rd time / strobilating 2nd time)	0	6.2	12.0	8.3	$\chi^2 = 0.755$	>0.05
	(0/12)	(1/16)	(3/25)	(1/12)		
Days to 1st strobilation	41.2 \pm 12.6	42.7 \pm 7.6	34.4 \pm 13.1	16.5 \pm 14.4	$F_{(3,114)} = 26.63$	<0.0005
Days from 1st to 2nd strobilation	12.75 \pm 2.00	13.56 \pm 7.26	19.61 \pm 9.22	24.07 \pm 9.46	$F_{(3,58)} = 5.84$	0.001
No. discs 1st strobilation	5.50 \pm 1.31	5.08 \pm 1.60	5.18 \pm 1.30	4.04 \pm 1.60	$F_{(3,114)} = 4.52$	0.005
No. discs 2nd strobilation	5.5 \pm 1.24	4.56 \pm 1.36	4.38 \pm 1.20	4.53 \pm 0.77	$F_{(3,58)} = 3.47$	>0.05
No. discs per strobilating polyp	8.25 \pm 3.51	7.13 \pm 3.63	8.45 \pm 3.13	6.62 \pm 3.06	$F_{(3,114)} = 1.913$	>0.05

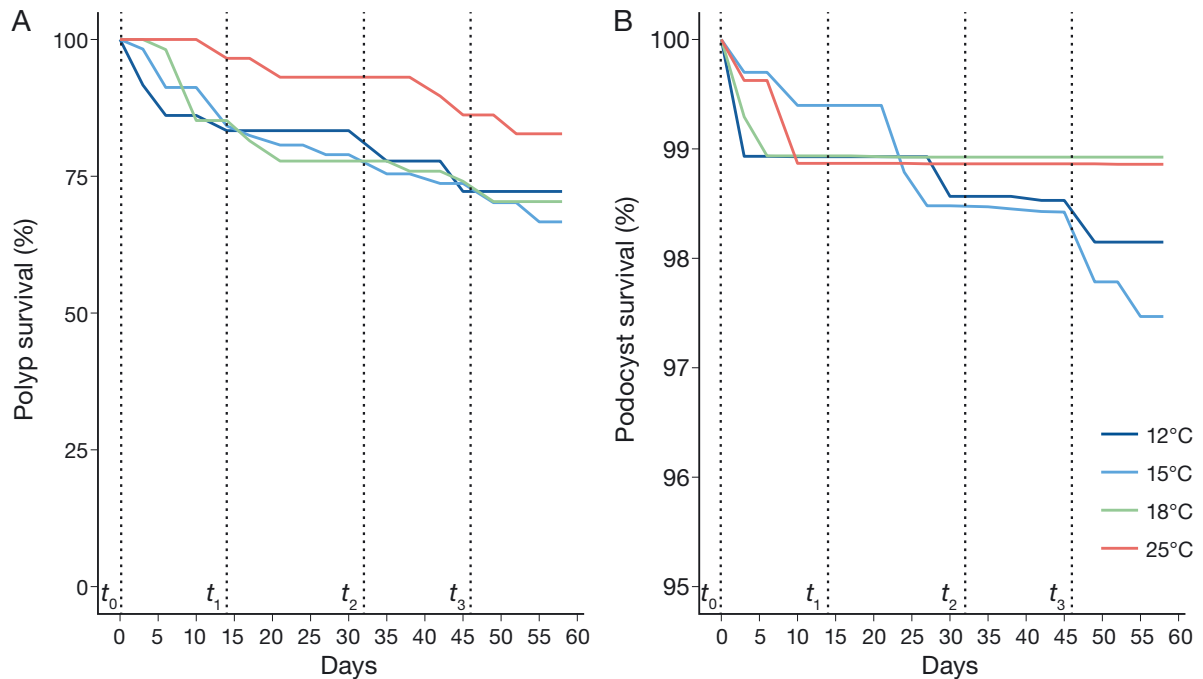


Fig. 5. Mean survival of *Rhopilema nomadica* (A) polyps and (B) podocysts during the 60 d changing-temperature experiment. Only polyps/podocysts present at the beginning of the experiment (t_0), not those formed during the experiment, were analyzed. Dotted lines at t_0 , t_1 , t_2 , and t_3 denote the different periods where the temperature was decreasing, constantly low, increasing, and constantly high

ment (0 and 20% survival at 12 and 18°C, respectively). Almost all podocysts survived throughout the experiment (min. survival: 97.6% at 15°C), and no correlation between podocyst survival and temperature was detected (Fig. 5B) ($\chi^2 = 2.298$, $df = 3$, $p = 0.5$).

Podocyst production rate (presented as new podocysts $\text{polyp}^{-1} \text{d}^{-1}$) during the temperature decrease phase was lower than the production rate during temperature increase (Fig. 6A). The control (constant 25°C) exhibited decreased podocyst production rates during the dates of temperature decrease for the other treatments (Days 0–14); therefore, we normalized the results to facilitate comparison between treatments and the 25°C control. The normalizing factor used was the ratio between mean podocyst production rates in the control during periods of increasing and decreasing temperature (factor = 1.9):

$$\text{Normalizing factor} = \frac{\text{Podocyst production rate } 25^\circ\text{C}_{\text{days } 32-46}}{\text{Podocyst production rate } 25^\circ\text{C}_{\text{days } 0-14}} \quad (1)$$

The normalized and non-normalized podocyst production rates were lower during the temperature decrease phase than when temperatures were increased (Table 3), thus rejecting our hypothesis of higher podocyst production rate during temperature decrease.

Excystment of podocysts occurred almost entirely during the temperature increase phase (98.2, 98, and 87.8% of excystments at 12, 15, and 18°C, respectively, occurred during temperature increase) (Fig. S3). To examine whether podocysts act as seeds that increase polyp populations when temperatures become favorable, we analyzed excystment during the temperature increase, dividing the podocysts into 2 groups: podocysts present at t_0 ('winter podocysts': those that experienced a temperature decrease followed by an increase) and podocysts produced during the temperature increase ('spring podocysts': those that experienced only a temperature increase). Excystment of the spring podocysts during temperature increase was significantly higher in each of the treatments (Fig. 6B) ($\chi^2 = 43-70$, $df = 1$, $p < 0.0005$ for all tests).

Strobilation was strongly affected by temperature and generally occurred between 22 and 25°C (Fig. 7). There was no significant difference between the various treatments (12, 15, 18, and 25°C) for most strobilation indices: % of 1st, 2nd, and 3rd strobilation, discs per strobilation event, and discs per strobilating polyp (Table 3). In the few cases where a significant difference was detected (timing of 1st and 2nd strobilation), Tukey's HSD test found the control group to be different from the other groups. The control (25°C) exhibited continued strobilation throughout

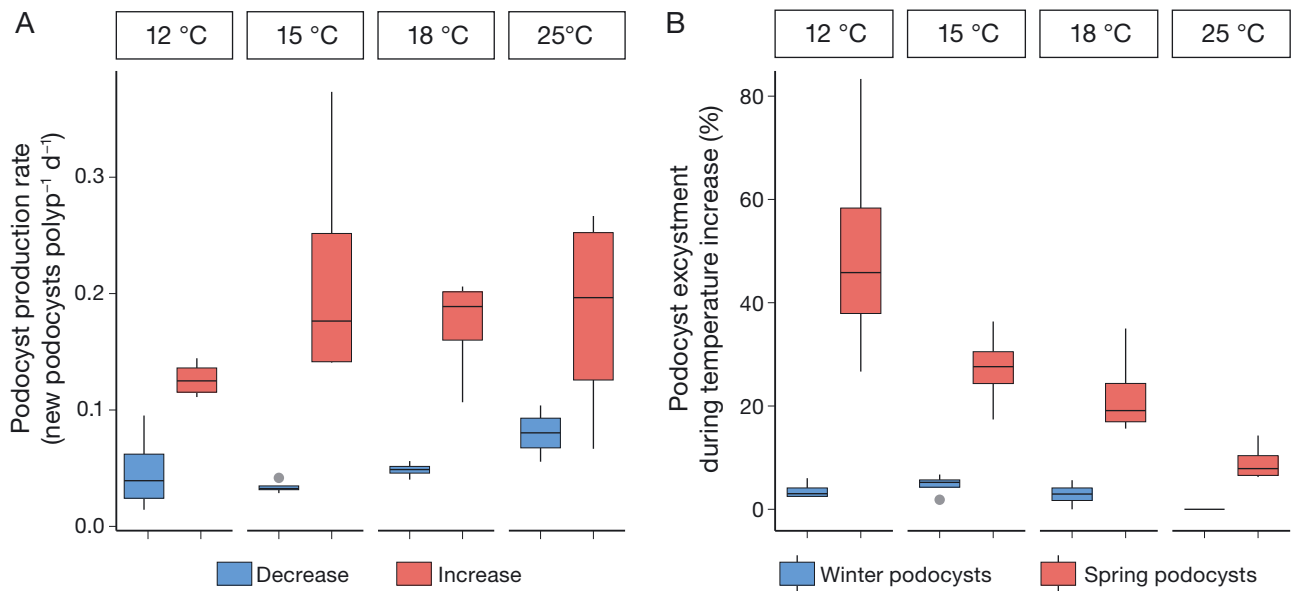


Fig. 6. (A) Podocyst production rate of *Rhopilema nomadica* polyps during the decreasing (Days 0–14, in blue) and increasing (Days 32–46, in red) temperature phases of the experiment ($n = 4$ slides treatment⁻¹). Values are presented as number of new podocysts polyp⁻¹ d⁻¹ and illustrated as boxplots including quartiles (box and whiskers), median (horizontal line), and outliers (point). (B) Excystment of *R. nomadica* podocysts during temperature increase to 25 °C (Days 32–46) from the various low-temperature treatments ($n = 4$ slides treatment⁻¹). Constant 25 °C serves as the control. Blue: 'winter podocysts': those produced during the temperature decrease phase (Days 0–14) and thus experienced a decrease, constant low, and increase in temperature (simulating a seasonal cycle leading to spring of the following year). Red: 'spring podocysts': those produced during the temperature increase phase (Days 32–46) and thus experienced only a temperature increase

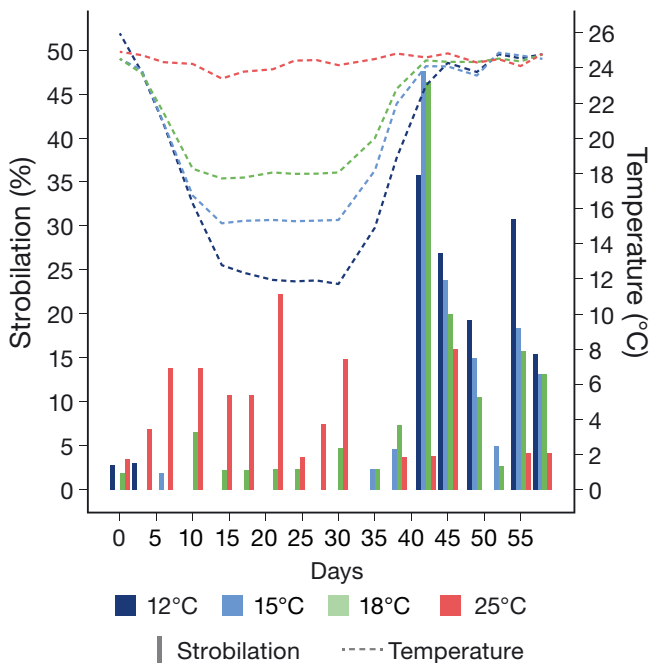


Fig. 7. Mean strobilation ratio of *Rhopilema nomadica* polyps during the 60 d experiment. Colors represent the different treatments. Dashed lines represent temperatures during the experiment timeline. Bars represent % of strobilating polyps at a specific time. Only polyps present at the beginning of the experiment were analyzed

the experiment, while the experimental 'winter' treatments strobilated immediately after temperature increased to over 22 °C (for the 12 °C treatment) and over 24 °C (15 and 18 °C treatments). In the 18 °C treatment, apart from rapid strobilation following the temperature increase, a very low strobilation rate (<2%) was evident even during the low-temperature period. Overall, strobilation occurred before, during, and after podocyst production.

4. DISCUSSION

The propagation of many temperate-water scyphozoan polyps and the occurrence of adult medusae are limited by low winter temperatures (Prieto et al. 2010, Purcell et al. 2012, Widmer et al. 2016). This response is not only species-specific, but also population-specific (Purcell 2007, Willcox et al. 2007) and is more evident in populations living close to their lower thermal limits (Lucas et al. 2012). This principle was applied to the case of *Rhopilema nomadica*, which was not expected to spread to the western Mediterranean due to its presumed intolerance to low winter temperatures (Lotan et al. 1994, Daly Yahia et al. 2013). Nevertheless, in the last 3 decades,

R. nomadica has been gradually expanding westward, reaching Sardinia (Balistreri et al. 2017) and Tunisia (Daly Yahia et al. 2013), and experiencing lower winter temperatures (12.2°C) than in the eastern basin (16.8°C) (Shaltout & Omstedt 2014). Therefore, we wanted to examine the effect of temperature on the benthic stages of *R. nomadica* and assess the feasibility that this species may form a steady, viable population in the western basin of the Mediterranean.

4.1. Temperature effect on polyps and podocysts

We found that increased temperatures are beneficial to polyp survival and asexual reproduction (Figs. 2 & 3), as reported in other scyphozoan species such as *Cotylorhiza tuberculata*, *Aurelia aurita*, *Chrysaora quinquecirrha*, and *C. fuscescens* (Willcox et al. 2007, Prieto et al. 2010, Pascual et al. 2015, Treible & Condon 2019) (Table 1). At higher temperatures (24 and 30°C), the survival of *R. nomadica* polyps was significantly higher than at the lower temperatures tested (12 and 18°C). Decreased vitality and increased mortality at lower temperatures (<13°C) were previously reported for *R. nomadica*, yet survival was not as low as found in our study and mortality began at lower temperatures (13 vs. 18°C for Lotan et al. 1994 and the current experiment, respectively). See further discussion in Section 4.5.

Podocyst production and excystment were the principal modes of polyp proliferation in *R. nomadica* and both increased with temperature in our experiments (non-significant results in the excystment at lower temperatures were probably the result of low numbers of podocysts that were produced). Higher temperatures have been shown to have a positive effect on the production of podocysts in other jellyfish species, such as *A. aurita*, *Cyanea nozakii*, *Chrysaora pacifica*, *Rhopilema esculentum*, and *Rhizostoma octopus* (Table 1; see references therein). The vast majority of *R. nomadica* polyps produced podocysts at all temperatures, aside from 12°C, and podocyst production continued throughout the experiment, including during strobilation periods. Production of podocysts at the same time as strobilation is rare among scyphozoans and, apart from *R. nomadica* (Lotan et al. 1992), has only been documented in *Lychnorhiza lucerna* (Schiariti et al. 2014). It was proposed that polyps need to transfer resources from one activity (podocyst production) to the other (strobilation), thereby precluding simultaneous asexual activities in these cnidaria (Brewer & Feingold 1991), but it appears there are exceptions to this strategy.

Excystment of *R. nomadica* podocysts commonly occurred within the first 2 wk after production, though most excysted within a few days of production (Fig. 4). A significantly higher excystment ratio among 'young' (<4 mo old) as opposed to 'older' podocysts was also observed in *A. aurita* and *Nemopilema nomurai* (Thein et al. 2012, Kawahara et al. 2013), yet immediate (within a few days) excystment has not been reported for any of the other species studied. The highest proportion of excystment (no. excysted/total no. of podocysts) in *R. nomadica* was 26% in the 30°C treatment, which is lower than that reported for *C. nozakii* (54%), *C. pacifica* (48%), and *A. aurita* (39%) (Thein et al. 2013) (Table 1). However, the proportion of excystment that we found in the other temperature treatments (8 and 12% at 18 and 24°C, respectively) was similar to that previously reported for *R. nomadica* (Lotan et al. 1992).

R. nomadica strobilation occurred only in the 24°C treatment, starting 12 d after temperatures stabilized and continuing throughout the experiment, with over 40% of the polyps continuing to a second strobilation. Earlier studies (Lotan et al. 1994) found similar ratios of strobilating polyps but reported that strobilation peaked between 18 and 22°C and that an increase in temperature to 24 or 26°C reduced strobilation rates. This difference may be attributed to the different conditions in which the polyps were kept before the beginning of the experiments.

4.2. The effect of changing temperatures on benthic stages of Scyphozoa

Since constant low temperatures proved to be unfavorable for polyp survival, podocyst production, excystment, and strobilation, we tested the response of the sessile life stages to a drop in temperature, simulating winter onset. Several previous studies have demonstrated the importance of temperature change (either increase or decrease) on podocyst production and excystment (Table 1; see references). In *A. aurita*, *C. nozakii*, and *Cyanea capillata*, podocyst production was positively correlated with temperature increase, whereas excystment occurred only with temperature decrease (Brewer & Feingold 1991, Thein et al. 2012, 2013). In contrast, podocyst production of *C. quinquecirrha* from the Chesapeake Bay increased when water temperatures cooled and excystment occurred when the temperature rose (Cargo & Schultz 1967, Cargo & Rabenold 1980). For many species, podocyst production and podocyst excystment are generally not simultaneous and occur

under different environmental conditions (Table 1; all species but *R. esculentum*). This is not the case for podocysts of *R. nomadica*. During the changing-temperature experiment, polyps and podocysts were subjected to a drop in temperature followed by a period of constant low temperature and then a temperature increase. In all treatments, extensive excystment (Fig. S3) and strobilation (Fig. 7) occurred only when temperatures increased beyond 22°C, peaking at 24–25°C, whereas podocyst production did not increase when the temperature was reduced (Fig. 6A). Therefore, unlike other species described above, podocyst production, excystment, and strobilation in *R. nomadica* all occurred simultaneously when temperatures increased. The temperature increase following cold ‘winter’ conditions mimics spring conditions in the Mediterranean as coastal water temperatures increase, in contrast to the cooling trend during the fall and winter. Thus, *R. nomadica* podocysts are apparently not produced in response to a decline in temperature, as low temperatures may result in polyp mortality (podocysts do not act as ‘seeds’ to maintain populations through winter conditions). Rather, they excyst soon after being produced in the spring, possibly to help boost the current season’s growing polyp population.

It appears that the strobilation process was not triggered by a change in temperature per se, but by exceeding a minimum temperature threshold (22°C). In the 25°C control treatment, temperatures did not change and polyps strobilated throughout the experiment (Fig. 7). In addition, in all treatments, strobilation started only after reaching the temperature threshold, and most strobilation indices (% strobilation, no. of discs polyp⁻¹, etc.) were not affected by the intensity of ‘winter’ temperatures (12, 15, and 18°C) or by the range of temperature change prior to reaching the temperature threshold (Table 3). In a previous study on *R. nomadica*, water temperature affected only the ratio of strobilating polyps and not the number of ephyrae released polyp⁻¹ (Lotan et al. 1994). It may well be that in the natural environment, the cue to strobilation during seasons of temperature change is a result of interactions with other factors, such as seawater chemical composition, light, and food supply.

4.3. The role of podocysts

Podocysts are thought to serve 4 main purposes (Brewer & Feingold 1991, Arai 2009): (1) increase polyp population, (2) enable survival during periods

of harsh conditions, (3) protect against predation by nudibranchs, and (4) compete over substrate. The strategy and mechanisms through which these purposes are achieved differ from species to species. We suggest that all of the above roles may be fulfilled by *R. nomadica* podocysts, although, contrary to our initial hypothesis, increasing the polyp population for the immediate season appears to be the major role, whereas maintaining the population through harsh conditions is secondary.

In both of our experiments, mature polyps began producing podocysts almost immediately, and podocyst production increased with temperature, reaching 0.23 podocysts polyp⁻¹ d⁻¹. A similar podocyst production rate of up to 0.11–0.39 polyp⁻¹ d⁻¹ has been reported in *A. aurita* (Thein et al. 2013), *C. nozakii* (Thein et al. 2013, Feng et al. 2015b), *R. esculentum* (Guo 1990), and *R. nomadica* (Lotan et al. 1992). Excystment in *R. nomadica* occurred mainly within the first 2 wk of podocyst production, reaching a maximum of 23% excystment (at 30°C) out of all podocysts present. Thus, podocyst production along with almost immediate excystment may increase polyp population dramatically within a short time during elevated temperatures, just before strobilation. Many podocyst-producing species have been known to form swarms (Arai 2009), but most of these swarm-forming species exhibit a multi-mode reproduction strategy, including the production of buds, stolons, podocysts, etc. Podocyst-producing mono-mode species are considered to have low reproduction rates since podocyst production is usually slower than budding and involves environmental stimuli for both production and excystment of podocysts (Schiariti et al. 2014). *R. nomadica* presents a unique example of a mono-mode medusa that proliferates rapidly using podocysts alone, rather than budding, forming massive swarms (Galil & Zenetos 2002).

In order to enhance survival, podocysts need to be more resilient than polyps (Arai 2009). In the changing-temperature experiment, temperature reduction resulted in mortality of ca. 30% of polyps, whereas podocyst mortality was only 3%. But survival of podocysts alone does not ensure these will re-establish a thriving polyp population. Other prerequisites include increased production of podocysts before the unfavorable season (as temperatures drop) and excystment of these podocysts to form polyps when temperatures rise. These responses were not observed in our experiments. Podocyst production did not increase when temperatures dropped (Fig. 6A), but rather when temperatures rose. Excystment increased immediately after

temperatures rose (Fig. S3); however, most of the excystment occurred in podocysts that had just been produced (spring podocysts), while <4% of podocysts that were present during the drop in temperature (winter podocysts) excysted in response to this temperature increase (Fig. 6B). We have monitored podocysts on glass slides for several years and, despite natural fluctuations in eastern Mediterranean SSTs (16–32°C), most of these remained alive but never excysted (H. Dror unpubl. data). Thus, we propose that each year, only a small proportion of podocysts produced in the previous year(s) will excyst and join the population of newly formed polyps.

Finally, laboratory and field observations on the predation of polyps by nudibranchs and other natural predators have shown that while hundreds of polyps may be consumed by a single nudibranch each day, podocysts are not (Cargo & Schultz 1967, Arai 2009, Takao et al. 2014). In laboratory experiments, *Phidiana militaris*, a common aeolid nudibranch in the eastern Mediterranean, was offered polyps and podocysts of *R. nomadica* on glass slides. The nudibranch devoured hundreds of polyps, but despite the presence of hundreds of podocysts adjacent to the polyps, none of these were consumed (H. Dror unpubl. data).

It is thought that podocysts may also play a role in competition over substrate (Brewer & Feingold 1991). This may be true for podocysts that excyst immediately or soon after production but is less relevant for podocysts that act to increase the polyp population in the following season since they may be covered by other fouling organisms by that time. More studies regarding interactions of podocysts with other organisms are needed to better understand their function; for instance, examining whether the presence of predators that consume polyps might inhibit excystment.

4.4. Dynamics of *R. nomadica* in the eastern Mediterranean

Based on the seasonal occurrence of adults (Edelist et al. 2020) and the patterns of asexual reproduction observed in this study, the seasonal life cycles of *R. nomadica* in the eastern Mediterranean are described below.

Our experiments indicate that increased water temperatures during spring may stimulate podocyst production and excystment. Thus, during May and June, podocysts produced by polyps that survived the winter may excyst within a few days. A small

number of last season's podocysts may also excyst at that time, but these are generally few and are likely to be fairly insignificant. The water temperature in the eastern Mediterranean usually exceeds 22°C by mid-late April, which should stimulate strobilation, as shown in our results. It is notable that Lotan et al. (1994) reported a strobilation peak for *R. nomadica* between 18 and 22°C and a decline in strobilation at higher temperatures, contrary to our findings. The release of ephyrae in springtime would explain the peak in annual swarms of medium-sized medusae (10–30 cm) in July (Edelist et al. 2020, 2022). This supposition is based on the assumption that ephyrae released during spring develop into mature medusae within ca. 3 mo, as described for *A. aurita* (Möller 1980) and *N. nomurai* (Kawahara et al. 2006). The location of the polyps that establish these outbreaks is still unknown, but particle-release models combined with ocean current velocity models suggest that they are located along the northern coasts of Egypt (Edelist et al. 2022). Sexual reproduction presumably occurs mostly during the summer swarming events in June and July. Planulae released thereafter metamorphose into polyps within a few days, and 2–3 wk later, polyps become mature. In this way, during August and September, a new generation of polyps is formed and immediately starts to produce podocysts. Polyps continue to strobilate and produce podocysts, and these continue to excyst throughout the summer. During winter, polyps struggle to survive at low temperatures and asexual reproduction is minimal.

4.5. Invasion capacity into the western Mediterranean

Whereas it has been proposed that the Indo-Pacific *R. nomadica* has managed to invade the eastern Mediterranean Sea due to the warming of this region, our results show that this species can endure the range of temperatures prevailing in the western Mediterranean as well. Decreased polyp survival was observed at the lower temperatures (12, 15, 18°C) compared to the higher temperatures (24 and 30°C) in both experiments, but differences between the 2 experiments are noteworthy: ca. 70 vs. 0% survival at 12°C and ca. 70 vs. 20% survival at 18°C for the changing- vs. constant-temperature experiments, respectively. Since most of the mortality occurred during the temperature-adjustment phase, the fact that we only tested short durations at low temperatures does not seem to be the reason for this difference. Rather, we ascribe the lower fitness of the po-

lyps in the constant-temperature experiment to the fact that they were produced in winter (which is not the natural season for reproduction), as opposed to the polyps used in the changing temperature experiment, which were produced in summer. Nonetheless, the polyps were viable and actively reproduced, as described above, but these polyps may have had a disadvantage at the lower temperatures tested.

In the changing-temperature experiment, there was no significant difference in survival between the various winter temperatures (12, 15, and 18°C). In the Bizerte Lagoon, Tunisia, where a viable reproducing population of *R. nomadica* has been reported, winter temperatures drop to 13°C (Balistreri et al. 2017), while in the eastern Mediterranean, winter coastal water temperatures generally do not drop below 18°C. Thus, the results of our experiments along with the existence of a population in the cold Bizerte Lagoon during winter suggest that temperature may not be the limiting factor for polyp vitality in the western basin. Moreover, it has been proposed that polyp populations originating from habitats that experience a wide temperature range, such as the Levant, are more robust to temperatures outside their range than stenothermal populations and are more likely to succeed in expansion of their range (Pascual et al. 2015). On the other hand, the lower summer temperatures in the western basin compared to the eastern basin may limit the extent of temperature-dependent podocyst production, thereby limiting medusa swarms. As for strobilation, we have shown that the number of ephyrae released (and other strobilation indices) is not affected by low winter temperatures as long as a threshold of 22°C is exceeded. Summer water temperatures in the western Mediterranean may reach 25°C (Lejeusne et al. 2010, Shaltout & Omstedt 2014); thus, the number of ephyrae produced by each polyp in the western basin should be similar to that produced in the east. Although we did not study the effect of temperature on planula settlement, we conducted multiple successful fertilizations in a temperature range of 17–24°C. Therefore, we believe that temperatures in the western Mediterranean may also be suitable for planula production, settlement, and growth.

Over the last few decades, a general warming trend has been observed in the Mediterranean in both the eastern and western basins and from shallow to deep water (Lejeusne et al. 2010, Shaltout & Omstedt 2014, Ozer et al. 2017). Many of the tropical species introduced into the Mediterranean have been confined to the Levantine waters for a long time, but the rapid warming of the Mediterranean is

promoting their accelerated spread westward and northward (Occhipinti-Ambrogi 2007, Coll et al. 2010, Zenetos & Galanidi 2020). Many alien species, including algae, seagrasses, invertebrates, and fish, have recently been recorded and are gradually becoming more common in the colder, northwestern Mediterranean. Examples of these Lessepsian migrants include *Siganus luridus* (fish) reaching the Gulf of Lions (Lejeusne et al. 2010), *Etrumeus golanii* (fish) recorded in the Alboran Sea, *Pteragogus trispilus* (fish) found in the Aegean and Algerian basins (Zenetos & Galanidi 2020), *Brachidontes pharaonis* (bivalve) detected in western Sicily (Sarà et al. 2008), and *Halophila stipulacea* (seagrass) reported in the Tyrrhenian Sea (Gambi et al. 2009). Climatic models predict that the Mediterranean Sea will continue to be heavily affected by climate change (Shaltout & Omstedt 2014). Like other warm-water species, thermophilic jellyfish are positively affected by global warming conditions (Boero et al. 2016). The expected increase in water temperatures will likely result in milder winters and longer spring seasons, which will improve winter survival of polyps and broaden the reproductive periods of these jellyfish. This will enable them to expand further and increase their reproductive success, providing them with a significant advantage over native species (Boero et al. 2008, 2016, Coll et al. 2010). In a recent study, *R. nomadica* was reported amongst the highest-scoring species to expand its range to more northern locations and become invasive in the Mediterranean under predicted climate change conditions (Killi et al. 2020). We anticipate that *R. nomadica* will continue spreading into the western Mediterranean and will establish a viable, reproducing, possibly swarm-forming population in that region as well.

4.6. Conclusions

We have shown that increased temperatures stimulate asexual reproduction via podocysts in *R. nomadica*. Podocysts were also affected by the trajectory of temperature change: when temperatures decreased, asexual reproduction was minimal, while increasing temperatures lead to the onset of extensive podocyst production and excystment. Thus, most asexual reproduction occurs during spring and summer in the eastern Mediterranean. These results also elucidate the role of podocysts in the life history of *R. nomadica*. Podocysts that are produced in response to a temperature increase excyst within a few days, whereas only a small percent excyst in the following season. There-

fore, we suggest that podocysts of *R. nomadica* serve as a means of increasing the immediate season's polyp population and less as a means of maintaining populations through adverse conditions. Moreover, strobilation in *R. nomadica* does not require a change in temperature, but rather a temperature exceeding a thermal threshold; occurring at temperatures $>22^{\circ}\text{C}$. Finally, since winter temperatures in the western Mediterranean are less than optimal for *R. nomadica* but still within their physiological limits, and summer temperatures enable asexual reproduction and strobilation, temperature may ultimately not serve as a limiting factor in the spread and success of *R. nomadica* in the western Mediterranean. The predicted continuing increase in SST in the Mediterranean therefore likely enables a further expansion of its range and an increase in performance in the western basin.

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