Management of Biological Invasions (2023) Volume 14, Issue 3: 561–577

Research Article

Treating ballast waters to limit *Mnemiopsis leidyi* access to new habitats

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Citation: Budiša A, Burić E, Blecich P, Matulja T, Millotti G, Iveša N, Djakovac T, Najdek M, Mičić M, Paliaga P (2023) Treating ballast waters to limit *Mnemiopsis leidyi* access to new habitats. *Management of Biological Invasions* 14(3): 561–577, https://doi.org/10.3391/mbi.2023.14.3.12

Received: 5 July 2022
Accepted: 19 January 2023
Published: 10 April 2023

Thematic editor: Katherine Dafforn

Abstract

Discharging untreated ballast waters supports the spread of invasive species. One of the most successful ballast-water mediated biological invasions was a transatlantic transport of the ctenophore *Mnemiopsis leidyi* to many Eurasian seas, where it has significantly impacted local ecosystems. To prevent its spread to new areas, we studied the survival of different *M. leidyi* life stages exposed to several eradication techniques. We looked closely into the technical applicability of an onboard thermal treatment by calculating its duration and the required energy to perform it. The duration was considered as a sum of the time to heat ballast tanks by using the engine's waste heat for two types of ships and *M. leidyi*'s eradication. The calculated duration of the proposed treatment allowed us to estimate a minimum travel length undertaken by a specific type of ship to eliminate *M. leidyi* successfully. The travel length determines the appropriate technique and minimal requirements to eliminate *M. leidyi* successfully and, thus, can serve as a guideline for a management plan. In conclusion, the proposed onboard treatment would be efficient on smaller ships and minimum distances of 200–300 km depending on the season but not on the short routes where other treatment techniques, e.g. exposure to ultrasonic cavitation or microwave radiation, should be considered.

Key words: marine invasive species, ballast water management treatment, *Mnemiopsis* survival, onboard thermal treatments, Adriatic Sea

Introduction

Nowadays, approximately two-thirds of traded goods are shipped worldwide by sea (Kumar and Hoffmann 2002), transporting ~ $3.5 \cdot 10^9$ t of ballast water annually (Endresen et al. 2004). Ballast waters ensure ships' buoyancy, stability, and manoeuvrability (Werschkun et al. 2014) but can also carry organisms into new ecosystems (Carlton 1985; Williams et al. 1988). Therefore, their adequate management and treatment are becoming progressively urgent to prevent or at least reduce the intensity of biological invasions mediated by ballast waters. One of the well-known ballast-water mediated biological invasions was the transatlantic transport of the ctenophore

Mnemiopsis leidyi Agassiz, 1860 to the Eurasian seas, which disrupted local ecosystems resulting in the collapse of fisheries (Kideys et al. 2005).

Mnemiopsis leidyi is one of the world's worst invaders (Lowe et al. 2000). After arriving in the Black Sea, this holoplanktonic and hermaphroditic species has dramatically increased its population and spread by currents and ballast waters to the neighbouring basins (Shiganova et al. 2019). *Mnemiopsis leidyi*'s adaptability to a vast range of environmental conditions, such as wide temperature and salinity ranges and the tolerance to low concentrations of oxygen (O_2) (Shiganova et al. 2019), coupled with a non-selective diet (Colin et al. 2010) exceptionally high fecundity (Reeve et al. 1989) and lack of its predators, lead to one of the most successful ballast water-mediated biological invasions because even after years *M. leidyi* is still present in the most of the invaded area (Shiganova et al. 2019).

Ballast waters also likely offered the means for the arrival of *M. leidyi* to the Adriatic Sea. The first record occurred in 2005 in the Gulf of Trieste (Shiganova and Malej 2009) – an area of intensive maritime traffic. Since 2016 *M. leidyi* has consistently appeared in the northern and western but not in the southern Adriatic – in direct contact with the rest of the Mediterranean (Budiša et al. 2021). The only exception was a brief appearance in 2017 in the Port of Ploče (south-east Adriatic) (D. Lučić *pers. comm*.) – a cargo port receiving ships from the northern/western Adriatic. In addition, a preliminary molecular study (Baričević et al. 2018) linked Adriatic populations to those in the Black Sea. In the north of the basin, seasonal *M. leidyi* blooms covering tens of km^2 exert multiple effects on the pelagic ecosystem by influencing plankton communities and the carbon pool along the coast (Budiša et al. 2021; Paliaga et al. 2021).

In this context, the geomorphology of the Adriatic represents a compliance problem with the International Convention (IMO 2004) because ballast exchange allowed at ≥ 200 m depth and never closer than 50 NM of the land is applicable within a very limited area and could significantly slow down ship transport. Thus, according to Markovina et al. (2007), due to its dimensions, the Adriatic is inadequate for ballast water exchange. This means that it is essential to effectively treat ballast waters onboard/in harbours for transport ships in the Adriatic. Moreover, the management standards stating that < 10 smaller (< 50 µm) viable organisms per mL and bigger per m^3 have to be discharged (Vorkapić et al. 2016) is insufficient for such a resilient species. Therefore, to prevent the spread of *M. leidyi* within the Adriatic and other nearby seas, we considered preventive measures to eliminate it from ballast waters.

There are plenty of ballast water treatments. The primary onboard separation techniques include various physical methods, e.g., self-cleaning filter systems (pores size 40 µm), allowing smaller organisms to enter (Werschkun et al. 2014). Secondary techniques include various mechanical and chemical methods or their combination. The most common physical methods are ultrasound, UV-light, thermal, magnetic and electrical treatments (Vorkapić et al. 2016). Another efficient technique is deoxygenation which involves bubbling nitrogen (N_2) or other inert gases to supersede O_2 and eliminate organisms while protecting tanks against corrosion (Tamburri et al. 2002). A combination of several treatments is used most of the time (Balaji and Jaakob 2011).

This research is focused primarily on thermal treatments since they offer the advantage of using the engine and backup boiler-generated waste heat (Balaji and Jaakob 2011) to increase the temperature in tanks without the repercussions of undesirable by-products (Rigby et al. 2004), a non-renewably sourced energy is utilized more efficiently (Biswas et al. 2018). Unlike other treatments, the application of thermal treatment does not require advanced technological improvements, which renders its implementation more attainable. Moreover, to recommend an adequate treatment against *M. leidyi*, we tested the effects of temperature, microwaves, ultrasonication and deoxygenation on its survival. We combined the results of *M. leidyi* survival using thermal and deoxygenation treatments with preliminary technical calculations to assess the feasibility of an onboard treatment using engine coolant water. Finally, we propose suitable treatments for different travel distances and ship types.

Materials and methods

Mnemiopsis leidyi *survival*

Adult *M. leidyi* specimens (7 cm oral-aboral length) were carefully collected from the sea with a ladle and placed in a collection tank (40 hL) with recirculating seawater (21 ± 0.5 °C, salinity 37) at a 12:12 h light regime until exposed to different treatments.

Firstly, we placed 30 adult specimens in each of three enclosed 100 L tanks containing filtered seawater (industrial filters, pore size 0.7 μm) at 21 °C (*in situ* marine temperature) to obtain their survival time in a stagnant tank without any treatment (NT) (Figure 1A). In the second experiment, food (*Artemia salina* larvae), water circulation and air bubbling were added to simulate ideal conditions (IC) and measure the longest survival of *M. leidyi* (Figure 1B).

In the third experiment, we investigated the effects of thermal treatment (TT) on adult *M. leidyi*. We placed 30 adult ctenophores in each of three open tanks (100 L), with no circulation, containing non-filtered seawater collected from the same area where *M. leidyi* was present and monitored its survival time at 21, 30, 35, 40, 45 and 50 °C (Figure 1C).

The following experiment was aimed to investigate the effects of hypoxic and anoxic conditions, i.e., deoxygenation treatment (DT), on *M. leidyi* survival. We vigorously bubbled N_2 into the tanks while measuring its values with a probe (Hanna Instruments® multiparametric probe) up to 20%

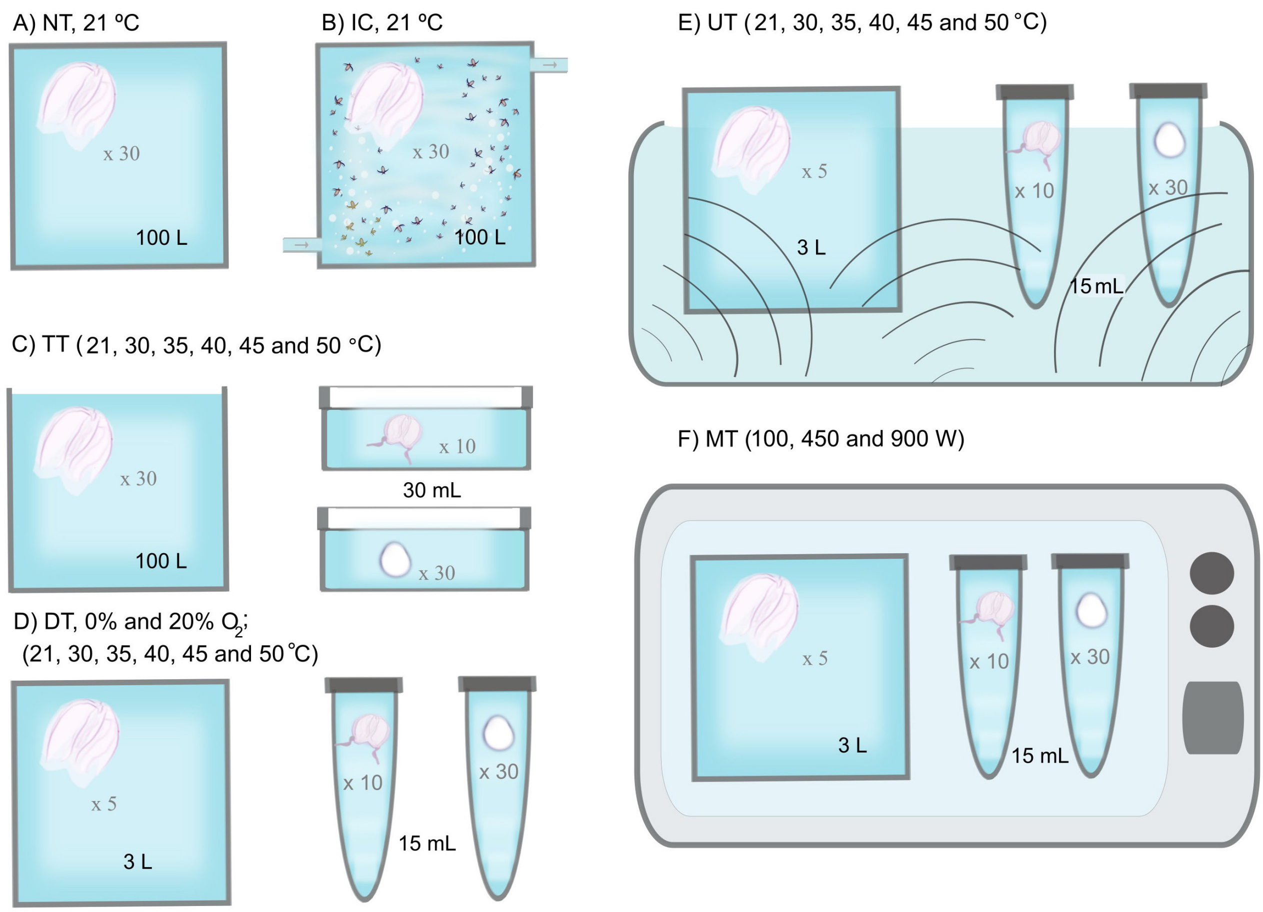

Figure 1. Experimental set-up for testing survival in A) stagnant closed tanks (NT), B) under ideal conditions with available food, air bubbles and water circulation (IC), C) thermic treatment in oxic, D) hypoxic and anoxic conditions (DT), E) thermal and ultrasound treatment (UT) and F) exposure to microwave radiation (MW). Treatments A-E were set in triplicates while F was repeated 6x.

O_2 saturation for hypoxia and 0% for anoxia. After reaching the desired saturation, we placed 30 adults into the three 100 L enclosed tanks to prevent O_2 from entering and monitored their survival times. The experiment was repeated 3x at the same six temperatures (Figure 1C, D).

In the fifth experiment, we studied the effects of ultrasounds and temperature (UT) on *M. leidyi*. In this case, we used smaller, 3 L buckets with five specimens to fit into the ultrasonic bath (VWR® Scientific 750D), sonic power of 360 W and frequency from 38.5–40.5 kHz. The experiment was repeated 3x at the same six temperatures (Figure 1E).

We also investigated the microwave (MT) heating effects on adults by placing a bucket (1.5 L) with filtered seawater (0.7 µm pore size Whatman™ GF/F) and five specimens into a microwave oven. As for the ultrasonic bath, the aliquots of water used were restricted by the oven's dimensions. We tested their survival at different powers, 100, 450 and 900 W, repeating it 6x.

In all the experiments for estimating the survival time of adult *M. leidyi*, specimens were considered dead when their body lost integrity and began to turn into a brownish gelatinous mass (Supplementary material Figure S1 C, F,).

To measure the juvenile phases' survival times, we applied the same test conditions as in TT, DT, UT and MT. Firstly, eggs and the larvae were obtained by placing 10 adults into 3 L buckets containing filtered seawater (zooplankton mesh, pore size 50 μm) and leaving them for 24 h to allow reproduction. After removing the adults, we applied reverse filtration (Holm-Hansen et al. 1970) to the remaining water. The sample was centrifuged (200 rpm, 5 min), decanted, and concentrated to 50–100 mL. Then, randomly selected intact *M. leidyi* eggs and larvae were isolated under a stereomicroscope (20–50x) and used for the experiments. The seawater used in all the experiments was pre-filtered (zooplankton mesh, pore size 20 μm). Each treatment was set in triplicates using 30 eggs/10 larvae produced by different adults.

For the TT, we incubated specimens in Petri dishes containing 15 mL of pre-filtered seawater at the six selected temperatures, while for deoxygenation, the specimens were incubated in 15 mL plastic test tubes containing anoxic/hypoxic pre-filtered seawater (see DT). To prevent the entry of O_2 in DT, we opened sample tubes only at the time of adults' decay. If the specimen were still viable, we extrapolated the actual time to decay. The same setup was repeated to test select ultrasonication (see UT) and microwave (see MT) effects. The larvae and eggs (Figure S1A, B, D, E) were considered decayed when their structure started to degrade, and the larvae were not motile.

Onboard thermal treatment to eradicate M. leidyi *from ballast waters*

We proceeded in four steps to assess the feasibility of an onboard thermal treatment of ballast waters to eliminate *M. leidyi*. The first consisted of defining an equation to describe the heating of the ballast waters considering the losses from the ballast tanks toward the Sea and air. In the second step, we chose two different ship designs as models for estimating the heating of their ballast waters to certain temperatures. In the third step, we analysed the heating of the ballast waters of our model ships in different temperature conditions combined with different desired ballast water temperatures. After verifying the heating capacities in the various conditions, the fourth step consisted in calculating the times necessary for reaching certain temperatures in the ballast waters and applying the treatment to eliminate *M. leidyi*. In this step, we considered using a thermal treatment alone and a combined thermal/deoxygenation treatment. For each treatment, we calculated the energy required and within what travel distance the treatment could achieve its results. In all the calculations, we assumed that the ships were, moving at a typical cruise speed of 15 kn (Wang et al. 2007).

Heating of ballast waters

To describe the total heat to warm up the water in the ballast tanks to the desired temperature, we have to consider a term that accounts for the replacement of heat losses from the ballast tank water to outer air and seawater and one for heating the tank to the desired final temperature (Eq. 1), i.e.:

$$\dot{Q}_{\mathrm{tot}} = \dot{Q}_{\mathrm{r}} + \dot{Q}_{\mathrm{h}} \quad \text{(Eq. 1).}$$

The heating capacity for the replacement of heat losses can be expressed as:

$$\dot{Q}_{\mathrm{r}} = \sum_{i=1}^{n} U_{\mathrm{i}} \cdot A_{\mathrm{i}} \cdot (T_{\mathrm{in}} - T_{\mathrm{out}}) \quad \text{(Eq. 2),}$$

where the overall heat transfer coefficient (U-value) of the i^{th} element wall of the ballast tank is denoted by U_{i} (W/m^{2}K), the corresponding heat transfer surface area is A_{i} (m^2), and the temperature difference between internal tank water and the outer seawater is $T_{\mathrm{in}} - T_{\mathrm{out}}$ (K). The overall heat transfer coefficient is calculated from the convective heat transfer coefficients at the inner and outer tank walls, h_{in} and h_{out} (W/m^{2}K), the thickness δ (m), and the thermal conductivity λ (W/mK) of the tank wall material as:

$$U_{\mathrm{i}} = \left(\frac{1}{h_{\mathrm{in}}} + \frac{\delta}{\lambda} + \frac{1}{h_{\mathrm{out}}}\right)^{-1} \quad \text{(Eq. 3).}$$

The capacity for heating the ballast tank water to the desired final temperature can be expressed as:

$$\dot{Q}_{\mathrm{h}} = \frac{V_{\mathrm{w}} \cdot \rho_{\mathrm{w}} \cdot c_{\mathrm{w}} \cdot (T_{\mathrm{f}} - T_0)}{t} \quad \text{(Eq. 4),}$$

where V_{w} (m^3) is the water volume in the ballast tank, ρ_{w} (kg/m^3) is the density of water and c_{w} (J/kgK) is the specific thermal capacity of water.

The difference between the final and the initial ballast tank water temperatures is $T_{\mathrm{f}} - T_0$ (K); thus, the internal tank water temperature ranges from the initial to the final temperature during the thermal treatment process, $T_0 \leq T_{\mathrm{in}} \leq T_{\mathrm{f}}$. The time duration of the ballast water heating is t (s). The physical properties of water are calculated using the CoolProp library for fluids properties embedded in MSExcel.

The physical properties of water change with temperature and, thus, change the heat transfer coefficients at the ballast tank walls. An iterative assumption-correction procedure was implemented to obtain the real tank wall temperatures at the inner and outer surfaces. In the first iteration step, the inner and outer tank wall temperatures are assumed and used to calculate the physical properties of water and obtain the heat transfer coefficients and the heat flow rates. These values are then used to update the tank wall temperatures until convergence between all heat flow rates is achieved. The calculation proceeds with a temperature step of 1 K, starting from the initial tank water temperature T_0 to the final tank temperature T_{f}. This procedure is repeated for all the tank wall surfaces.

Depending on the case, the convective heat transfer coefficients are calculated from heat transfer correlations for natural and forced convection available in the textbook of Cengel and Ghajar (2020). A list of equations used for calculations is given in Table 1.

Table 1. Heat transfer correlations for natural and forced convection.

Convention				Eq.
$Nu = \frac{h \cdot l}{\lambda} \begin{cases} f(Gr, Pr) & - \text{ natural} \\ f(Re, Pr) & - \text{ forced} \end{cases}$	*Nu* – Nusselt number *Gr* – Grashof parameter *Pr* – Prandtl parameter	*Re* – Reynolds parameter *l* – characteristic length (m) *h* – heat transfer coefficient (W/m^2K)		(5)
a) $Gr = \frac{g \cdot l^3}{(\mu/\rho)^2} \cdot \frac{\rho - \rho_\infty}{\rho}$, b) $Re = \frac{w \cdot l \cdot \rho}{\mu}$, c) $Pr = \frac{\mu \cdot c}{\lambda}$	*g* – gravitational acceleration ($9.81\ m/s^2$) *w* – fluid flow velocity (m/s) μ – dynamic viscosity (Pa·s) *c* – specific heat capacity (J/kgK) ρ_∞ – fluid density at T_∞ (kg/m^3)	ρ – density (kg/m^3) Evaluated at T_f (film temperature) $= 0.5\ (T_w + T_\infty)$ T_w – wall temperature T_∞ – temperature of undisturbed flow		(6)
Natural convection			Parameter range	
External, vertical surface	$Nu = \left\{0.825 + \frac{0.387 \cdot Ra^{1/6}}{[1 + (0.492/Pr)^{9/16}]^{8/27}}\right\}^2$		$10^4 \leq Ra < 10^{13}$ $Ra = Gr \cdot Pr$	(7)
External, horizontal surface: - Upper surface of a hot plate or lower of a cold plate	$Nu = 0.54 \cdot Ra^{1/4}$ $Nu = 0.15 \cdot Ra^{1/3}$		$10^4 \leq Ra < 10^7$ $10^7 \leq Ra < 10^{11}$	(8.1) (8.2)
- Lower surface of a hot plate or upper of a cold plate	$Nu = 0.27 \cdot Ra^{1/4}$		$10^5 \leq Ra < 10^{11}$	(8.3)
Internal, vertical rectangular enclosure (double hull side tank)	$Nu = 0.18 \left(\frac{Pr}{0.2 + Pr} Ra\right)^{0.29}$		$1 \leq H/B < 2$	(9.1)
	$Nu = 0.22 \left(\frac{Pr}{0.2 + Pr} Ra\right)^{0.28} \left(\frac{H}{L}\right)^{-0.25}$		$2 \leq H/B \leq 10$	(9.2)
Internal, horizontal rectangular enclosure (double bottom ballast tank)	$Nu = 1 + 1.44 \left(1 - \frac{1708}{Ra}\right) + \left(\frac{Ra^{1/3}}{18} - 1\right)$		$Ra < 10^8$	(10)
External, horizontal tube	$Nu = \left\{0.6 + \frac{0.387 \cdot Ra^{1/6}}{[1 + (0.559/Pr)^{9/16}]^{8/27}}\right\}^2$		$Ra < 10^{12}$	(11)
Forced convection				
Laminar flow, flat plate	$Nu = 0.664 \cdot Re^{1/2} \cdot Pr^{1/3}$		$Re < 5 \cdot 10^5$	(12.1)
Turbulent flow, flat plate	$Nu = 0.037 \cdot Re^{4/5} \cdot Pr^{1/3}$		$5 \cdot 10^5 < Re < 10^7$	(12.2)

Model ships type I and II

Two typical ship designs often used for transport in the Adriatic Sea were considered for the analysis, i.e. ship *type I* with only a horizontal tank and ship *type II* with horizontal and vertical side tanks (Figure S2).

The principal dimensions of horizontal tanks are width (D), length (L) and distance between inner and outer shell (H_1), and for vertical tanks are the height of the water column (H_2), the height of air fill (H_3) and distance between inner and outer shell (B_1). The heat transfer at the surfaces of the tanks is determined from the overall heat transfer coefficients (Eq. 2–3), and convective heat transfer coefficients are evaluated from Nusselt number correlations reported in Table 1. For instance, the heat transfer coefficient at the inner shell of the double-bottom ballast tank (U_8) is determined from the heat transfer coefficient for external natural convection on the upper surface of a hot plate (Eq. 8.1 or 8.2) and the heat transfer coefficient for internal natural convection. In Table 1, we also listed a series of assumptions of ships' geometry. The ballast tank geometry was assumed from the Technical specification for the RO-PAX ship, courtesy of Uljanik Shipyard (Pula, Croatia) (2015).

Heating of ballast waters for model ships

In this step, we analysed the heating of ballast waters for ships *type I* and *II* in conditions typical for the Mediterranean Sea. In the context of heating ballast waters, we considered the worst-case scenario the one in the winter (sea and air temperatures of 15 °C and 10 °C respectively) and the best one in the summer (25 °C for both the water and the air). The most likely scenario was the one in the autumn (20 °C for the water and 15 °C for the air) because then *M. leidyi* shows its maximum proliferation in the Adriatic and has the greatest chance of entering the ballast tanks. For each scenario, we analysed the heating capacity (*Q*) and heating energy consumption (*E*) of the ballast waters to achieve a thermal (at 45 °C and 50 °C) and a combined thermal and deoxygenation treatment (at 35 °C and 45 °C). For all calculations, we assumed the cargo space to be empty (filled with air) and the total ballast volume to be 288 m^3 for ship *type I* and 648 m^3 in *type II*, according to the specifications given in Table 1.

Required durations and travel lengths

In the final step, we calculated the total treatment times for the ballast waters by summing the times necessary to heat the ballast waters to the treatment temperatures and the maximum survival times of *M. leidyi* specimens at those temperatures. The calculations were done for the thermal (TT) and combined deoxygenation treatments (DT). Based on the obtained times in the various case scenarios, by multiplying them by the assumed speed of the vessels, we obtained the minimum distances that would be required for the treatments to be effective. We explored different case scenarios corresponding to different duration of thermal treatments.

Results and discussion

Mnemiopsis leidyi survival

Until today, several onboard ballast treatments were proposed to remove different organisms. For instance, a study showed that a short exposure (1 min) to heat (< 60 °C) is enough to eliminate < 80% of phytoplankton, crustacean zooplankton, and bacteria, including some fish eggs (Balaji et al. 2017). However, information about *M. leidyi*'s survival is scarce, and information on its eradication is generally lacking. Our results (Table 2) from the ballast tank simulations (NT, IC) set *M. leidyi* survival up to a month. The survival in control simulating ideal conditions (IC) exceeded 30 days, but the longest survivals in comparable conditions recorded in the Aquarium of Pula were in the range between 60 and 90 days (M. Mičić *pers. comm.*), in line with survival times estimated for the much colder (3 °C) Baltic Sea (Javidpour et al. 2020a). Although an older study reports that the maximum age of ballast water containing a living organism was 41 days

Table 2. *Mnemiopsis leidyi* survival thresholds (start of decay, 50% and 100% of specimen decayed) in various treatments (NT – no treatment, IC – ideal conditions, TT – thermal, DT – deoxygenation, UT – ultrasound and MT – microwave treatment).

Specimen decay		Adults			Larvae	Eggs
Treatment		Start	50%	100%	100%	100%
NT	21 °C	5–7 days	11–15 days	17–22 days	[a]	[b]
IC	21 °C, food, air	8–9 days	15–18 days	31–34 days	[a]	[b]
TT	21 °C	5–7 days	10–13 days	20–22 days	[a]	[b]
	30 °C	4–6 days	10–12 days	10–20 days	[a]	[b]
	35 °C	7–10 h	14–16 h	20–22 h	24 h	24–28 h
	40 °C	5–5.5 h	6–7 h	7.5–8 h	8.5 h	11-12 h
	45 °C	1 h	2.5–3 h	4–4.5 h	5.5 h	~ 6.5 h
	50 °C	30 min	~ 70 min	85–90 min	1.5 h	2.5 h
DT	21 °C, 20% O_2	19 h	24–25 h	25–26.5 h	26.5 h	48 h
	30 °C, 20% O_2	3.5–4.5 h	6–6.5 h	8–8.5 h	8.5 h	18 h
	35 °C, 20% O_2	2.5–3 h	3.5–4 h	5.5–6 h	9 h	16 h
	40 °C, 20% O_2	1.5 h	2.5–3 h	4–4.5 h	5 h	8 h
	45 °C, 20% O_2	1.5–2 h	2–2.5 h	2.5–3 h	3 h	5.5 h
	50 °C, 20% O_2	15 min	45–60 min	75 min	75 min	3 h
	21 °C, 0% O_2	5 h	7–7.5 h	8–9 h	9 h	9 h
	30 °C, 0% O_2	3.5–4.5 h	4.5– 5 h	5.5–6.2 h	6.2 h	7.5 h
	35 °C, 0% O_2	3–3.5 h	3.5–4 h	4.2–4.7 h	4.7 h	6 h
	40 °C, 0% O_2	2 h	2.5–3 h	3.2–3.5 h	3.5 h	3.5 h
	45 °C, 0% O_2	1–1.5 h	1.7–2 h	2.2–2.5 h	2.5 h	2.5 h
	50 °C, 0% O_2	15 min	25–30 min	36–45 min	45 min	50 min
UT	21 °C	61 min	81 min	98 min	110 min	147 min
	30 °C	50 min	77 min	85 min	90 min	108 min
	35 °C	25 min	43–46 min	55 min	62 min	77 min
	40 °C	25 min	40–44 min	50 min	50 min	62 min
	45 °C	23 min	25–26 min	30 min	30 min	40 min
	50 °C	11 min	13–14 min	17 min	17 min	37 min
MT	100 W (0.8 ± 0.2 °C/min)[*]	34–39 min (55.9–60 °C)	38–41 min (60–62.3 °C)	40–43 min (60.8–63 °C)	45 min	65 min
	450 W (2.5 ± 0.2 °C/min)[*]	15 min (55.8–58.1 °C)	16–17 min (60.5–64 °C)	16–19 min (60.5– 68.1 °C)	25 min	30 min
	900 W (7.6 ± 0.4 °C/min)[*]	5 min (56–60 °C)	5–6 min (58–72 °C)	6–7 min (67.5–74.3 °C)	7 min	10 min

[a] Rapid hatching of larvae occurs from 12–20 h at 20–31 °C (Baker and Reeve 1974).
[b] It was reported that 13 days are sufficient for the development of reproducing adults from the hatching of the eggs (Baker and Reeve 1974; Kremer and Reeve 1989).
[*] Temperature increases from an initial 21 °C during the microwave treatment are given in brackets.

(Smith et al. 1996), *M. leidyi*'s resistance to low dissolved O_2 concentrations (Decker et al. 2004), the ability to regrow damaged body parts (Bading et al. 2017) and overcome long starvation periods (Javidpour et al. 2020a, b) underlines the importance of its eradication from ballast tanks.

We selected temperatures up to 50 °C because they are commonly applied to study the survival of different planktonic organisms bigger than bacteria which would require temperatures closer to pasteurization for their eradication (Quilez-Badia et al. 2008). In TT, adults' survival was the longest at 21 °C. At 30 °C – specimens survived for approximately 20 days, which is not a sufficiently effective temperature if we consider that the voyages that in the early 1980s transported the first specimens from the western Atlantic to the Black Sea lasted 22 days (Carlton 1996). Monitoring larval and eggs' intact structural integrity up to 30 °C was not applicable because of hatching and maturing. On the other hand, the shortest survival occurred at 50 °C, 1.5 h for adults and larvae and 2.5 h for eggs. A somewhat better thermal resistance > 50 °C was already reported in the literature for fish eggs in comparison to some fully developed zooplankton species, e.g. copepods (Balaji et al. 2017), while crustacean eggs have managed to preserve their viability on > 40 °C for 2 days (Rigby et al. 2004).

Nowadays, combined treatments are also explored to obtain superior results. For instance, a combination of chlorination and ballast water exchange turned out to be very useful in eradicating macroplankton fraction (Paolucci et al. 2015). Here, we chose the thermal effects coupled with deoxygenation because it does not require toxic chemicals and provides additional benefits as an anticorrosion agent (Tamburri et al. 2002). The results (Table 2) indicate that the lack of O_2 (DT) set the decay of adult *M. leidyi* within a day and was ~ 2x as fast during anoxia. Ultrasonication sets the survival within a few h, while microwave exposure even within an h. Overall, above 40 °C, adults' survival was < 10 h, somewhat longer for larvae and 12 h for eggs. Eggs represented the most enduring life stage in all the treatments, but their viability was unaccounted for, defining their decay solely as a loss of structural integrity. Thus, as reported for some techniques (Osman et al. 2016), the inside damage could set the decay earlier.

Of all the treatments, microwave exposure was the most efficient, especially when temperature increased rapidly, eliminating every life stage of *M. leidyi* within an h which is in line with the reported mortality for other zooplankton species exposed to an equivalent amount of heat (Balaji et al. 2017). Earlier studies have also demonstrated the efficient removal of zooplankton cysts and larvae stages by using 5x higher microwave powers (Boldor et al. 2008).

Ultrasonication combined with temperatures > 40 °C gave similar results. And although the literature reports that the best results using ultrasonic cavitation that would limit zooplankton's survival to a few seconds are achieved by using high-powered ultrasonic intensities (Brizzolara et al. 2006), those results typically referred to an experimental set in a smaller batch. An additional concern regarding UT is the long exposure, as it could potentially damage the coating of tank walls (Richards et al. 1990). Moreover, the use of microwaves or ultrasound techniques requires expensive additional onboard equipment available on the market or in a prototype stage but has not been consistently installed and employed yet. In this research, we focus on assessing the applicability of alternative approaches in the thermic treatment of ballast waters that rely on the existing ship infrastructure and energy sources, with the addition of simple, low-tech components such as tubes (Biswas et al. 2018) and additional insulation to the ballast tanks (IACS 2021). The objective of TT would be to reach temperatures of > 40 °C, setting the survival threshold under 10 h. or, if feasible, even at higher temperatures of 50 °C which would ensure the elimination of *M. leidyi* within 2.5 h.

Table 3. Duration of ballast water treatment (TT and DT, anoxia) in variable conditions.

Case scenario	Treatment	Seawater temperature (˚C)	Ambient air temperature (˚C)	Ballast water temperature (˚C)	Treatment duration (h)
1.1 Worse	Temperature	15	10	45	6.5
1.2 Worse	Temperature	15	10	50	2.5
1.3 Worse	Temperature, N_2	15	10	35	6
1.4 Worse	Temperature, N_2	15	10	45	2.5
2.1 Most likely	Temperature	20	15	45	6.5
2.2 Most likely	Temperature	20	15	50	2.5
2.3 Most likely	Temperature, N_2	20	15	35	6
2.4 Most likely	Temperature, N_2	20	15	45	2.5
3.1 Best	Temperature	25	25	45	6.5
3.2 Best	Temperature	25	25	50	2.5
3.3 Best	Temperature, N_2	25	25	35	6
3.4 Best	Temperature, N_2	25	25	45	2.5

Alternatively, the thermic treatment could be complemented with the addition of N_2 in order to deoxygenate the ballast waters and eliminate *M. leidyi* faster (Table 2) or with much higher energy efficiency at lower temperatures. Our data show that releasing untreated ballast waters containing *M. leidyi* within less than 20 days will likely convey live specimens to the receiving waters. In addition, if a portion of water gets exchanged during the voyage, bringing food and O_2 to the ballast tanks, even voyages lasting a couple of months might ensure *M. leidyi*'s survival.

Onboard thermal treatment to eradicate M. leidyi *from ballast waters*

We focused on TT by making use of the engine's waste heat because it is an important concept in the shipping industry as a way to utilize non-renewable energies more efficiently by lowering their consumption rate and obtaining additional benefits (Biswas et al. 2018; Gude 2019). Here, we explore the feasibility of *M. leidyi*'s eradication. In Table 3, different case scenarios corresponding to different duration of thermal treatments are given. We assumed the cargo space to be empty (filled with air) and the total ballast volume to be 288 m^3 for ship *type I* and 648 m^3 for ship *type II* (see Figure S2).

The relationship between the temperature in water tanks, heat transfer coefficients ($U_{2,7,8}$) and heat losses ($\dot{Q}_r$) for the double bottom tank surfaces shows that *U*-values increase with the ballast temperature as a consequence of increasing temperature differences between tank wall and water that intensifies natural buoyancy in the tank (e.g., *type I*/1.2 case, Figure S3). The highest *U* is at the vertical sidewall of the double bottom tank (U_2) – a consequence of forced convection heat transfer at the external seawater side, and the lowest at the inner shell of the tank (U_2), if we assume air-filled cargo space, thus low heat transfer properties. Increases in $\dot{Q}_r$ become faster at higher tank temperatures – a consequence of larger temperature differences and higher *U*-values (Eq. 2), e.g. $\dot{Q}_r$=0.40 MW (30 °C), 0.84 MW (40 °C) and 1.39 MW (50 °C). The minimum heating capacity for thermal treatment should be sufficient to replace heat losses in ballast tanks, $\dot{Q}_{tot} = \dot{Q}_r$, (Eq. 1). When this is applied to, e.g. *type I*/case 1.2 (Figure S4), it demonstrates initially fast heating (23.6 h to reach 49 °C) because of a surplus of heating capacity for the water heating itself ($\dot{Q}_h$) that is followed by heat losses ($\dot{Q}_r$) progressively slowing the process (additional 33 h to 50 °C). Including the time for eradication, the process becomes very long (59.1 h) and requires excessive energy (E) consumption (82.74 MWh). To speed up heating and reduce consumption, we need $\dot{Q}_{tot} > \dot{Q}_r$. In practice, the eradication starts already above 40 °C, and *M. leidyi* could be eliminated within 20 h, which would require 28 MWh.

TT appears to be a promising solution, although the application of low temperature, as tested here, may be limited by the time required for the process (Mesbahi et al. 2007). Thus, we also explored a different approach by limiting the duration of the heating and increasing $\dot{Q}_{tot}$. For instance, a process of 10 h (heating 7.5 h and eradication 2.5 h) would require 2.26 MW to compensate for heat losses (1.39 MW) and speed up the heating. In this case, E consumption (20.43 MWh) is 4x lower than previously discussed. Similarly, it can be calculated for the other cases (Table 3), e.g., the process at 45 °C requires 14% less energy (1.89 MW) but takes 14 h, increasing the total consumption (21.33 MWh) slightly. Indeed, the total consumption depends on the final temperature and speed of the heating, while energy consumption also depends on *M. leidyi* eradication. In Figure 2, $\dot{Q}_{tot}$ and E are given for all the cases and show that $\dot{Q}_{tot}$ increases with the increased tank-seawater temperature difference, while E depends on the eradication time. Besides, combining thermal and deoxygenation treatments would speed eradication, as shown in the combined deoxygenation and thermic treatment experiment and would reduce both $\dot{Q}_{tot}$ and E. Compared to *type I*, $\dot{Q}_{tot}$ and E for ship *type II* are over 2x higher due to the bigger tanks (2.25x) and greater heat losses. The lowest values for *type II* are calculated for case 3.3 ($\dot{Q}_{tot}$ = 1.27 MW, E = 12.57 MWh), and the highest for the 1.2 ($\dot{Q}_{tot}$ = 4.78 MW, E = 42.28 MWh). However, for cases 1.1, 1.2, 2.4 and 3.1, $\dot{Q}_r > \dot{Q}_{tot}$ and requires >2 MW to achieve the desired temperature.

To provide a realistic scenario and verify the feasibility of the proposed treatments, we considered $\dot{Q}_{tot}$ = 2.0 MW accounted from the ship-built specifications: two diesel generator sets, each with an electric capacity of 1368 kW and an emergency diesel generation set (280 kW) (Uljanik Shipyard 2015). Out of the available 3.0 MW, ⅔ could be used for heating and the rest for other energy consumers.

In Figure 3, the duration of treatments and E are compared and show that treatment slows as the heating time (T_h) increases with the tank-seawater temperature difference, e.g. for the ship *type I*, T_h = 9.2 h in case 1.2 and 1.8 h in case 3.3. The consumption increases with T_h, e.g., for the ship *type I*, E=21.8 MWh in case 1.2 but only 5.2 MWh in case 3.3. In all cases for *type I*, the total thermal treatment time is finite, meaning that the fixed 2.0 MW exceeds $\dot{Q}_r$. For *type II*, cases 1.1, 1.2, 2.4 and 3.1 were omitted

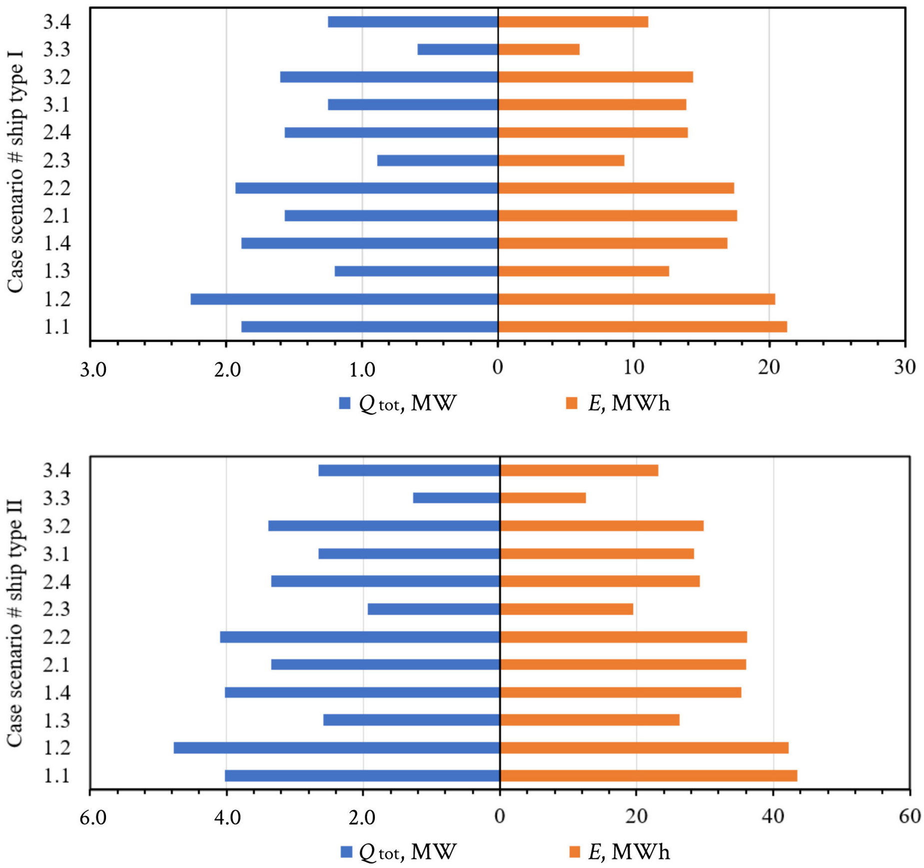

Figure 2. Heating capacity ($\dot{Q}$) and heating energy consumption (E) for ballast water thermal treatment in ship A) *type I* and B) *type II* (maximum heating time 7.5 h).

as unfit considering parameters from Table 3, while the rest shows longer T_h than in *type I*.

To further check for the feasibility of an onboard thermic treatment, we considered that the heat supplied by the engines could be distributed in the form of saturated steam flowing through tubes in the ballast tanks. The estimated tube length is indeed technically feasible (Figure S5).

However, even if both systems can be constructed, the treatments could only be used throughout the year for ship *type I*. The fastest treatment for the worst scenario (case 1.2) that simulates winter conditions can be concluded within 11 h or 9 h if combined with deoxygenation (case 1.4). In the best scenario simulating summer conditions, the eradication can be attained within 9 h (case 3.2) or by 7 h if combined with deoxygenation (case 3.4). Moreover, in the autumn (or spring), the fastest eradication can be achieved within 9.5 h (case 2.2) and within 8 h when combined with N_2 (case 2.4). Overall, combined treatments reduce treatments' duration from 16 to 25%. In addition, the thermal treatments are more energy-consuming than those combined (Figure 3) as the higher temperatures shorten *M. leidyi*'s survival (Table 2). The most energetically efficient approach and the 2nd fastest is the combined treatment at 35 °C (case 2.3).

The calculated treatment times, in combination with the average cruising speed of 15 kn (Wang et al. 2007), help us estimate minimum voyage lengths

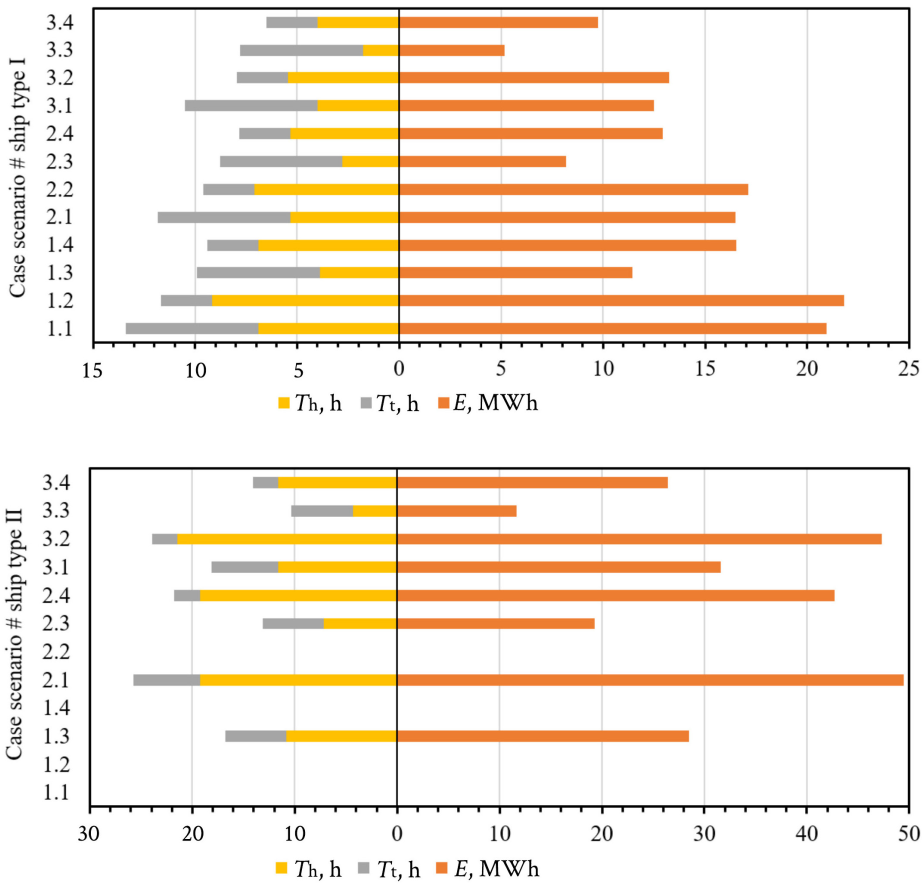

Figure 3. Thermal treatment time; T_h – ballast heating up time, T_t – *M. leidyi*'s eradication, and heating energy consumption (E) for A) *type I* and B) *type II* (2.0 MW fixed heating capacity).

where proposed treatments could be applied. For thermal treatments, the worst scenario (case 1.2) requires a distance of 318 km, and the best case is 208 km (3.2), while the combined treatment is 249 km (case 1.4), while for the best case, it would be 180 km (case 3.4). For the most likely scenarios, travelled distances should be between 222 km (case 2.4) and 263 km (case 2.2). Our results show that the proposed treatments could be applied on long-range oceanic cruises and numerous medium-range voyages, such as the ones within the Mediterranean and other epicontinental basins. Because the proposed treatments would take too long for short routes, the treatment could start earlier while the ship is docked or by prolonging the travelling. Alternatively, other treatments, such as ultrasonication or microwave radiation (Table 2), should be considered. However, when considering voyages outside of the Mediterranean basin, it needs to be considered that TT may be less effective for ships traversing areas where ocean temperatures are very low (< 15 °C) as this could increase the heating time or reduce the final temperature in ballast tanks (Rigby et al. 1999). Moreover, considering that *M. leidyi's* blooms spread by natural forcing (Paliaga et al. 2021) and cover an overwhelmingly large area, e.g. the whole northern Adriatic (~ 22–35 · 10^3 km^2) (Budiša et al. 2021), we would like to point out to the ineffectiveness of treatments for close-range voyages. Within the Adriatic, the proposed onboard treatments might be more useful for longer distances, e.g. connecting Port of Venice and Ploče (~ 500 km) and preventing so *M. leidyi* from settling in another enclosed and likely eutrophic area.

Conclusions

We demonstrated a preliminary attempt to evaluate the efficiency of different ballast water treatments to eradicate all life stages of the invasive comb jelly *M. leidyi*. Our laboratory experiments combined with technical estimates indicate several ways to eradicate this invasive ctenophore and reduce its global spreading. We proposed different treatments applied to two ship types travelling different distances to ensure that there would be no viable specimens. For ship *type I*, the thermal and deoxygenation treatments were successful in all seasons, while for ship *type II*, the effectiveness was limited and, therefore, new approaches should be considered (e.g. ultrasonication, microwave radiation). This study can prove useful to management efforts to prevent the adverse effects of introducing this ctenophore to new habitats. However, further research on combined techniques using thermal control and other physical treatments is needed to provide a more concrete management framework for *M. leidyi*'s eradication and successful control of its spread.

Acknowledgements

We are grateful to the Aquarium Verudela from Pula (Croatia) for cultivating and supplying *Artemia salina* larvae. We thank Josip Dujmović and Virna Dalino Polo for their assistance in the calculations. We would also like to extend our thanks to the reviewers for their suggestions and overall improvement of the manuscript. Finally, we would like to acknowledge the financial support of the project "Monitoring of the state of transitional and coastal waters in the northern Adriatic".

Authors' contribution

A.B.: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, roles/writing – original draft; E.B.: sample design and methodology, investigation and data collection, data analysis and interpretation, roles/writing – original draft; P.B.: sample design and methodology, data analysis and interpretation, roles/writing – original draft; T.M.: sample design and methodology, data analysis and interpretation, roles/writing – review and editing; G.M.: sample design and methodology, roles/writing – review and editing; N.I.: roles/writing – review and editing; T.D.: roles/writing – review and editing; M.N.: sample design and methodology, roles/writing – review and editing; M.M.: sample design and methodology, roles/writing – review and editing; P.P.: research conceptualization, sample design and methodology, investigation and data collection, data analysis and interpretation, roles/writing – original draft.

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Supplementary material

The following supplementary material is available for this article:

Figure S1. Life stages of ctenophore *M. leidyi*.
Figure S2. Simplified cross-sections of ships *type I* and *II*.
Figure S3. Heat transfer coefficients and heat losses for ship *type I*.
Figure S4. Heating process for ship *type I*.
Figure S5. Steam tube length in ship *types I* and *II*.

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