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Effects of temperature and salinity on the invasive *Mnemiopsis leidyi* (Ctenophora) in the Venice lagoon

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1 INTRODUCTION

1.1 Invasive species and *Mnemiopsis leidyi* origins

A biological invasion is a human-made process in which a species is introduced into a novel geographic region or environment either accidentally or deliberately, in which the species proliferates and persists **(Ricciardi, 2012).** These species are referred to as alien, exotic, and non-native when referred to outside of the area their native habitat **(Colautti, 2004).** Usually, these species are not able to thrive in their new environmental conditions and if they are successful in establishing a new population, this population is constrained to a fine geographic region **(Ricciardi, 2012).** In the occurrence that a biological invasion leads to a well-established population that continues to spread aggressively geographically and/or has a strong effect on the environment the species is referred to as invasive **(Keller et al., 2011).** In the recent century the ctenophore *Mnemiopsis leidyi* Agassiz, 1865 has joined this list of the 100 worst invasive species (IUCN) and has continued its aggressive spreading throughout European and Mediterranean waters causing negative environmental and economic impacts to is new geographical habitats (Fig. 1) (**Shiganova, 1998; Costello et al., 2012, Lowe et al., 2000)**.

Figure 1 the picture shows two Mnemiopsis leidyi captured from the southern Venice lagoon

M. leidyi is native to the temperate western parts of the Atlantic Ocean having a high latitudinal spread including the eastern coast of North America and Mexico and the southeastern part of South America including Brazil, Uruguay and Argentina **(Costello et al., 2012)**. This ctenophore species thrives in the offshore coastal waters

of the above-mentioned areas and has a limited dispersal to the surrounding areas due to wind currents, bottom topography, temperature and salinity **(Lehtiniemi et al., 2012, Harraldsson et al., 2013).**

First introduced in the early 1980s in the Black Sea, *M. leidyi* was unintentionally transferred in the ballast waters of transatlantic fishing vessels **(Shiganova, 1998; Costello et al., 2012)**. Ballast waters are waters that ships intake from the leaving port as stabilization against wind, waves and overall buoyancy conditions when transporting and unloading cargo **(Shiganova, 1998)**. This intake of ballast water could have come from a number of ports along the coast of northern and southern Americas where *M. leidyi* naturally occurs. When the ship returned to the Black Sea to load more cargo it would empty the ballast water tanks into the surrounding environment releasing *M. leidyi* too **(Shiganova, 1998)**. A study has confirmed due to genetic similarities that the invasive Eurasian ctenophore populations originated firstly from the Gulf of Mexico specifically the Tampa Bay region and spread to the Black Sea first followed by the Caspian Sea **(Ghabooli et al., 2010)**. The second invasion arose from the northern native distribution range of *M. leidyi* (Narragansett Bay) to the Baltic Sea **(Ghabooli et al., 2010)**. However, the high genetic diversity of the invasive populations suggests there was either a large primary inoculation group into the new geographical range or multiple introductions of these ctenophores into the novel area **(Ghabooli et al., 2010).**

After the first introduction into the Black Sea there was a rapid population explosion (300 individuals per cubic meter) observed in the summer months however, during the winter months the numbers of ctenophores decreased dramatically (4 individuals per cubic meter), prompting the question if cold temperature was a limiting factor to their survival and invasiveness **(Kube et al., 2007)**. A study performed during the second introduction of ctenophores into the Baltic Sea showed that the majority of individuals sampled between January and May 2007 were juveniles less than 1mm in total body length and although the number of ctenophores drop to <1 individual per cubic meter, they were able to survive the cold temperatures of Baltic Sea by staying well below the halocline level in stratified Baltic Sea specifically the southwestern and central regions **(Kube et al., 2007)**. However, the question still persists in shallower water climates such as the Venice lagoon with maximum depth 21.5 meters and average depth 10.5m where escape below the halocline level (25-100m) is not possible, if temperature or salinity can be a limiting dispersal factor for these invasive ctenophores or if reintroduction through trans-Atlantic ballast waters is paramount to a successful stable introduction into the European waters **(Costello et al., 2012, Amos et al., 2017, Zirino et al., 2014).**

From the Black Sea the ctenophore population then spread to the Sea of Marmara and following that it expanded into the whole Mediterranean Sea in the 1990s specifically through Aegean Sea **(Costello et al., 2012).** Today *M. leidyi* can be found throughout the coastal waters of Europe including the Netherlands, Spain, France and Italy with a well-established population in the Venice Lagoon (**Costello et al., 2012; Piccardi et al., 2024).**

1.2 Life history, biological traits and invasive capabilities

M. leidyi is an holoplanktonic species that spends most of its life floating and drifting with the currents throughout the upper layer of the water column above the halocline (10-20M) **(Main, 1928; Haraldsson et al., 2013).** *M. leidyi* mainly preys on planktonic species, larvae and eggs that tend to be distributed in the upper layer of the water column due to the light availability **(Budiša et al., 2021)**. Due to the tendency of *M. leidyi* to remain in the upper layer of the water column these ctenophores can be an easy target to be transported in the ballast water of international transport ships **(Shiganova, 1998)**. Even a few individuals of *M. leidyi* or parts of an individuals have the ability to establish a new population in a novel environment **(Finenko et al, 2006)**. *M. leidyi* can regrow from fragments larger than a quarter of the individual's size which usually ranges from 7-12cm, so even a small fragment has a potential to regrow into a full individual and can then become reproductively active and proceed to populate a new area **(Doyle, 1984)**. This possibility of a few individuals to establish a new population is due to being a self-fertilizing hermaphroditic species **(Shiganova et al., 2001).** For self-fertilization sperm are released into the water column followed by eggs which are then fertilized (**Sasson et al., 2018).**

M. leidyi has three distinctive life stages presenting different diets and prey capture mechanisms **(Sullivan and Gifford, 2004).** The tentaculate stage, referred to as cydippid larvae, (larval stage) occurs when the animal is hatched until it reaches about 4mm; known as the tentaculate stage because the animal feeds by dragging its tentacles passively behind it; when prey is encountered the tentacles are retracted towards the body and the prey is ushered into the mouth **(Sullivan and Gifford, 2004).** Following the tentaculate stage there is a transitional stage usually ranging from size of 4mm-6mm in body length in which *M. leidyi* still possesses its tentacles for feeding but also begins to develop small oral lobes that will eventually transition into the primary feeding strategy of this animal. During this time the tentacles are slowly reabsorbed by the body while the oral lobes increase in size and by 6mm the tentacles have been completely reabsorbed leaving only the oral lobes **(Sullivan and Gifford, 2004).** Following the transition stage there is the adult stage during which auricles

begin to develop and *M. leidyi* moves to a more active feeding approach propelling through the water. Encountered prey becomes trapped in a mucosal lining on the feeding lobes in a structure called the labial folds and then cilia move the prey towards the mouth. At 12-14mm the animal is completely developed with fully formed auricles, oral lobes, and cilia (**Sullivan and Gifford, 2004).** *M. leidyi* will rarely allow something other than prey into its labial folds unless the food is attached to the dirt; when dirt is trapped by the lobes it is caught up in mucus and then expelled behind the animal **(Finenko et al., 2006)**. When too much dirt is present, *M. leidyi* will refuse to uptake new food and will produce excess mucus as well as perform a full body contraction to expel the foreign and non-food particles out behind the organism **(Main, 1928; Sullivan and Gifford, 2004).** It is at this stage that *M. leidyi* reaches sexual maturity and can begin reproduction (**Sullivan and Gifford, 2004)**.

As a simultaneous self-fertilizing hermaphroditic species *M. leidyi* is able to successfully establish entirely new populations from just a small number of individuals due to the high fecundity rate **(Jaspers et al., 2015, Shiganova et al., 2001)**. With optimal environmental conditions, *M. leidyi* is capable of reproducing with over 13,500 eggs per day per individual and able to reproduce daily, meaning in the span of a week there can be over 80,000 new eggs released into the water column by a single individual **(Sasson and Ryan, 2016; Malej et al., 2017).** Larvae can hatch within 48 hours and reach sexual maturity in optimal conditions in about 4 weeks **(Sasson and Ryan, 2016, Ramon-Mateu et al. 2022)**. There is also evidence that suggests that *M. leidyi* is capable of releasing gametes during what is thought to be the juvenile stage of development at about 13 days termed "dissogeny" decreasing the time from hatching to being able to release more gametes into the water column by half **(Ramon-Mateu et al. 2022, Sasson and Ryan, 2016).**

Previous research has linked *M. leidyi* spawning to the availability of light in the environment, potentially controlling at what time of the year these organisms are reproductively active based on light availability **(Lehtiniemi et al., 2012).** A study focused on starvation and reproduction rates showed data suggesting that *M. leidyi* produced the most gametes in the overnight and dark period rather than during the day **(Jaspers et al., 2015).** The most current findings of *M. leidyi* has shown that they possess light exposure cues for spawning **(Sasson and Ryan, 2016)**. After several hours in darkness, they will spawn when introduced to a light source **(Jaspers et al., 2015)**. It has been previously speculated that during summer months there may be an increase in the amount of spawning compared to the winter months due to the duration of sunlight throughout the day **(Sasson and Ryan, 2016).** In the winter months there may be a potential decrease in reproduction rates due to the shorter days and less sunlight cues for the *M. leidyi* to reproduce **(Sasson and Ryan, 2016).**

Reproduction rates are also highly influenced by temperature conditions and food resources **(Shiganova, 2020, Piccardi et al., 2024).** When temperature is low and food become scarce *M. leidyi* are capable of decreasing body size to withstand colder climates and food scarcity **(Shiganova, 2020).** The size of these ctenophores is a large predictor of their reproductive capabilities as well as the temperature and salinity of the environment they have adapted to, higher salinities are associated with a smaller length of the individual compared to lower salinities however when water salinity exceeds 40 PSU their length begins to decrease **(Shiganova, 2020).** A case study in the Venice lagoon has shown, that the size of the ctenophore's blooms in the following year is highly correlated to the previous year's temperature **(Piccardi et al., 2024).**

When comparing self-fertilization of *M. leidyi* to that of paired fertilization there is no difference **(Jaspers et al., 2011)**. Paired fertilization offered no more gametes produced than when the individuals were left to reproduce in tanks alone. When pair fertilization occurs only one of the two individuals releases eggs and the other releases sperm rather than both individuals releasing both sperm and eggs **(Finenko et al., 2006; Jaspers, Møller & Kiørboe, 2011; Sasson and Ryan 2016)**. When *M. leidyi* were transported accidentally to new waters with smaller breeding groups compared to their native areas this may have caused an inbreeding depression that may have had a large impact on the population genomics of these newly inhabited areas **(Sasson and Ryan, 2016).** However, *M. leidyi* have been shown to self-reproduce for multiple generations with very little genetic variations that would imply a large inbreeding coefficient **(Shiganova, 2020).**

Primarily known as a predatory species there is speculation into whether *M. leidyi* is specifically carnivorous or can also be herbivorous **(Jaspers et al., 2015)**. Although is it possible for the species to survive being fed only with phytoplankton this species exhibits larger size and growth rate when fed with microzooplankton **(Rapoza, Novak, and Costello, 2005).** These ctenophores feed on the larvae of several species including Echinodermata, copepods, Bivalvia and Cladocera **(BUDIŠA et al., 2021).** Feeding on these species can have severe impacts higher up the trophic levels, out competing other species such as anchovies for the microplankton and larvae available in the marine environment **(BUDIŠA et al., 2021).** The impact on anchovies can have a bottom-up effect with larger species including other types of larger commercial fish all the way to dolphins and other cetaceans that rely on anchovies as a source of food **(Shiganova et al., 2001).** With high fecundity rates, fast life cycles and fast maturation rates *M. leidyi* is incredibly effective in consuming entire populations of planktonic species and commercial larvae such as oysters and clams **(Jaspers et al., 2015).**

M. leidyi has few natural predators in its invaded regions with its main predator being jellyfish **(Costello et al., 2012).** The specific species that is able predate and eat *M. leidyi* is the jellyfish *Chrysaora quinquecirrha* **(Purcell and Cowan, 1995).** In addition to *C. quinquecirrha* a ctenophore species named *Beroe ovata* is an exclusive predator to *M. leidyi* **(Volovik and Korpakova, 2004; Alekseenko et al., 2018; Shiganova et al., 2003).** In a case study on the Sea of Azov, fisheries were showing noticeable decline in yields of herring and grey mullet following the introduction of *M. leidyi* **(Volovik and Korpakova, 2004).** As a potential solution for the rampant invasion of *M. leidyi* in a case study of the introduction in the Black Sea of *B. ovata* there was observed a significant recovery of the pelagic food chain **(Alekseenko et al., 2018; Volovik and Korpakova, 2004).** Following experimental evaluations of the salinity and temperature tolerances of *B. ovata* there was speculation on whether *B. ovata* should be introduced to the nearby Caspian Sea to remedy the infestation of *M. leidyi* there and help restore the pelagic food chain **(Volovik and Korpakova, 2004).** In the case study of the Black Sea introduction of *B. ovata* there were few observed negative pelagic and ecosystem side effects and it was then proposed that *B. ovata* be introduced to the Caspian Sea which contains similar water dynamics to the Sea of Azov as a remedy to the infestation of *M. leidyi* **(Alekseenko et al., 2018; Volovik and Korpakova, 2004).** With few naturally occurring predators of *M. leidyi* if the primary reproductive period after introduction to a new environment is successful then a new population of these ctenophores can be established quickly with very little predatory limiting factors to its growing population, environmental factors instead are the largest limiting factors, specifically temperature and salinity, to the distribution into novel areas **(Purcell and Cowan, 1995)**. Even the natural predators to *M. leidyi* like *B. ovata* are heavily influenced by the temperature and salinities conditions in which they are introduced **(Volovik and Korpakova, 2004).**

There has been previous speculation that there are chemical agents that can play a role in the survival of *M. leidyi* **(Bilio and Niermann, 2004).** Offshore oil leaks and chemical dispersant leaks into the environment have raised the question of if *M. leidyi* can withstand exposure. In a case study on the effects of Corexit® 9500A chemical dispersant, crude oil (WAF), and dispersed crude oil (CEWAF) there were lethal and sublethal effects observed in *M. leidyi* **(Peiffer and Cohan, 2015).** Although these chemicals have the ability to diminish their respiratory and reproductive success and therefore to eradicate *M.* leidyi, there has been very little research done on the impacts to other species and the ecological impact to the entire Mediterranean ecosystem when involving chemical agents **(Bilio and Niermann, 2004)**. The use of these agents could lead to wipe outs of entire sections of the food web and working ecosystem that the habitat itself cannot recover from **(Chang et al., 2014)**. For this reason, there needs to be further research into how these chemical agents can impact

not only the survival and invasive capabilities of *M. leidyi* but all the organisms directly surrounding *M. leidyi* in the food web and the overall environment in the Mediterranean, Black, Baltic and North Seas **(Bilio and Niermann, 2004).**

1.3 The Venice Lagoon

The Venice Lagoon is situated between the land and the Adriatic Sea in northeastern Italy and offers a complex ecosystem unique to this specific location **(Ravera, 2000).** There are 3 inlets into the Venice lagoon that connect the lagoon to the sea: Chioggia, the southernmost inlet, Lido in the northernmost section and Malamocco in the middle **(Zirino et al., 2014).** With an average depth throughout the shallow parts of the lagoon of one meter it is highly subjected to temperature and salinity variations following the seasons, tidal shifts and rainfall events **(Ravera, 2000; Zirino et al., 2014).** In the northernmost section of the lagoon especially in very shallow areas, salinity is highly influenced by the amount of river runoff and has a lower salinity than the central and southern regions **(Zirino et al., 2014).** The tidal areas of the Venice lagoon that are more inland have less salinity in the water due to the fresh water runoff from the land, this salinity gradient increases with depth of the canals as the Adriatic Sea is much more saline that these brackish rivers **(Zirino et al., 2014).**

The Venice lagoon plays a vital role in the clamming economy of the northern Adriatic Sea **(Rossetto, 2000).** The Venice lagoon is the widest lagoon system in the entire Mediterranean and the estuary waters utilized by many local fish species as a nursing ground, and the mud flats are home to *Ruditapes philippinarum* an invasive clam **(Rossetto, 2000; Silvestri et al., 2006).** With the Northern Adriatic Sea accounting for one third of the Mediterranean's fishing production this region has high socio-economic value to the surround region and the continent as a whole **(Rossetto, 2000).** The main commercial species fished in the lagoon in order of revenue generated are: Boyer's sand smelt €1.01 million; cuttlefish €500,000; crabs €500,000; shrimps €300,000; Flounder €100,000; and eels €81,000 with more revenue generated from artisanal fishing **(Rossetto, 2000).** When tides are low locals use the mud flats for collection of *T. philippinarum* that now with improved capture methods is a commercially successful venture **(Silvestri et al., 2006).**

The Water Framework Directive has divided the lagoon into 14 different geospatial regions and 4 different major habitats **(Directive, 2000; Rossetto, 2000).** These habitats include tidal flats, seagrass meadows, manila clam banks and macroalgal beds **(Directive, 2000; Rossetto, 2000).** Turbidity and water depth play a significant role in the distinction of these habitats ranging from 0.1m-25m in depth

and 10-30FNU for turbidity **(Rossetto, 2000).** *M. leidyi* has been shown to be greatly affected by turbid waters with higher turbidity showing a reduction in feeding **(Main, 1928; Sullivan and Gifford, 2004).** This prompts the question of where *M. leidyi* can be found in the Venice lagoon based on water turbidity, salinity, temperature and oxygen concentration and if changes in these values are associated with the presence/lack of *M. leidyi* in the water **(Main, 1928; Sullivan and Gifford, 2004).**

1.4 Impacts of *Mnemiopsis leidyi* in the Venice Lagoon

M. leidyi blooms were first recorded in the Venice lagoon in 2005 and very little knowledge of their impacts on the local artisanal small-scale fishery is available **(Piccardi et al., 2024; Malej et al., 2017).** Previous studies in other invaded areas have shown that *M. leidyi* affects the anchovy population as it competes for planktonic food source **(Budiša et al., 2021)**, further moving up the chain to cetaceans, pinnipeds and large fish that rely on the smaller animals such as the anchovy population as their primary food source **(Budiša et al., 2021).** *M. leidyi* also has impacts on the oyster and clam populations in these areas. *M. leidyi* cannot hold onto actively escaping prey such as copepods, it will release them in mucus before suffering damage to its lobes **(Budiša et al., 2021)**. However, oyster and clam larvae do not actively try to escape from the ctenophore instead some close up within their shell making them an easier target for *M. leidyi* to propel toward its oral opening **(Sullivan and Gifford, 2004)**. With the clam species of *R. philippinarum* being a highly sought commercial catch in the Venice lagoon it begs the question of whether the *M. leidyi* invasion will have a negative effect on the catch rate.

Following the first blooms of *M. leidyi* a decline in fishery production was observed in the Southern Lagoon of Venice. Nets of local fishers were filled more than 50% volume with the invasive ctenophore and even sometimes completely filled with *M. leidyi.* This study also showed how the previous year's temperature was a strong indication of the size of the ctenophore bloom for the following year. With a decrease in fishing landing there can also be a predicted bottom-up effect in the economic sector for these local fisheries. Swarms of ctenophores will crowd the nets and reduce the space available for the target fish species. This in turns causes less yield per catch and longer fishing expeditions to reach the same quotas. In this way the *M. leidyi* population in the Venice lagoon directly impacts the fishing communities in the surrounding areas **(Piccardi et al., 2024)**. This not only effects how much the fisher is able to sell to market but in turn will also affect the availability to consumers for purchase **(Alberini et al., 2007)**. The dwindling availability for consumers will in turn affect price of the sea food and will further cause economic backlash to the region.

What was once a readily available and cost effect protein source will become a delicacy due to the difficulties of net trawling in a lagoon full of ctenophores **(Silvestri et al., 2006)**.

The economic and ecological impacts of *M. leidyi* are not only limited to current fishing expeditions but in the further eradication attempts of the ctenophores **(Monti et al., 2021)**. Current options to solve the *M. leidyi* invasion are either to introduce a new predator of the species such as *B. ovata* which may eradicate *M. leidyi,* introduce oil or chemical surfactants, or manipulate the salinity and temperature of the Venice lagoon, all of these options may come with other ecological downfalls not yet observed **(Diciotti et al., 2016).** In the case of *B. ovata* as a potential solution to eradicate the *M. leidyi* population there are further limitations to viability due to the salinity and temperature ranges present in the Venice lagoon **(Volovik and Korpakova, 2004).** *B. ovata* has optimal temperature and salinity conditions that are similar to that of the Sea of Azov and the Caspian Sea which are remarkably colder than parts of the Venetian lagoon **(Volovik and Korpakova, 2004).**

1.5 Global warming and salinity effects on the invasiveness of *Mnemiopsis leidyi*

Temperature and salinity have been shown as a high indicator of the reproductive success of *M.* leidyi **(Haraldsson et al., 2013; Piccardi et al., 2024).** Originally found in the warmer regions of the easter side of the Americas, cold water may be a limiting factor in the dispersal areas of other major bodies of water **(Volovik and Korpakova, 2004).**

Due to global warming, there has been a significant increase of Sea Surface Temperature (SST) of 1.75°C/decade in the Venice lagoon **(Amos et al., 2017).** In addition to an increase in temperature melting ice caps are causing a global water level increase predicted to be between 17-53cm in the Venice lagoon by the year 2100 **(Carbognin et al., 2010).**

Previous studies have showed higher survival for *M. leidyi* in conditions with higher temperature and higher salinity **(Haradlsson et al., 2013).** With the Venice lagoon showing trends of higher temperature and higher salinity there is a need for more research on if these invasive ctenophores will thrive in these changing conditions.

2 Aims for this study

As an invasive species to the Venice Lagoon *M. leidyi* raises concerns about the future of the health and stability of the pelagic food chain in the area. The destruction of the pelagic food chain could have a bottom-up effect that is costly to the fisherman and communities that rely on the Venice Lagoon. With global warming increasing the temperature of the Oceans and Seas there is a need to observe how this invasive species will tolerate these environmental changes.

The aims of this study are, firstly, to investigate the presences of *M. leidyi* in the lagoon in areas at different temperatures and salinity by sampling locations in the northern, central and southern lagoon. The second aim was to investigate the tolerances of *M. leidyi* to different salinity and temperatures in an experimental environment to determine the duration of their survival under those conditions.

The first aim was accomplished by taking water parameters (temperature, salinity, and dissolved oxygen) of the northern, central and southern lagoon at varying depths over a period of the year from spring to winter months, and monitoring abundance of *M. leidyi* to highlight any correlation between species abundance and temperature and salinity conditions.

For the second aim, we captured individuals of *M. leidyi* and relocated them to experimental tanks under different temperature and salinity treatments. Their survival was then monitored in the different environmental conditions.

Based on the evidence in this study we can potentially predict how *M. leidyi* will respond to the environmental changes brought on by global warming and the potential repercussions for the environment based on their presence as well as the economic effect on local fisheries.

3 MATERIALS AND METHODS

3.1 Collection Site

Samplings were carried out at 3 different sites in the lagoon: northern, central and southern (Fig. 2). In each sampling site 15 transects were selected (Fig. 3 labeled A to O). Transect A was the most inland and while O was located closest to the sea. Each transect had a start and an end labeled 1 and 2 on the maps; this corresponding to the 5 min bongo net trawling (Fig. 3). The different sampling transects had an average distance of 200m among them. The mapping of the coordinates into google earth allowed for the boat to return to the same sample collection sites every month (May-September).

At the start and at the end of each transect (checked using the boat navigation system to the coordinates mapped on google earth), environmental parameters (temperature (°C), oxygen saturation in percentage and dissolved oxygen, salinity PSU) were taken using a multiparameter probe "HANNA". Depth was recorded using the depth gauge in the console of the boat and recorded in meters, additionally using the flowmeter present in the mouth of the bongo net the spins were also recorded. Then, the data taken at the start and end of the collection trawl was averaged to infer the water parameters and depth throughout the transect. In addition, if caught, the number of ctenophores for each trawling period was also recorded.

Upon arrival back to the hydrobiological station lab, all the caught ctenophores were pooled together and a random sample of 100 *M. leidyi* was taken from the overall collection and measured for length and width of the animals. If the samplings were less than 100 animals, then all lengths and widths were recorded. Animals were then killed using a UV Lamp Filtriacquashop 6 Watt (2 L/min) and discarded. This produced 616 individuals measured from the field in total, the lengths and widths of these individuals were plotted correlations among measures calculated. The measures highly correlated ($r=0.942$, $P<0.05$, $N=616$) therefore only width was used as indicator of ctenophore size.

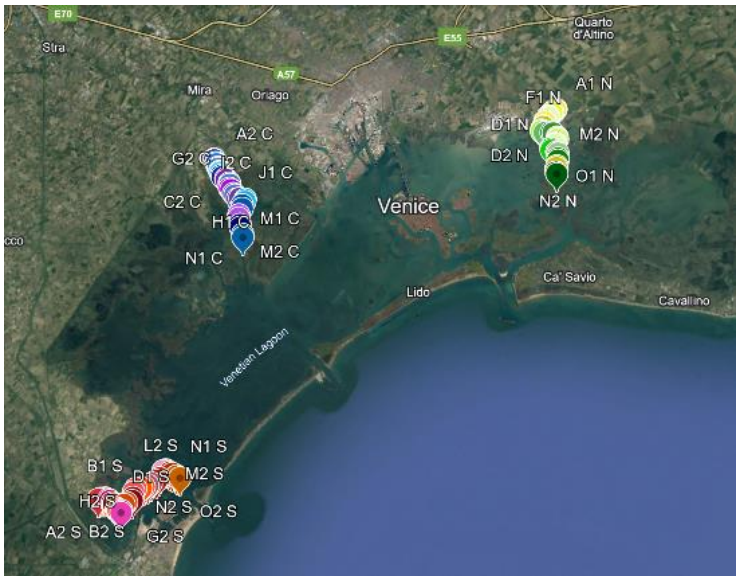

Figure 2 Picture created using Google Earth of the three sampling sites in the northern (green/yellow), central (blue/purple) and southern (pink/orange) sectors of the Venice lagoon.

Figure 3 (left) The 15 transects (A-O) of the northern lagoon, (center) The 15 transects (A-O) of the central lagoon, (right) The 15 transects (A1-O) of the southern lagoon. A blue and yellow star indicates the position of the Hydrobiological station of the University of Padua where the experiments were conducted.

3.2 Bongo Net

M. leidyi were captured along the transects using a dual Bongo net with an 18cm diameter opening and 30cm length plastic compartment followed by two attached nets, one collection net with the mesh size of 200µm the other mesh net size of 300µm. At the end of the cone shaped mesh nets is a secondary plastic container that is clasped onto the net with metal screws and washers that can be detached to observe the organisms caught. The plastic containers had two buoys attached to keep the net horizontal and suspended. To keep the bongo net suspended in the upper 1 meter of the water surface it was fixed to a wooden pole attached to the bow of the boat (Fig. 4). After the net was positioned, the samplings were conducted with the boat moving backwards at minimum speed (1.2knots) to avoid entanglement of the bongo net with the propeller and much of the water turbulence caused by the engine.

Bongo nets (Fig. 4) are used to capture planktonic species and other small organisms based on the mesh size of the net, as a by-product the bongo net also captures organisms smaller than the 18cm opening including ctenophores, medusae, seahorses, needle fish, crabs, algae, sea grass and any other organisms or debris floating within the first meter of the surface of the lagoon, with turbid waters it can also accumulate sediment larger than 200/300µm from the water. This type of net would allow the capture of *M.* leidyi without damage to the organism due to the small mesh size.

A flowmeter was located in the mouth of the 300µm net with a rotor inside quantifying the spins of the rotor within the collection period to calculate liters of lagoon water passed through the net during each of the collection trials given by the formula:

Filtered Water = 0.3 * 2 * 0.025434 * Rounds recorded on the flowmeter.

Ctenophores per liter - CPL = Ctenophores Caught / Filtered Water

M. leidyi pass through the plastic 18cm opening of the net and through the initial 30cm of plastic compartment, travel down the cone shaped mesh net and remain in the bottom sector of the bongo net equipped with an orange plastic tube. At the end of the 5 min towing trial period each of the two orange plastic tubes were unscrewed from the net and *M. leidyi* as well as the other by-catch products were poured into a 20L collection bucket for examination. All by-catch was discarded over the side of the boat keeping only the ctenophores which were transferred into smaller 500ml collection containers. *M. leidyi* were carried in these buckets for a maximum of 2 hours travel back to the Hydrobiological “Umberto D’Ancona” (Chioggia) lab.

Figure 4 The picture on the left shows an up close view of the opening of the bongo net with flowmeter in the mouth of the 300µm net, the bongo net is folded over the side of the boat and the wooden suspension pole to also show both ends of the net including the orange plastic containment unit at the bottom for which the catches aggregated. The picture on the right depicts the bongo net being trawled off the bow of the boat while idling backwards for collection of target species M. leidyi.

3.3 Experimental set up

In order to evaluate the tolerance to different temperatures and salinities of *M. leidyi* in the Venice lagoon, an experiment was set up. Temperatures chosen for this experiment were: 18°C, 28°C and 32°C. The coldest temperature of 18°C is the average temperature at the hydrobiological station in the month of November before the decrease of temperature in the winter months **(Sea Temperature info, 2022)**. Of the warmer temperatures 28°C is the upper limit temperature in which *M. leidyi* is reproductively active as well as having feeding and growth rates acceptable for reproduction **(Rowshantabari et al., 2012)**. The final warmest temperature of interest is 32°C which is the maximum recorded temperature at the hydrobiological station during the warm summer months in the southern lagoon **(Garcia et al., 2022)**.

The values chosen for salinity are 10PSU, 20PSU, 30PSU and 37 PSU. In the northern Venice Lagoon salinity has the lowest values ranging between 10-20PSU so both extremes of this range were used for the lower salinity tolerances **(Zirino et al., 2014).** The PSU of 30 is the average salinity in the central and southern Lagoons of Venice. The 37PSU is the maximum PSU that has been recorded in the southern lagoon of Venice and was used as the upper limit for the experiment **(Zirino et al., 2014)**.

For the experiments, 48 tanks were used; 16 designated for the hot conditions of 32°C, 16 designated for the warm conditions of 28°C and 16

designated for the cold conditions of 18°C. For the hot condition air conditioning unit was set to 32°C however with the air conditioner even at 32°C water temperature only reached 28°C so a small aquarium heater was placed into the reservoir tank to maintain the water at 32°C. For the warm conditions (28°C) the containers were placed into the same air-conditioned room as the hot tanks, but they did not have an aquarium heater in the water. For the cold condition, tanks were placed into a separate room with an air conditioning unit set to 18°C (Fig. 5). Temperature in the reservoir tank was monitored and stabilized for 48 hours before the start of each trial. In each temperature group 16 containers were housed in reservoir tanks with 4 containers in each reservoir tank, the containers were given a label A,B,C, or D (Fig. 5).

Figure 5 the picture shows the experiment set up of the 16 collection tanks in the cold condition (18°C); each larger reservoir tank contained 4 smaller tanks inside labeled A,B,C, and D based on the salinity conditions. This experimental design was also repeated in a warm room for the 28°C tanks and the 32°C tanks. The 32°C tanks contained an aquarium heater in between the two suspension bricks in the bottom of the reservoir tank to increase the temperature

In each of the four containers water at the four different salinities was added. All water was first filtered through a vacuum filtration unit with a filtration disc of 1 µm or less to remove potential bacteria from the sea water. To obtain the different

PSU of the filtration water, fresh water was added into a bucket and a multiparametric probe “HANNA” was used to check the salinity until the correct value for each treatment was reached. Water was aerated using an aquarium aeration system for at least 1 hour before being added to the smaller containment tanks. Tanks marked A received 37PSU treatment, tanks B received 30PSU treatment, tanks C received 20PSU treatment and tanks D received 10PSU treatment. This allowed 4 replicates to run simultaneously per temperature condition; 4(A) tanks per temperature condition, 4 (B) tanks per temperature condition, 4 (C) tanks per temperature condition and 4 (D) tanks per temperature condition.

The ctenophores were randomly assigned to one of the 12 (3 temperature x 4 salinity values) possible treatment groups. Prior to their placement in their experimental tanks, they were briefly put in a petri dish (<15seconds) to obtain length and width measurements with a metric ruler and then placed into a 12-compartment tray corresponding to the 4L tank they would be placed in (Fig. 6). All animals began the experiment at 35PSU and 25°C mimicking the conditions of the environment in which they were caught in (late June from the southern lagoon). Organisms used for the experiment were caught off the dock of the Hydrobiological Station of the University of Padua using a fine mesh aquarium net attached to a pole and then placed into a clean 20L bucket filled with sea water also obtained from the dock. Temperature was changed within 24 hours following their placement into their respective temperature-controlled rooms and salinity was changed at a rate of 5PSU every 12 hours until the target salinity per experimental condition was reached. To change the salinity the animals were removed from their 4L containers using a 250mL beaker, the water was exchanged with new water 5PSU closer to the target salinity and the animal was replaced into the container. Animals in A tanks were brought to PSU 37 in one water change. Animals in tanks B were brought to PSU 30 in one water change. Animals C took 4 water changes, one every 12 hours to reach the target salinity of 20PSU. Animals in D tank took the longest to reach their target salinity over the course of 3 days going from 35 to 30 to 25 to 20 to 15 to 10 every 12 hours. Each time these animals were removed from their D tanks and their water was exchanged all other animals in tanks A, B, and C were removed as well and their water exchanged with new water at the target salinity so that every one of the 48 animals over the 3 days period were removed, water changed, and replaced. After the three days the animals were no longer removed from their tanks and instead the water was circulated twice a day with a small pipette swirled gently around the top of the tank to provide movement of the ctenophore and to mimic the sea current. Once placed into their experimental tanks, every 12 hours temperature, salinity and survival checks were conducted regardless of whether the tanks were in the process of reaching their target salinity and temperature or actively at their target salinity and temperature.

Figure 6 PhD student Filippo Piccardi with gloved hands and a petri dish obtaining length and width measurements of the ctenophores before their random assignment to their experimental tank (A-D) in each of the temperature conditions.

3.4 Survival Protocol

Checks for *M. leidyi* survival were conducted at two different times throughout the day 12 hours apart; one in the morning between 09:00-10:00 and once in the evening between 21:00-22:00 Italian time. Multiparametric probe "HANNA" was used to check temperature and salinity at the same time. To take the measurements the water was firstly illuminated from underneath with a flashlight or from the side as illuminating from above makes the animal harder to see with the eye. Once the animal was located it was noted if it is on top of the water column or sinking to the bottom and if movement of the cilia was active, this is a reliable indication of life status of the organism **(Tamm, 2014)**. If no ciliation is noted and the animal is on the bottom of the tank with a deflated or melted appearance the animal is noted as dead. Once the viability of the animal has been noted the probe was put into the tank farthest away from the organism as possible to not disturb or injure them. Temperature, salinity, date and time were recorded into a master data sheet. This process was repeated in all tanks noting the alive/dead animals, temperature and salinity. After probing and recording was completed, a pipette was used to gently swirl the surface of tank creating an artificial current to mimic the natural environment of the animals in the lagoon.

Once a day, after probing and taking of measurements and swirling are finished all ctenophores were fed about 10-15ml solution of the *Artemia salina* grown in the warm room (32°C). All feedings came from the same population of *Artemia* shrimp in which the supply of artemia was renewed every 5-7 days. Using a syringe filled with 10-15ml of artemia solution checking first that the artemia are swirling in their tank and not completely dead and then distributed the allotted amount into each

experimental tank. To grow artemia: about 2g of refrigerated artemia eggs were placed into a 200g beaker and the rest of the beaker was filled with filtered sea water. The artemia take 2-3 days to hatch and begin swimming so a secondary tank of artemia was reserved so that the ctenophores will have nightly access to food.

Once the animal was confirmed dead the water was UV treated with an UV Lamp (Filtriacquashop 6 Watt (2 L/min)) to kill any eggs or larvae potentially present, the container was rinsed and dried. If the animal continuously survived throughout the one-week experiment, then the same process of water treatment was used for that of the living animal. No living *M. leidyi* were discarded without firstly being subjected to UV treatment to kill them.

3.5 Data analysis

Data analysis was conducted using a combination of Excel Spreadsheets, Google Earth mapping, and R coding. Plots for the collection locations were mapped in Google Earth showing the northern, central and southern locations.

Data from the in-field observations was collected in a master Excel spread sheet and then inputted into R for graphical and statistical analysis.

Environmental data from the field and ctenophore abundance were compared among the three lagoon areas (northern, central and southern) and months using an ANOVA test, with area and month as fixed orthogonal factors and temperature, salinity and ctenophores per liter (CPL) as dependent variables. Post-hoc Tukey test was then applied for CPL.

Experimental data were analyzed applying an ANOVA test with salinity and temperature as fixed orthogonal factors and hours of survival as dependent variable.

To test if there were any difference in size among animals in different treatments, a two-way ANOVA test was conducted (with salinity and temperature as fixed orthogonal factors). No differences in size were found (all $P>0.05$) therefore, there are no statistically significant differences in the widths of the organisms in each of the experimental treatment condition, as they were randomly selected and designated to a treatment after measurement.

4 RESULTS

4.1 Observational Field Data Results

A two way ANOVA test was used to test any difference in environmental parameters among the northern, central and southern lagoon sample sites and sampling months. Temperature was significantly differeng among areas and months and also the interaction if these two factors was significant (Table 1).

Table 1 Two-way ANOVA test comparing temperatures among the northern, central and southern sampling sites labeled "Area" and months April, May, June, July/August and September labeled "month". Degrees of freedom sum (Df Sum), the square mean (Sq mean), the square (Sq) and F and P values, multiple asterisks highlight significant p-values.

	Df Sum	Sq Mean	Sq	F value	Pr(>F)
Area	2	99	49.4	57.41	< 0.0001 ***
month	4	4277	1069.3	1243.06	< 0.0001 ***
Area:month	8	97	12.1	14.03	< 0.0001 ***
Residuals	210	181	0.9		

Salinity was significantly different among areas and months (Table 2).

Table 2 Two-way ANOVA test comparing salinities among the northern, central and southern sampling sites labeled "Area" and months April, May, June, July/August and September labeled "month". Degrees of freedom sum (Df Sum), the square mean (Sq mean), the square (Sq) and F and P values, multiple asterisks highlight significant p-values

	Df Sum	Sq Mean	Sq	F value	Pr(>F)
Area	2	3397	1698.7	34.785	<0.0001 ***
month	4	566	141.6	2.899	0.023 *
Area:month	8	444	55.4	1.135	0.341
Residuals	210	10255	48.8		

Differences in ctenophore per liters across the three sampling sites (Northern, Central, Southern lagoon) were graphed in a boxplot separated by months (Fig. 7). No ctenophores were collected in April and May while in June only 9 ctenophores were caught across all three sample sites combined. The most total ctenophore per liters catch rate of all areas combined occurred in July/August, followed by September and then June. In September there was a highest catch rate in the Southern lagoon compared to any other month.

The number of ctenophores per liter (CPL) was significantly different among areas, months and their interaction (Table 3). Only the months June, July/August and September were included as they were only months with recorded catches of ctenophores.

Table 3 Two-way ANOVA test comparing ctenophores per liter (CPL) among the northern, central and southern sampling sites labeled "Area" and months April, May, June, July/August and September labeled "month". Degrees of freedom sum (Df Sum), the square mean (Sq mean), the square (Sq) and F and P values, multiple asterisks highlight significant p-values.

	Df Sum	Sq Mean	Sq	F value	Pr(>F)
Area	2	126.4	63.2	3.083	0.0493 *
month	2	1062.7	531.4	25.915	<0.0001 ***
Area:month	4	316.3	79.1	3.857	0.0054 **
Residuals	126	2583.6	20.5		

Following the ANOVA test a post-Hoc test was performed to determine which specific Month/Area interactions different in terms of CPL. Within the southern lagoon there were statistically significant differences in CPL between the months June and September. Within the northern lagoon there were statistically significant differences in CPL between the months July/August and June, and June and September. Within the central lagoon there were statistically significant differences in CPL between the months July/August and June, and July/August and September. Within just the month parameter post-Hoc test was performed using only months June, July/August and September as these months were the only months with recorded catches of ctenophores to generate a ctenophore per liter (CPL) ratio. Only statistically significant differences are reported in Table 4 all other contrasts had p-value>0.05.

Table 4 post-Hoc test comparing Ctenophore per Liter catch rates across the three sampling sites of the lagoon LN (northern lagoon), LC (central lagoon), LS (southern lagoon) compared to months of catch (June, July/August, September) for statistically significant differences. Results displayed with standard error (SE), degrees of freedom (df), t ratio (t.ratio) and p-value (p.value). Only statistically significant differences were reported in this table all other interactions had p-value>0.05.

contrast	estimate	SE	df	t.ratio	p.value
LC July/August - LS July/August	6.23015	1.65	126	3.768	0.0075
LC July/August - LC June	8.69461	1.65	126	5.258	<.0001
LC July/August - LN June	8.70099	1.65	126	5.262	<.0001
LC July/August - LS June	8.67222	1.65	126	5.245	<.0001
LN July/August - LS July/August	5.22645	1.65	126	3.161	0.0494
LN July/August - LC June	7.69091	1.65	126	4.651	0.0003
LN July/August - LN June	7.69729	1.65	126	4.655	0.0003
LN July/August - LS June	7.66852	1.65	126	4.638	0.0003
LC June - LN September	-7.44487	1.65	126	-4.503	0.0005
LC June - LS September	-5.58613	1.65	126	-3.378	0.0262
LN June - LN September	-7.45125	1.65	126	-4.506	0.0005
LN June - LS September	-5.59251	1.65	126	-3.382	0.0259
LS June - LN September	-7.42247	1.65	126	-4.489	0.0005
LS June - LS September	-5.56374	1.65	126	-3.365	0.0273

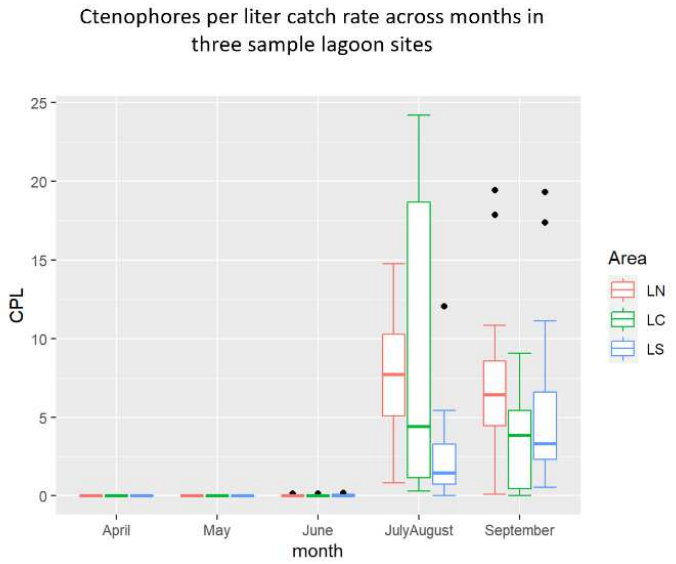

Figure 7 Box plots show median (thick line), Interquartile range (box), and singular lines showing the maximum and minimum ctenophores per liter (CPL) caught across the 3 transects in the northern lagoon (LN), central lagoon (LC) and southern lagoon (LS) in different months. Outliers are shown with black dots.

Considering the highest number of ctenophores present in July-August, we analyzed the trend in their occurrence along the transects and in relation to environmental parameters in this period. A higher number of ctenophores were present in the sampling sites closer to the sea in the southern and central lagoon, while no clear patterns emerged for the northern lagoon (Fig. 8).

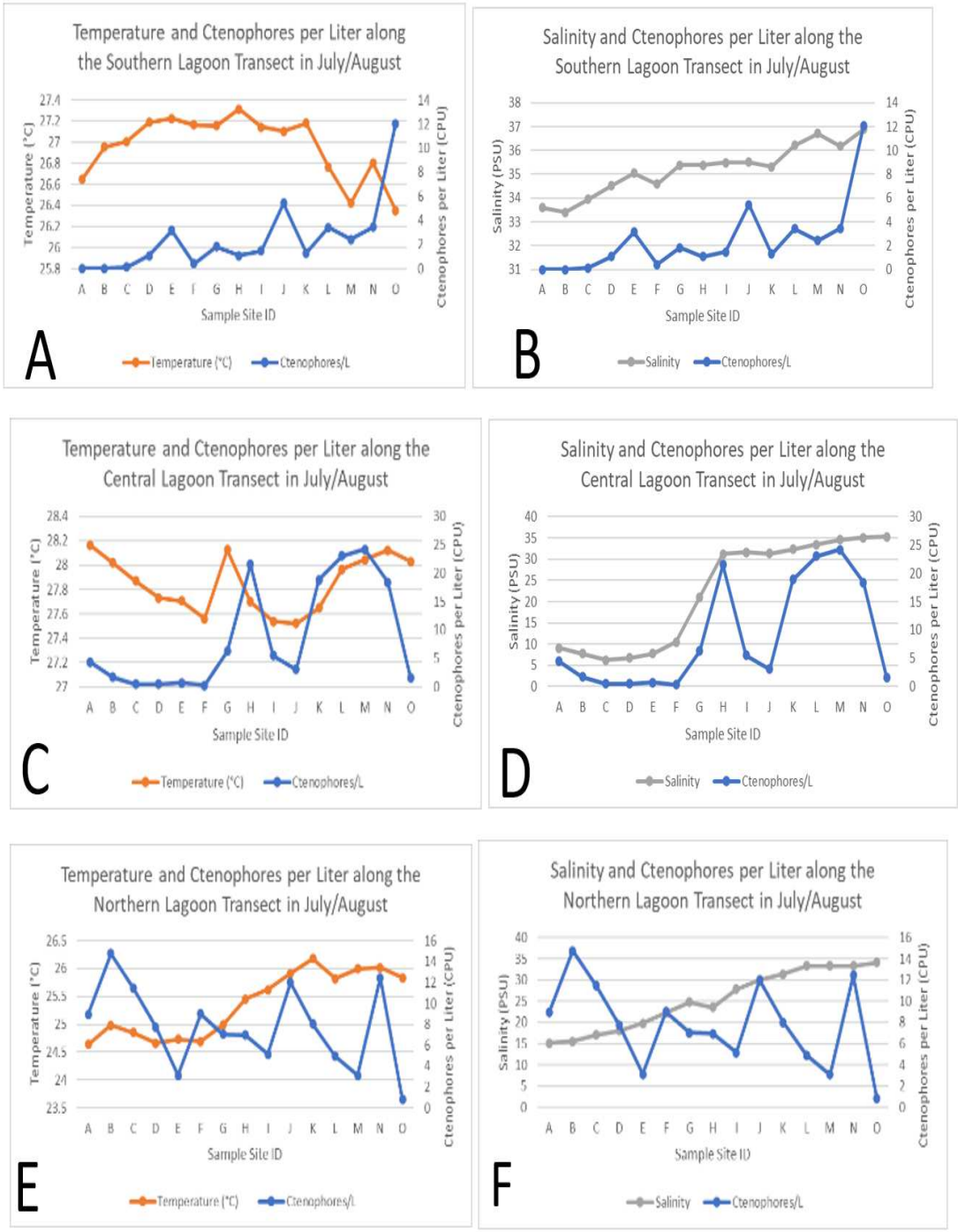

Figure 8 Ctenophores per liter (CPL, blue line) in each sampling along the transect in the southern (A, B), central (C, D) and northern (E, F) lagoon in relation with temperature (A, C, E, orange line) and salinity (B, D, F, grey line)

4.2 Experiments on the survival at different temperature and salinities

Experimental data of the ctenophores (N=214; 17 in 32°C/37PSU, 15 in 32°C/30PSU, 15 in 32°C/20PSU, 15 in 32°C/10PSU, 33 in 28°C/37PSU, 18 in 28°C/30PSU, 18 in 28°C/20PSU, 19 in 28°C/10PSU, 16 in 18°C/37PSU, 16 in 18°C/30PSU, 16 in 18°C/20PSU, and 16 in 18°C/10PSU) in relation to temperature and salinity conditions were plotted in bar graph form using R coding software (Fig. 9).

A 2-way ANOVA test was used to determine statistical significance in the survival hours of the ctenophores in the laboratory experiment in relation to temperature, salinity and the interaction of temperature and salinity. Salinity treatments, temperature treatments and the interaction of salinity and temperature on treatments were all statistically significant (Table 6)

Table 6 Two-way ANOVA test testing the effect of temperature and salinity (Streat) on hours of survival. Degrees of freedom sum (Df Sum), the square mean (Sq mean), the square (Sq) and F and P values, multiple asterisks highlight significant p-values

Contrast	Df Sum	Sq Mean	Sq	F value	Pr(>F)
Temperature	2	46517	23259	29.347	<0.0001 ***
Streat	3	39392	13131	16.568	<0.0001 ***
Temperature:Streat	6	21416	3569	4.504	0.0002 ***
Residuals	202	160095	793		

The highest survival in hours occurred in the group subjected to 37PSU and 28°C with over 50% of the individuals surviving longer than 108 hours (the experiment ran for 144 hours at maximum). Throughout the 3 remaining different salinity groups (30PSU, 20PSU, 10PSU) the cold 18°C experimental group had higher survival in hours compared to any other group. In each salinity experimental group, the lowest survival was observed in the hot 32°C temperature group. The largest variation is survival hours occurred in the 30PSU and 28°C experimental group. The lowest variation in survival hours occurred in 10PSU and 32°C experimental group. The overall trend across the 30PSU, 20PSU, and 10PSU groups show the 18°C survival with the highest hours followed by the 28°C, and then the 32°C. The difference to this trend is exhibited in the 37PSU treatment group where 28°C shows the highest survival in hours followed by the 18°C, and subsequently the 32°C group.

Table 7 reported values of the different salinity treatment, temperature treatment, minimimum survival within the treatment in hours, median survival within the treatment in hours, maxium survival within the treatment in hours and the interquartile range of survival within the treatment in hours. This data was used to construct the boxplot in Figure 8.

Salinity Treatment (PSU)	Temperature Treatment (°C)	Minimum Survival (hours)	Median Survival (hours)	Maximum Survival (hours)	Interquartile Range
37	18	36	90	108	45
37	28	24	108	144	48
37	32	12	60	72	48
30	18	36	90	108	45
30	28	12	66	108	57
30	32	12	48	72	36
20	18	12	90	108	45
20	28	12	48	72	33
20	32	12	36	60	18
10	18	12	78	108	39
10	28	12	24	72	24
10	32	12	36	72	18

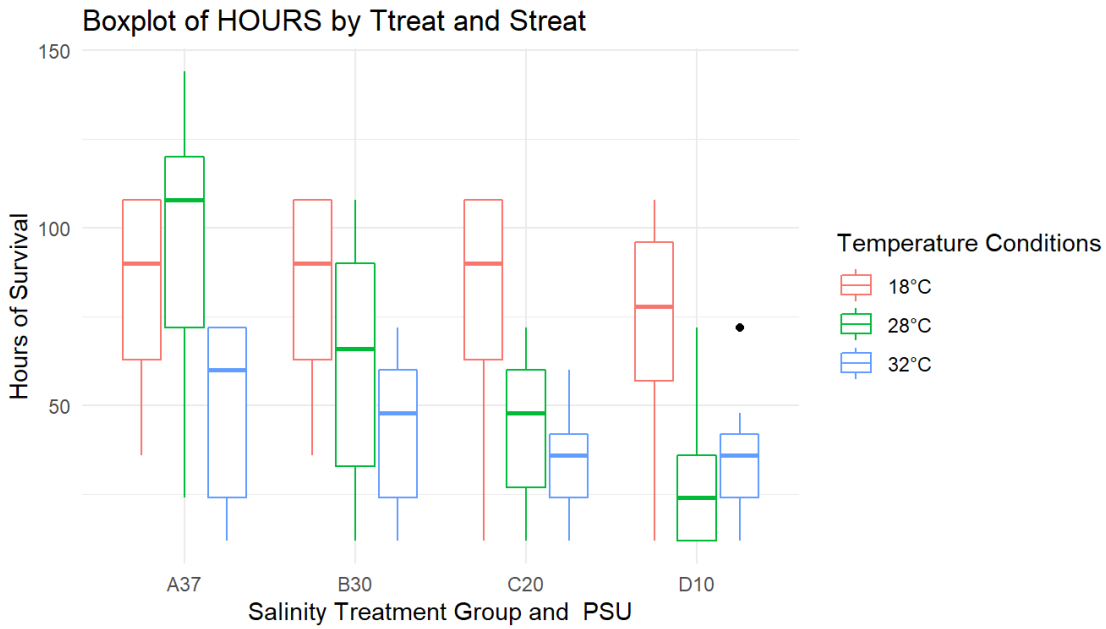

Figure 9 Box plot of Temperature treatment (Ttreat) and Salinity treatment (Streat) showing median (thick line), Interquartile range (box), and singular lines showing the maximum and minimum survival in hours of experimental groups at varying temperature and salinity conditions, outliers shown with black dots. Along the X-axis are the salinity conditions of each experimental group corresponding to the letters and salinity conditions used to label the individual trials within the experiment; A-37PSU, B-30PSU, C-20PSU, D-10PSU. Each salinity treatment (A,B,C,D) has

within its sector 3 separate bar graphs showing the temperature conditions of the subsequent temperature experimental group; 18°C (red), 28°C (green), and 32°C (blue). The y-axis depicts the hour of survival for the ctenophores within the 12 different experimental trials.

5 DISCUSSION

As a highly invasive species with a recent population establishment in the Venice lagoon there is much to be explored as to why the ctenophore *Mnemiopsis leidyi* has had such a successful establishment. In this study the patterns of abundance of *M. leidyi* in relation to environmental parameters in the Venice lagoon was depicted. This study also provided novel data regarding the tolerance of these ctenophores to temperature and salinity in an experimental setting. As the first experiment exploring tolerances of *M. leidyi* in the Venice lagoon the results from this study offers valuable insight into the potential occurrence of these ctenophores within the Venice lagoon. This data can be used in collaboration with previous environmental impact studies as well as future studies to follow the effects of these organisms within the Venice lagoon.

5.1 Field data

In situ observations of *M. leidyi* in the Venice lagoon revealed new information of the presence of these ctenophores within the lagoon in relation to temperature and salinity. Both temperature and salinity differed significantly between sample areas and the months of sampling. With the capture of ctenophores happening between June and September temperature may be linked to the initial bloom of ctenophores in the lagoon. Temperature has been previously supported as an indicator of bloom presence in a case study in the southern lagoon of Venice, in which it has been demonstrated how the warming temperature of the previous year influences the *M. leidyi* bloom size of the subsequent year **(Piccardi et al., 2024)**. In this study the presence of *M. leidyi* in the lagoon firstly occurred between the sampling month of May and June, in June less than 10 ctenophores were captured throughout all sampling sites with the majority of catches occurring in July/August. At some point between June and July/August hypothetically there would be the optimal temperature that favors massive reproduction events throughout the lagoon. Although the central lagoon had the highest ctenophore per liter catches (CPL) it also had the greatest variation in CPL across the transect. It was the colder northern lagoon that showed an average of the higher CPL compared to the other sites. There is a scarcity of other research on the temperature tolerances of these ctenophores in a warm climate such as the Venice lagoon. The observance of these ctenophores in warmer locations (26-28°C) may potentially exhibit a local adaptation of this species to high temperature.

In terms of salinity, we expected that the northern lagoon would have the lowest salinity values due to the location and fresh water river runoffs. Average salinity across the transects was, indeed, lowest in the northern lagoon respects to the other areas. However, when comparing individual transect points the central lagoon at the beginning of the transect had lower salinity values than any of the northern lagoon points when sampled in July/August with a bigger salinity gradient from the beginning of the transect to the end. Overall, the southern lagoon had the highest salinity and the lowest gradient of the three locations. Previous literature has shown a trade off in egg production in relation to salinity variations **(Jaspers et al., 2011).** The study showed egg production was greatest at 33PSU with a negative trend as salinity decreased. Even at salinities above 6PSU there was egg production although reduced from the maximum egg production possible by this species **(Jaspers et al., 2011)**. The previous data indicates that these ctenophores do reproduce at low salinities, due to this low salinity areas may be less suitable for them. Our data supports these assumptions as the higher CPL catches occurred at higher salinity transect points in general compared to lower salinity locations and transect points. However, we did observe substantial CPL catches in the central lagoon during July/August at salinities lower than 10PSU at transect points A, B, and C.

If *M. leidyi* relied solely on temperature and salinity conditions of the environment for population dispersal we would expect to see the CPL catch rate following the trends of the salinity and temperature gradient. However, there is little correlation in the transects between the temperature and salinity trends and the CPL trend lines, instead the CPL trendline is one resembling a sinusoidal curve with 2-4 peaks depending on the site transect. A potential explanation for the sinusoidal curve of the ctenophores could be based on the tides in the Venice lagoon. This phenomenon appears much like how a beach wrack is deposited in a line or zone along the sea shore during high tide **(Orr et al., 2005).** When water conditions are calm *M. leidyi* remains in the upper layer of the water column instead of finding refuge in deeper water, this makes the larger individuals highly susceptible to tidal currents **(Miller, 1974).** The Venice lagoon presents a tidal structure with 2 peak waves in tidal height within a 24-hour period **(Bellafiore et al., 2008).** Considering sampling in the three sites was carried out on calm days so the boat could easily traverse the waves, the distribution of ctenophores observed follows the sinusoidal curve present in the tides over a 24-hour period. The tides could promote a pushing of *M. leidyi* through the shallower channels establishing a larger front of ctenophores followed by a lull and then another large front. This is observed in a large spike in CPL followed by the next two sampling points having lower CPL catches and then a second wave is encountered, etc. **(Orr et al., 2005).**

5.2 Experimental data

The experimental testing of *M. leidyi* present clear results in the interaction of temperature and salinity tolerances on the organisms' ability to survive. The experimental tests showed a consistently high survival with temperature treatment of 18°C across all salinity conditions. The only condition that had a greater survival was the treatment of 28°C and high salinity of 37PSU which had over 50% of the experimental group surviving longer than the colder temperature counterparts. This leads us to predict that conditions are most optimal in the lagoon at 28°C and 37PSU, **(Finenko et al., 2006),** however the species is able to survive at any of the salinity ranges as long as the water temperature is cooler.

With the values from the experimental section there is further support that there should be reduced numbers of ctenophores in lower salinity waters as all temperature groups had the lowest survival at salinity of 10PSU. This experimental data would suggest that ctenophores should not be present at low salinities. Yet in the environmental observations ctenophores were found at salinities lower than 10PSU. This result appears to contradict the experimental data but there may be other factors, not considered in this study, that might influence ctenophore presence. Among the possible factors tide may play a crucial role as explained in the field data discussion. Indeed, considering that the ctenophores did have some survival in the low-salinity experimental conditions, it could be that with the tides coming twice a day, that when the ctenophores are pushed inland, they can survive being pushed into the less saline waters for some hours before being pulled back out to the deeper and more saline channels with the following low tide.

6 CONCLUSION

The experimental data shows that temperature and salinity play important roles in the survival of *M.* leidyi, yet the environmental data shows presence of these organisms that contradict the experimental results. Experimental data and literature suggest that there should not be a large presence of these ctenophores at lower salinity conditions **(Jaspers et al., 2011).** However, finding them in the lagoon at lower salinity conditions justifies the need for more research into the salinity tolerances of these animals or if there is something else about the water dynamics that was outside the scope of this study.

With global warming trends favoring a rise in temperature and a rise in salinity in the Venice lagoon **(Amos et al., 2017; Carbognin et al., 2010)**, the experimental data suggests a higher survival rate**.** This increased survival rate is supported until 28°C with a decreased survival rate at temperatures of 32°C and above. A previous study showed that with an increase in temperature there is a correlation to the increase in bloom size **(Piccardi et al., 2024)**, in this study with an increase in temperature there is a correlation to an increase in survival hours within the experiment. But the data in this study may suggest this is not linear but a logistic relationship, and eventually there is a tipping point at 28°C in which temperatures beyond this point are too hot for the individuals to be able to thrive.

In terms of temperature these ctenophores could be using the colder northern lagoon as a refuge, because although they experimentally favored the 28°C conditions compared to the 18°C conditions there was a decrease in survival when temperature surpassed the 28°C mark towards 32°C. In the recent future there may be an increase in *M. leidyi* blooms throughout the Venice lagoon until the average temperature routinely surpasses the optimal 28°C for the species.

With a predicted increase in ctenophore blooms across the lagoon this could mean in the coming years that there will be greater and greater negative impacts upon the fishing community and that more research should be conducted on how there could be possible eradication attempts of this species from the lagoon. Regarding the results obtained future experiments could be focused on the interaction of the tides present in the lagoon with the presence of ctenophores. In addition, water turbidity could be studied more thoroughly along the transects in relation to ctenophore survival as *M. leidyi* has shown to have reduced feeding in turbid waters **(Main, 1928; Sullivan and Gifford, 2004).** Another potential avenue for further study could be in the presence of juvenile ctenophores (<1mm) along these transects in the three sample sites to determine the presence of reproductive sites throughout the Venice

lagoon and the tolerances of salinity and temperature of these juvenile organisms in an experimental setting. There is also little research into predator species such as *B. ovata* survival rates in a warm climate like the Venice lagoon and if introduction of this species into the lagoon can mitigate potential future damage caused by large blooms of *M. leidyi.*

7 Supplemental Graphs

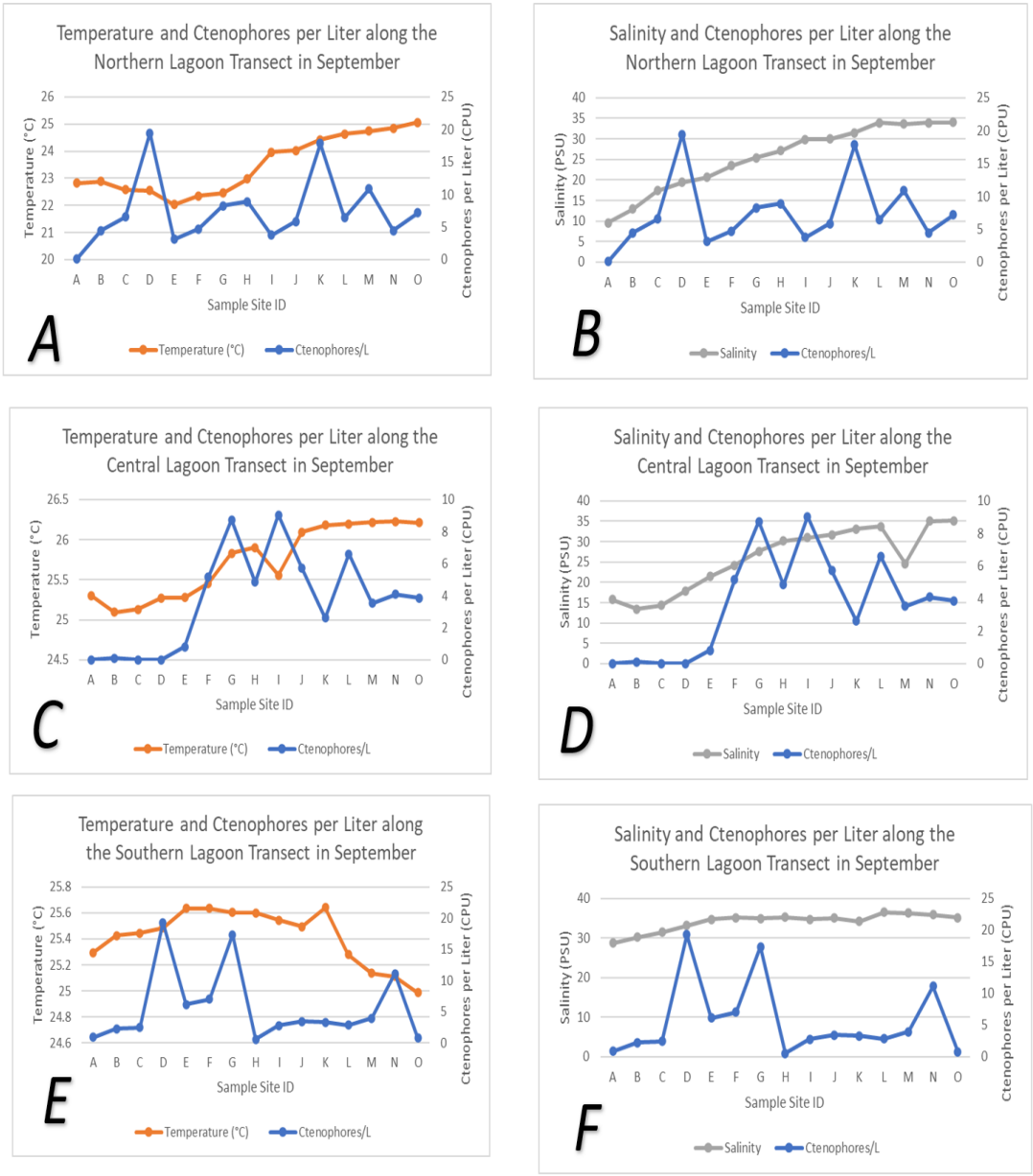

Figure 10 Ctenophores per liter (CPL, blue line) in each sampling along the transect in the northern (A, B), central (C, D) and southern (E, F) lagoon in relation with temperature (A, C, E, orange line) and salinity (B, D, F, grey line)

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