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DOCTORAL THESIS

Population dynamics of gelatinous organisms and their environmental connections

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Dinámica poblacional de organismos gelatinosos y su conexión medioambiental

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No water, no life.
No blue, no green.

Silvia Earle, Oceanographer

SUMMARY

Gelatinous organisms are key members of the marine environment and have been around circa 540-520 million years. It is well known that blooms (sudden outbreak of one species which comes to dominate the plankton for a finite period) are natural phenomena, which occur seasonally in many marine ecosystems. However, swimming can be perceived as a “dangerous” activity in the presence of jellyfish blooms and this can negatively affect the tourist trade. The economic damage is evident and can be seriously detrimental when tourism is the main source of income, as is the case in many coastal economies in southern Spain. In recent decades, the proliferation of jellyfish in coastal waters appears to have increased in both frequency and intensity, producing negative ecological, social and economic impacts. In some regions, these blooms of gelatinous organisms have been connected to anthropogenic practices such as overfishing, pollution, eutrophication, translocation, climate change, and ocean acidification. Historically, due to a lack of interest in the ecological role of gelatinous organisms, there is a clear gap of knowledge about their taxonomy, life cycle, ecology and ecosystem services. Therefore, when initiating new research on a species in a

specific region, the absence of prior data needs to be interpreted with caution; it may be misinterpreted as a lack of the species historically.

The main objective in this Doctoral Thesis is to better understand the spatio-temporal distribution, environmental drivers of populations of gelatinous macroplankton occurring in the southern Iberian Peninsula and contextualise this at a local and regional level. In order to archive the objective proposed, a combination of literature review, field study and experimental works have been performed.

The first four chapters are dedicated to the rediscovery of the little-known jellyfish *Rhizostoma luteum*. This large scyphomedusa was described for the first time in 1827 by Quoy and Gaimard under the name *Orythia lutea* within the Straits of Gibraltar (southern Iberian Peninsula). Prior to 2013, only four reports of this jellyfish exist along the Iberian coast. These reports are never further east than the Strait of Gibraltar. We suggest that the reasons why *Rhizostoma* went scientifically unnoticed for more than 60 years are: 1) the confusion over its identity and 2) the lack of scientific studies, rather than an absence of this large jellyfish. By using photographic and video evidence through citizen science initiatives, we have updated its geographic range to the Bay of Biscay (northeastern Atlantic Ocean) and to the Coast of Murcia and the Balearic Basin (northwestern Mediterranean Sea). Moreover, we have fully described its life cycle for the first time. It resembles that of its congeners, but can be distinguished by two unique features. Firstly, the female *R. luteum* is a brooding species unlike the others. Secondly, the predominant type of strobilation is monodisc (one ephyra per strobila). The result of our experiments suggests that they can thrive over a wide range of temperatures and salinities, which may predispose them to cope better with ongoing environmental changes. Besides *R. luteum,* we identified three other large jellyfish which can be sporadically observed in the northern Alboran Sea: *Rhizostoma pulmo*, *Catostylus tagi* and *Drymonema dalmatinum*.

In the fifth chapter, we report on the first scientific report of *D. dalmatinum* from the western Mediterranean since 1873. This rare Semaeostomeae was first described in 1880 by Haeckel from the Adriatic Sea (eastern Mediterranean).

Chapter six is dedicated to the holoplanktonic scyphozoan *Pelagia noctiluca.* The mauve stinger is considered to be one of the most common and notorious species in the Mediterranean Sea. In spite of its proliferation causes significant negative socio-economic effects on the sector of fisheries and tourism little is known of its interannual, seasonal fluctuations and population dynamic from the Alboran Sea (southwestern Mediterranean). Since 2007, the mauve stinger *P. noctiluca* has been annually present in the Alboran Sea. Between July 2012 and December 2016 we recorded its presence in almost all the months. By far, the greatest abundance of jellies was during summer 2012 and 2015. Its presence near the coast during these two summers can be attributed to two factors. The presence of strong westerly winds, "*Poniente*", favouring the near coast upwelling mechanism in this region and which injects nutrient-rich water to an oligotrophic system. Afterwards a change to light easterly breeze "*Levante*", which push the jellyfish near the coast. However, to predict *P. noctiluca* fluctuation over the years a longer dataset is necessary, as our 4 ½ years show a great interannual and seasonal fluctuation recording the presence/absence of this jellyfish. Young jellies were present (<3.5 cm) from May-October and larger medusae (>15 cm) were registered during winter, this being one of the biggest sizes of *P. noctiluca* from the western Mediterranean Sea.

In the final chapter, we report of two beaching events for the calycophoran siphonophore *Abylopsis tetragona* (Otto, 1823). We show that mass stranding can be correlated to external stress factors like strong easterlies, preceded by upwelling mechanism activated by strong westerlies in the Strait of Gibraltar.

Finally, this Doctoral Thesis confirms the importance of citizen science programs as a tool to improve knowledge of natural phenomena, both regular and exceptional, whilst also describing past/present occurrences of gelatinous organisms. Therefore, we urge the need to regularly monitor gelatinous organisms through systematic, robust monitoring programs. Furthermore, citizen science initiatives need to be coordinated at local, regional and international level. In ever-changing environments, records of species at local, regional and across broad spatial scales are essential for decision-making processes in biodiversity conservation.

RESUMEN

Los organismos gelatinosos son miembros clave del ambiente marino y han estado presentes más de 540-520 millones de años. Es bien sabido que las floraciones (brote repentino de una especie que llega a dominar el plancton durante un período finito) son fenómenos naturales que ocurren estacionalmente en muchos ecosistemas marinos. Sin embargo, cuando el uso recreativo de las playas puede ser percibido como una actividad "peligrosa" en presencia de la proliferación de medusas, puede afectar negativamente al sector turístico. El daño económico es evidente y puede ser muy perjudicial cuando el turismo es la principal fuente de ingresos, como es el caso de muchas economías costeras del sur de España. En las últimas décadas, la proliferación de medusas en las aguas costeras parece haber aumentado tanto en frecuencia como en intensidad, produciendo impactos ecológicos, sociales y económicos negativos. En algunas regiones, estas floraciones de organismos gelatinosos se han relacionado con prácticas antrópicas como la sobrepesca, la contaminación, la eutrofización, la translocación, el cambio climático y la acidificación de los océanos. Históricamente, debido a la falta de interés en el papel ecológico de los organismos gelatinosos, existe un claro vacío de conocimiento sobre su taxonomía, ciclo de vida, ecología y servicios de los ecosistemas. Por lo tanto, cuando se inicia una nueva investigación sobre una especie en una región específica, la ausencia de datos previos debe ser interpretada con cautela.

El objetivo principal de esta Tesis Doctoral es comprender la distribución espacio-temporal y los factores ambientales de las poblaciones de macroplancton gelatinoso que se producen en el sur de la Península Ibérica y contextualizarlos a escala

local y regional. Para alcanzar el objetivo propuesto, se ha realizado una combinación de revisión bibliográfica, estudio de campo y trabajos experimentales.

Los primeros cuatro capítulos están dedicados al estudio de la medusa *Rhizostoma luteum*. Esta escifomedusa de gran tamaño fue descrita por primera vez en 1827 por Quoy y Gaimard bajo el nombre de *Orythia lutea* dentro del estrecho de Gibraltar (sur de la península Ibérica). Antes de 2013, sólo existen cuatro registros científicos de esta medusa a lo largo de la península ibérica y nunca se han registrado en el mar de Alborán. Sugerimos que las razones por las que esta Rhizostomeae pasó científicamente desapercibida durante más de 60 años son: 1) la confusión sobre su identidad y 2) la falta de estudios científicos, más que la ausencia de esta medusa. Mediante el uso fotografías y de vídeos a través de iniciativas de ciencia ciudadana, hemos actualizado su área de distribución geográfica al golfo de Vizcaya (noreste del océano Atlántico) y a la costa de Murcia y la cuenca Balear (noroeste del Mar Mediterráneo). Además, por primera vez hemos descrito su ciclo de vida, que se parece al de sus congéneres, pero se puede distinguir por dos características únicas. En primer lugar, la hembra *R. luteum* tiene los huevos fertilizados con ella a diferencia de las demás. En segundo lugar, el tipo predominante de estrobilación es el monodisco (una éfira por pólipo). El resultado de nuestros experimentos sugiere que pueden prosperar en un amplio rango de temperaturas y salinidades, lo que puede predisponerlos a adaptarse mejor a los cambios ambientales en curso. Además de *R. luteum*, identificamos otras tres medusas de tamaño considerable que se pueden observar esporádicamente en el norte del mar de Alborán: *Rhizostoma pulmo*, *Catostylus tagi* y *Drymonema dalmatinum*. Este último, perteneciente a la familia Semaeostomeae, fue descrito por primera vez por Ernst Haeckel en el mar Adriático, y se aborda en el quinto capítulo.

El capítulo seis está dedicado al escifozoo holoplanctónico *Pelagia noctiluca.* Esta medusa luminiscente está considerada como una de las especies más comunes y notorias en el mar Mediterráneo. A pesar de que su proliferación provoca importantes efectos socioeconómicos negativos en el sector pesquero y turístico, poco se sabe de sus fluctuaciones estacionales e interanuales y de la dinámica poblacional en el mar de Alborán (suroeste del Mediterráneo). Desde 2007, *Pelagia* está presente anualmente en el mar de Alborán. Entre julio 2012 y diciembre 2016 registramos su presencia en casi todos los meses del año. La mayor abundancia de medusas fue registrada durante el

verano de 2012 y 2015. Su presencia cerca de la costa durante estos dos veranos puede atribuirse a dos factores. La presencia de fuertes vientos del oeste, "Poniente", que favorecen el mecanismo de afloramiento costero cercano en esta región y que inyecta agua rica en nutrientes a un sistema oligotrófico. Después un cambio a la ligera brisa de este "Levante", que empuja a las medusas cerca de la costa. Las medusas jóvenes estuvieron presentes (<3,5 cm) de mayo a octubre y se registraron medusas más grandes (>15 cm) durante el invierno, siendo éste uno de los tamaños más grandes de *P. noctiluca* del Mediterráneo occidental.

En el capítulo final, se reportan dos eventos de varamiento para el sifonóforo *Abylopsis tetragona* (Otto, 1823). Demostramos que la llegada masiva a las costas puede correlacionarse con factores de estrés externos como el fuerte "Levante", precedido por un mecanismo de afloramiento activado por el fuerte "Poniente" occidental en el estrecho de Gibraltar.

Finalmente esta Tesis Doctoral confirma la importancia de los programas de ciencia ciudadana como una herramienta para mejorar el conocimiento de los fenómenos naturales, tanto regulares como excepcionales, a la vez que se describen las ocurrencias pasadas/presentes de organismos gelatinosos. Se sugiere la necesidad de monitorizar regularmente los organismos gelatinosos a través de programas de monitorización sistemáticos y robustos. Además, las iniciativas de ciencia ciudadana deben coordinarse a nivel local, regional e internacional. En entornos en constante cambio, los registros de especies a escala local y regional son esenciales para los procesos de toma de decisiones en la conservación de la biodiversidad.

CONTENTS

1. INTRODUCTION

General Introduction

Humans always had a fascination with medusas, these mystic creatures leaving in the deep of the oceans. In 1870, the French novelist Jules Verne described the captivating encounter with those marvellous and colourful invertebrates in his epic roman *Vingt mille lieuse sous les mers* - Twenty Thousand Leagues Under the Sea. Although nowadays, there is a less romantic view of this marine invertebrates, as to the grand majority they are considered a nuisance, “pollution” or even seen as “pest”. These bias views are being heavily accentuated by the media, which mostly report about jellyfish having a negative socio-economic impact on coastal (beach) oriented tourist economies. Nonetheless, these negative perceptions have hinder knowledge regarding their taxonomy, life cycle, ecology and ecosystem services (Brotz et al. 2012; Graham et al. 2014).

The general term gelatinous zooplankton lumps approximately 2000 species, including groups such as jellyfish (cnidarian medusae), ctenophores (comb jellies), and pelagic tunicates (Condon et al. 2012a). Throughout this Doctoral Thesis, the term gelatinous organism is referred to the phylum Cnidaria, and the term jellyfish (is used interchangeably with jellies), refers solely to the scyphomedusae, also commonly known as “true jellies”.

Gelatinous zooplankton outbreak (blooms) are a natural phenomena that occur seasonally in many marine ecosystems (CIESM 2001; Hamner and Dawson 2009;

Condon et al. 2012a, 2012b) and have been around for about 540-520 million years as fossil records of mass stranding have been found from the Cambrian period (Hagadorn et al. 2002; Young and Hagadorn 2010).

Their bad reputation as "lower animal" is partly due that until recently it was assumed that jellyfish were a trophic dead end. Though, recent studies have revealed quite the contrary (Hays et al. 2018 and references theirein), as the results of various studies have shown that jellyfish are important nutritional contribution to the diet of wide range of pelagic mesopredators and apex predators the bluefin tuna (*Thunnus thynnus*), little tunny (*Euthynnus alletteratus*), spearfish (*Tetrapturus belone*), and swordfish (*Xiphias gladius*), Loggerhead sea turtles (*Caretta caretta*), ocean sunfish (*Mola mola*) (Cardona et al. 2012) and for the larvae of the European eel (*Anguilla anguilla*) (Ayala et al. 2018) in the Sargasso Sea. A study from the SW Atlantic Ocean showed that 39 of 107 species of fish consumed jellyfish of which 23 fish species were previously undocumented as consumers of gelatinous organisms (Diaz Briz et al. 2017). Besides two species of albatross (McInnes et al. 2017) and four species of penguin (Thiebot et al. 2017) were reported to consume jellyfish.

The scientific community is still debating if there is a real worldwide increase of gelatinous zooplankton from their historical levels (Mills 2001; Condon et al. 2012a, 2012b; Gibbons and Richardson 2013; Duarte 2012; Purcell, 2012). This seems like an easy question to answer, however, its response is more complicated then one would think. Mostly, because of a historical lack of interested in the ecological role of jellyfish (Graham 2001), there is a shortage of long-term data set (defined as longer than 20 years) (Purcell et al. 2007; Condon et al. 2012a). Due to the scarcity of historical data, there may exist a paradigm based on perception, as one might refer only to the observer's immediate past and ignores reports of recurrent blooms in the more distant past. Curiously, in media reports outbreak of jellyfish stayed unreported for decades however as mention above not in the grey literature or older scientific reports (e.g. Verne, Hemmingway, Haekel). As proposed by Condon and coauthors (2012a), the perceived increase in the number of gelatinous organisms blooms may be a case of "shifting baseline syndrome". This theory was first proposed for the fisheries-related studies by Pauly (1995) and consists in that each generation of marine scientists accept as a baseline the stock-size and species compositions that occurred at the beginning of their career, and used this to evaluate changes. Resulting in a gradual shift of the baseline perception, therefore, using inappropriate reference points. Brotz and coauthors (2012) used the baseline of 1950 and found that population appear to be increasing in the large part of the world's coastal ecosystems. Most of the reported increases appear linked to human activities, but the mechanisms involved are still poorly understood. Perceptual biases are also present when starting to study one species for the first time in a specific region, the absence of prior data may be misinterpreted as a complete absence of the species over time (Condon et al. 2012a and references therein).

Citizen science is a powerful tool for ecological research and evolves the public participation in scientific research (Silvertown 2009; Dickinson et al. 2012). Since the last century digital cameras, the Internet and smartphones have been vital to the development of citizen science, and in particular the validation of questionable observations through digital photos. Since 2008 more than 1700 peer-reviewed journal articles have published using citizen science data (Chandler et al. 2017). Citizen science is a useful tool especially in the case of gelatinous organisms because of the character of the bloom events; it is just not realistic to think that scientist will be on the spot on time. Furthermore, traditional net sampling is often ineffective for the fragile gelatinous organism, since they can be damaged or destroyed (Hamner et al. 1975). Therefore, the use of photographic/video material is an excellent alternative and blooms or beaching events are often very striking and therefore easy to spot by beach users.

Thus, in an ever-changing environment, and especially in the framework of climate changes, it is important to know which species were/are present in a specific region, to understand their population dynamics and whether their outbreaks are cyclic or in response to climate changes.

Historical Background

Rhizostoma luteum

This large jellyfish from the Family of the Rhizostome was first described as *Orythia lutea* (Latin: *lutea*, yellow) 1826 by the French naturalists Quoy and Gaimard (1827) during a scientific cruise on the exploration ship of the French Navy Astrolabe. They described various small medusae collected in May from the Strait of Gibraltar, three near Ceuta and six from the Bay of Algeciras. All were young medusae as the largest specimens were only two thumbs of diameter "*le plus grand n'avait que deux pouses environ de diamètre*". Hence, the name *lutea*, as juvenile medusa has yellowish-golden colour.

Until summer 2012 *R. luteum* was a forgotten species, and even some scientist doubted its occurrence, believing it might be a young *Rhizostoma pulmo* or a local variation (Agassiz 1862, Mayer 1910). This *Rhizostoma* was even described as very rare, as only on 7 occasions this medusa was collected and reported from a wide geographic range. Being Angola (west coast of Africa, Ranson 1949) its most southern report and the most northern from the mouth of the river Tagus (Tejo), near Lisbon, Portugal (Stiasny 1936). Only on four occasions, this jellyfish was reported from the Iberian coasts and never further east than the Strait of Gibraltar. The last time this jellyfish was collected and reported in a scientific publication was by Kramp (1959) were 5 medusae were sampled from various locations on the west coast of Africa between 1948 and 1953 hitherto, it disappeared from the scientific regards.

Therefore it is understandable, that in 2007 the question was raised whether the recognition of three species in the genus *Rhizostoma* was justified (Holst et al. 2007). Lilley and coauthors (2009) did a review of all known records from peer-reviewed and broader public literature of the genus *Rhizostoma* across western Europe, and they found no records of *R. luteum* during their research. Therefore, was not further mentioned in their review and because of the ambiguity between *R. pulmo* and *R. octopus* they stayed at the genus level.

In summer 2012 the beaching of various very large medusae in South Spain occupied several local and international social media (Prieto et al. 2013). First, it was believed to be a new species. However, it was then identified as *R. luteum*. In 2013 Prieto and coauthors performed a phylogenetic analysis on the mitochondrial cytochrome c oxidase I gene sequence in specimens from both stranding events confirmed the morphological classification, ratified that *R. luteum* differs from *R. pulmo* and *R. octopus.* Besides, sightings from the Alboran Sea (SW Mediterranean) were reported for the first time. Nevertheless, nothing was known about its life cycle and why there were no records for more than 60 years.

Drymonema dalmatinum

Drymonema dalmatinum was first described by the German zoologist Ernst Haeckel in 1880 from the Adriatic Sea (eastern Mediterranean), and has been reported only on one occasion from the western Mediterranean basin describing a single sample from material collected by the HMS Challenger expedition near Gibraltar in 1873 (Haeckel 1882). Interestingly, Malej and coauthors (2014) reported an increased frequency of sightings in the past decades from the Adriatic Sea hypothesizing that this might be due to an increase of its prey *Aurelia*, as this was observed in the Caribbean Sea (Williams et al. 2001).

Pelagia noctiluca

The mauve stinger *Pelagia noctiluca* is a holoplanktonic scyphozoan. It is widely spread in the Mediterranean Sea and occurs in both the western and the eastern basin. This Semaeostomeae is considered one of the most common species (CIESM 2001; Boero 2013) in the Mediterranean Sea and large blooms have been reported in the last decades (Gili and Pagès 2005, Daly Yahia et al. 2010, Kogovšek et al. 2010, Bernard et al. 2011). Goy and coauthors (1989) reported that over the past 200 years (1785-1985) blooms of *P. noctiluca* have occurred about every 12 years in the western Mediterranean basin and were related to an excess of anticyclonic metrological situations (high pressure) in particular during late spring- early summer. Though, in the last decades, there seem to have occurred a shift indicating that blooms appear more frequent in the western basin. In southern France (Ligurian Sea, NW Mediterranean) results indicate a long-lasting abundance of about 15 years between 1994-2008 (Bernard 2011), and since 1998 a higher frequency of blooms was as well reported from the Balearic Sea and the Gulf of Tunis (Daly Yahia et al. 2010). Interestingly, this recent potential change in the periodicity of blooms of *P. noctiluca* in the western

Mediterranean does not appear to be occurring in the eastern basin (Kogovšek et al. 2010).

Recent studies about the population dynamic of *P. noctiluca* showed its year-round presence from the Strait of Messina (southern Italy). It was reported that its growth rate was increased and it reached a bigger size at higher water temperature (Rosa et al. 2013) and had two reproduction peaks in autumn and spring (Milisenda et al. 2018). In the Ligurian Sea *Pelagia* were observed as well year-round, thought, mostly at night and 5 km offshore (Ferraris et al. 2012). *Pelagia* was reported to be by far the most abundant jellyfish in the Balearic Sea (Prieto, 2018) and the Catalan Sea (Gili and Pagès 2005; Sabatés et al. 2010; Canepa et al. 2014). Its highest concentrations was observed offshore over the shelf (Sabatés et al. 2018). Data collected from stranding events showed that they occurred in greater numbers along the northern coast and on beaches located closer to marine canyons (Canepa et al. 2014).

In the Alboran Sea (SW Mediterranean) the mauve stinger is as well reported to be the most abundant jellyfish (Licandro et al. 2010; Prieto and Navarro 2013; Aouititen et al. 2019). In southern Spain *Pelagia* has been observed annually between 2007 and 2012, its abundance was reported to the by far the highest during summer 2012 (Prieto and Navarro 2013). In a recent study from the Mediterranean coast of Morocco the mauve stinger has been observed between 2011 and 2017 (Aouititen et al. 2019).

Abylopsis tetragona

Abylopsis tetragona (Otto 1823) is an epipelagic siphonophore inhabiting temperate and warm waters of the Atlantic, Pacific and Indian oceans and the Mediterranean Sea (Alvariño 1971; Mapstone 2014). It constitutes a diverse and abundant group of gelatinous organisms that are still poorly understood, due to their fragility and their, mostly, oceanic distribution (Pugh 1989; Haddock 2004). Mass stranding events of gelatinous zooplankton organisms along shorelines are a natural and relatively common phenomenon strongly influenced by the direction and energy of prevailing winds and surface currents (Graham et al. 2001). Shore-based surveys have been used in various studies to record the occurrence of gelatinous organisms (Doyle et al. 2007; Houghton et al. 2007; Purcell 2009 and references therein; Prieto et al. 2013; Prieto et al. 2015) providing data on their relative distribution, abundance, seasonality, and interannual variations. Nevertheless, calycophoran siphonophore mass stranding events has never been reported until this Doctoral Thesis.

References

Agassiz L (1862) Contributions to the natural history of the United States of America. IV. Little, Brown & Company, Boston, p 151

Alvariño A (1971) Siphonophores of the Pacific with a review of the world distribution. Bull Scripps Inst Ocean 16:1–432

Aouititen M, Bekkali R, Nachit D, Luan X, Mrhraoui M (2019) Predicting Jellyfish Strandings in the Moroccan North-West Mediterranean Coastline. European Scientific Journal 15(2):72–84. doi: 10.19044/esj.2019.v15n2p72

Ayala DJ, Munk P, Lundgreen RBC, et al. (2018) Gelatinous plankton is important in the diet of European eel (*Anguilla anguilla*) larvae in the Sargasso Sea. Scientific Reports 8(1): 6156. doi: 10.1038/s41598-018-24388-x

Bernard P, Berline L, Gorsky G (2011) Long term (1981-2008) monitoring of the jellyfish *Pelagia noctiluca* (Cnidaria, Scyphozoa) on Mediterranean Coasts (Principality of Monaco and French Riviera). Journal of Oceanography, Research and Data 4:1–10

Boero F (2013) Review of Jellyfish Blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean 92, FAO, Rome, pp 54

Brotz L, Cheung WWL, Kleisner K, Pakhomow E, Pauly D (2012) Increasing jellyfish populations: trends in Large Marine Ecosystems. Hydrobiologia. doi: 10.1007/s10750-012-1039-7

Canepa A, Fuentes V, Sabatés A, Piraino S, Boero F, Gili, J.M. (2014) *Pelagia noctiluca* in the Mediterranean Sea. In: Pitt, K.A. & Lucas, C.H. (Eds.), Jellyfish Bloom. Springer, Netherlands, pp. 237–266. doi.org/10.1007/978-94-007-7015-7_11

Cardona L, Alvarez de Quevedo I, Borrell A, Aguilar A (2012) Massive consumption of gelatinous plankton by mediterranean apex predators. PLoS ONE 7(3): e31329. doi: 10.1371/journal.pone.0031329

Chandler M, See L, Copas K, Bonde AMZ, et al. (2017) Contribution of citizen science towards international biodiversity monitoring. Biological Conservation 213: 280–294. doi: 10.1016/j.biocon.2016.09.004

CIESM (2001) Gelatinous zooplankton outbreaks: theory and practice. CIESM Worshop series 14, Mediterreanan Science Commission, Monaco, 112 pp

Condon RH, Graham WM, Duarte CM, Pitt KA, Cathy H, et al. (2012a) Questioning the rise of gelatinous zooplankton in the world's oceans. BioScience 62(2):160–169. doi: 10.1525/bio.2012.62.2.9

Condon RH, Duarte CM, Pitt KA, et al. (2012b) Recurrent jellyfish blooms are a consequence of global oscillations. PNAS. doi: 10.1073/pnas.1210920110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1210920110

Daly Yahia MN, et al. (2010) Are the outbreaks of *Pelagia noctiluca* (Forskal, 1775) more frequent in the Mediterranean basin? ICES Co-operative Research Report 300: 8-14

Diaz Briz L, Sánchez F, Marí N, Mianzan H, Genzano G (2017) Gelatinous zooplankton (ctenophores, salps and medusae): an important food resource of fishes in the temperate SW Atlantic Ocean. Marine Biology Research 13(6): 630–644. doi:10.1080/17451000.2016.1274403

Dickinson JL, Shirk J, Bonter D, et al. (2012) The current state of citizen science as a tool for ecological research and public engagement. Frontiers in Ecology and the Environment 10(6): 291–297. doi: 10.1890/110236

Doyle TK, Houghton JDR, Buckley SM, Hays GC, Davenport J (2007) The broad-scale distribution of five jellyfish species across a temperate coastal environment. Hydrobiologia 579(1):29–39. doi.org/10.1007/s10750-006-0362-2

Duarte CM, Pitt KA, Lucas CH, et al. (2012) Is global ocean sprawl a cause of jellyfish blooms? Front Ecol Environ 11:91-97. doi:10.1890/110246

Ferraris M, Berline L, Lombard F, et al. (2012) Distribution of *Pelagia noctiluca* (Cnidaria, Scyphozoa) in the Ligurian Sea (NW Mediterranean Sea). Journal of Plankton Research 34(10): 874–885. doi: 10.1093/plankt/fbs049

Gibbons MJ, Richardson AJ (2013) Beyond the jellyfish joyride and global oscillations: advancing jellyfish research. Journal of Plankton Research 35: 929–938. doi: 10.1093/plankt/fbt063

Gili JM, Pagés F (2005) Les proliferacions de meduses. Bol Soc Hist Nat Baleares 48: 9–22

Goy J, Morand P, Etienne M, (1989) Long-term fluctuations of *Pelagia noctiluca* (Cnidaria, Scyphomedusa) in the western Mediterranean Sea. Prediction by climatic variables. Deep Sea Res. Part A 36:269–279

Graham WM (2001) Numerical increases and distributional shifts of *Chrysaora quinquecirrha* (Desor) and *Aurelia aurita* (Linné) (Cnidaria: Scyphozoa) in the northern Gulf of Mexico. Hydrobiologia 451: 97–111

Graham WM, et al. (2014) Linking human well-being and jellyfish: ecosystem services, impacts, and societal responses. Frontiers in Ecology and the Environment 12(9): 515–523. doi: 10.1890/130298

Haddock SHD (2004) A golden age of gelata: past and future research on planktonic ctenophores and cnidarians. Hydrobiologia 530–531: 549–556. doi.org/10.1007/s10750-004-2653-9 Harvey

Haeckel E (1880) System der Acraspeden. Zweite Hälfte des System der Medusen. Verlag von Gustav Fischer, Jena, pp 361–672

Haeckel E (1882) Report on the deep-sea medusae dredged by H.M.S. Challenger during years 1873-1876. Rep Sci Res HMS Challenger (Zoology) 4: 1-154

Hagadorn JW, Dott RH, Damrow D (2002) Stranded on a late cambrian shoreline: Medusae from central Wisconsin. Geology 30(2): 147–150

Hamner WM, et al. (1975) Underwater observations of gelatinous zooplankton: Sampling problems, feeding biology, and behavior. Limnology and Oceanography 20(6): 907–917. doi: 10.4319/lo.1975.20.6.0907

Hamner WM, Dawson MN (2009) A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages.

Hydrobiologia 616:161–191

Hays GC, Doyle TK, Houghton JDR (2018) A Paradigm shift in the trophic importance of jellyfish? Trends in Ecology & Evolution 33(11): 874–884. doi: 10.1016/J.TREE.2018.09.001

Holst S, Sötje I, Tiemann H, Jarms G (2007) Life cycle of the rhizostome jellyfish *Rhizostoma octopus* (L.) (Scyphozoa, Rhizostomeae), with studies on cnidocysts and statoliths. Marine Biology 151(5): 1695–1710. doi: 10.1007/s00227-006-0594-8

Houghton JDR, Doyle TK, Davenport J, Lilley MKS, Wilson RP, Hays GC (2007). Stranding events provide indirect insights into the seasonality and persistence of jellyfish medusae (Cnidaria: Scyphozoa). Hydrobiologia 589 (1):1–13. doi.org/10.1007/s10750-007-0572-2

Kramp PL (1959) Medusae mainly from the west coast of Africa. Mém Inst Sci Nat Belg 3:1–33

Kogovšek T, Bogunović B, Malej A (2010) Recurrence of bloom- forming scyphomedusae: wavelet analysis ofa 200-year time series. Hydrobiologia 645:81–96

Licandro P, Conway DVP, Daly Yahia MN, Fernandez de Puelles ML, Gasparini S, Hecq JH, Tranter P, Kirby RR (2010) A blooming jellyfish in the northeast Atlantic and Mediterranean. Marine Biology 6: 688–91.

Lilley MKS, Houghton JDR, Hays GC (2009) Distribution, extent of inter-annual variability and diet of the bloom-forming jellyfish *Rhizostoma* in European waters. Journal of the Marine Biological Association of the United Kingdom 89(1): 39–48. doi: 10.1017/S0025315408002439

Malej A, Vodopivec M, Lučič D, Onofri I, Pestoric B (2014) The lesser-known medusa *Drymonema dalmatinum* Haeckel 1880 (Scyphozoa, Discomedusae) in the Adriatic Sea. Annales Ser. Hist. nat, 9: 79–86

Mapstone GM (2014) Global diversity and review of Siphonophorae (Cnidaria: Hydrozoa). PLoS One 9:e87737. doi.org/10. 1371/journal.pone.0087737

Mayer AG (1910) Medusae of the world. III. The Scyphomedusae. Carnegie Institution of Washington, Washington

McInnes JC, Aldermann R, Lea MA (2017) High occurrence of jellyfish predations by black-browed and cambell albatross identified by DNA metabarcoding. Molecular Ecology 26(18): 4831–4845. doi: 10.1111/ijlh.12426

Milisenda G, Martínez-Quintana A, Fuentes V, Aglieri G, Bosch-Belmar M, Boero F, Piraino (2018) Reproductive and bloom patterns of *Pelagia noctiluca* in the Strait of Messina, Italy. Estuarine, Coastal and Shelf Science 201: 29–39. doi.org/10.1016/j.ecss.2016.01.002

Mills CE (2001) Jellyfish blooms: are populations increasing globally in response to changing ocean conditions ? Hydrobiologia 451: 55–68

Pauly D (1995) Anecdotes and the shifting baseline syndrome of fisheries. Trends in Ecology & Evolution 10(10):430. doi: 10.1016/S0169-5347(00)89171-5

Prieto L (2018) Diagnosis, prognosis, and management of jellyfish swarms. In: New Frontiers in Operational Oceanography: pp 737–758. doi:

10.17125/gov2018.ch28.737

Prieto L, Armani A, Macías D (2013) Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard , 1827 (Cnidaria : Scyphozoa : Rhizostomeae) on the Atlantic and Mediterranean coasts. Marine Biology 1827. doi: 10.1007/s00227-013-2293-6

Prieto L, Navarro G (2013) Avistamientos de medusas en el litoral andaluz. ISBN: 978-84-695-8463-7, Cadiz (Spain), pp 36. http://www.marinera-medex.eu/index.php?option=311&file=Prieto_Navarro_2013.pdf

Prieto L, Macías D, Peliz A, Ruiz J (2015) Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: a permanent invasion or a casual appearance? Sci Rep 5:11545. https://doi.org/10.1038/ srep11545

Purcell JE (2009) Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. Hydrobiologia 616: 23–50. doi: 10.1007/s10750-008-9585-8

Purcell JE (2012) Jellyfish and ctenophore blooms coincide with human proliferations and environmental perturbations. Annual review of marine science 4: 209–35. doi: 10.1146/annurev-marine-120709-142751

Pugh PR (1989) Gelatinous zooplankton - the forgotten fauna. Prog Underw Sci 14:67–78

Purcell JE, Uye S, Lo W (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans : a review. Marine Ecology Progress Series 350: 153–174. doi: 10.3354/meps07093.

Quoy JRC, Gaimard JP (1827) Observations zoologiques faites à bord de l'Astrolabe, en mai 1826, dans le Détroit de Gibraltar. Ann Sci Nat Paris 10:175–237

Ranson G (1949) Résultats scientifiques des croisières du navire école belge BMercator^ IV. II Méduses. Mém Inst Sci Nat Belg 2(33): 121–158

Rosa S, Pansera M, Granata A, Guglielmo L (2013) Interannual Variability, growth, reproduction and feeding of *Pelagia noctiluca* (Cnidaria: Scyphozoa) in the Straits of Messina (Central Mediterranean Sea): Linkages with temperature and diet. Journal of Marine Systems 111–112: 97–107. doi.org/10.1016/j.jmarsys.2012.10.001

Sabatés A, Pagès F, Atienza D, Fuentes V, Purcell JE, Gili JM (2010) Planktonic cnidarian distribution and feeding of *Pelagia noctiluca* in the NW Mediterranean Sea. Hydrobiologia 645: 153–165. doi: 10.1007/s10750-010-0221-z

Sabatés A, Salat J, Tilves U, Raya V, Purcell J, Pascual M, Gili JM, Fuentes V (2018) Pathways for *Pelagia noctiluca* jellyfish intrusions onto the Catalan Shelf and their interactions with early life fish stages." Journal of Marine Systems 187:52–61. doi.org/10.1016/j.jmarsys.2018.06.013

Silvertown J (2009) A new dawn for citizen science. Trends in Ecology and Evolution 24(9): 467–471. doi: 10.1016/j.tree.2009.03.017

Stiasny G (1936) *Rhizostoma luteum* (Quoy und Gaimard), im Tejo vor Lissabon nachgewiesen. Arch Mus Bocage 7:1–6

Thiebot J B, Arnould JPY, Gomez-Laich A, et al. (2017) Jellyfish and other gelata as food for four penguin species – insights from predator-borne videos. Frontiers in

Ecology and the Environment 15(8): 437–441. doi: 10.1002/fee.1529

Williams EH, Bunkley-Williams L, Lilyestrom CG, et al. (2001) A population explosion of the rare tropical/subtropical purple sea mane, *Drymonema dalmatinum*, around Puerto Rico in the summer and fall of 1999. Caribb J Sci 37: 127-130

Young GA, Hagadorn JW (2010) The fossil record of cnidarian medusae. Palaeoworld 19: 212–221. doi: 10.1016/j.palwor.2010.09.014

OBJECTIVES

The main objective of this Doctoral Thesis is to better understand the spatio-temporal distribution and environmental drivers of populations of gelatinous macroplankton occurring in the southern Iberian Peninsula and their connection at a local and regional scale.

To accomplish the main objective, the following specific objectives have been defined:

- To disentangle the historical status of *Rhizostoma luteum*
- To define the geographic distribution of *R. luteum*
- To reproduce the life cycle of *R. luteum* in the laboratory and describe all development stages of its early life stages
- To parameterize environmental control (thermal and salinity) on the early life stages and asexual reproduction of *R. luteum*
- To categorize other large jellyfish occurring in the Alboran Sea
- To determine the variability of the coastal proliferation of *Pelagia noctiluca* at high temporal resolution and to characterize its interannual and seasonal cycle at a local and regional scale
- To identify the environmental variables associated with *P. noctiluca* presence near the coast
- To estimate the growth parameters of *P. noctiluca*
- To identify the physical forcing on the mass beaching of *Abylopsis tetragona*

OUTLINE OF THE THESIS

The research conducted in order to achieve the objective proposed in this Doctoral Thesis has given rise to seven research articles, which are presented in **Part II** (Results). Four of these articles have been published (Chapter I, III, VI and VII), and one (Chapter IV) is under revision.

Apart from the introduction (**Part I**), this Thesis is organized as follow:

Chapter I – IV is dedicated to the rediscovery of the little-known jellyfish *Rhizostoma luteum*.

Chapter I focuses to disentangle the ambiguity between *R. luteum* and its sister species from the Mediterranean Sea *R. pulmo*, and another Rhizostoma (*Catostylus tagi*) common from the coast of Portugal. For that, a literature review of scientific and grey literature was performed, and photographic and videographic material obtained through a citizen science project were analyse.

The geographic range of *R. luteum* and its seasonal occurrence is updated in ***Chapter II*** using a dataset collected through a citizen science project, media reports and open access database.

Chapter III describes for the first time the life cycle of *R. luteum* from the planula to the young medusa. Its early life history is compared with its sibling species *R. pulmo* and *R. octopus*.

In ***Chapter IV*** laboratory experiments on the early life stages are conducted analyzing the effects of temperature and salinity on the early life stages and the consequences for the adult medusa population.

In ***Chapter V*** reports the observation of a single specimen of the large scyphozoan *Drymonema dalmatinum* from the NW Alboran Sea using photographic material obtained through a citizen science project.

Chapter VI is dedicated to the mauve stinger (*Pelagia noctiluca*). Its interannual and seasonal variations from the NE Alboran Sea is analyzed and its population dynamic is monitored over more than 4 years.

In the last ***Chapter (VII),*** two beaching events of the calycophoran siphonophore occurring on both sides of the Strait of Gibraltar and its correlation to local wind forcing are investigated.

Finally, **Part III** summarizes the main conclusions of this Thesis and future research.

STUDIES ASSOCIATED WITH THIS THESIS

Studies related to the contents of this Doctoral Thesis are summarized below. These have been published in indexed journals:

- **Kienberger K** and Prieto L (2018) The jellyfish *Rhizostoma luteum* (Quoy and Gaimard, 1827): not such a rare species after all. Marine Biodiversity 48:1455-1462. doi: 10.1007/s12526-017-0637-z. Impact factor: 2.077 ***(Chapter I)***
- **Kienberger K**, Riera-Buch M, Schönemann AM, Barsch V, Halbauer R, Prieto L (2018) First description of the life cycle of the jellyfish *Rhizostoma luteum* (Scyphozoa: Rhizostomeae). PLoS ONE 13(8). doi: 10.1371/journal.pone.0202093. Impact factor: 2.766 ***(Chapter III)***
- **Kienberger K** and Prieto L (2018) The first record of *Drymonema dalmatinum* from the northern Alboran Sea (western Mediterranean). Marine Biodiversity 48:1281. doi: 10.1007/s12526-016-0620-0. Impact factor: 2.077 ***(Chapter V)***
- Guerrero E, **Kienberger K,** Villaescusa A, Navarro G, Gili JM, Prieto L (2018) First record of beaching events for a calycophoran siphonophore *Abylopsis tetragona* (Otto, 1823) at the Strait of Gibraltar. Marine Biodiversity 49: 1587. doi: 10.1007/s12526-018-0926-1. Impact factor: 2.077 ***(Chapter VI)***

This paper is currently under review:

- **Kienberger K**, Prieto L, Halbauer R, Bartsch V, Navarro G (2019) Environmental forcing in early life stages of the scyphozoan jellyfish *Rhizostoma luteum* (Quoy and Gaimard 1827). Marine Biology. Under review ***(Chapter IV)***

These papers are under preparation:

- **Kienberger K**, Prieto L, Navarro G. An update of the geographic distribution of the large jellyfish *Rhizostoma luteum* (Cnidaria: Scyphozoa) from the Northeast Atlantic and the Mediterranean Sea. ***(Chapter II)***
- **Kienberger K,** Navarro G, Prieto L. Interannual variability and population dynamic of *Pelagia noctiluca* (Forsskål, 1775) (Scyphozoa: Semaeostomeae, Pelagiidae) in the northern Alboran Sea, SW Mediterranean Sea. **(Chapter VI)**

The following publication has been presented in international conference:

- Beaching of calycophoran siphonophores (Hydrozoa, Cnidaria) in the areas of Algeciras Bay and Tarifa, Gibraltar Strait. Guerrero E, **Kienberger K,** Villaescusa A, Prieto L, Navarro G, Gili JM. 8th Workshop of the Hydrozoan Society 2015, Ischia, Italy.
- Citizen science: a tool to the past and present occurrence of the sporadic *Rhizostoma luteum* in the NE Atlantic and Alboran Sea. **Kienberger K** & Prieto L. 5th International Jellyfish Bloom Symposium 2016, Barcelona, Spain.
- High temporal resolution study all year-round of *Pelagia noctiluca* in the NE Alboran Sea. **Kienberger K**, Navarro G, Prieto L. 5th International Jellyfish Bloom Symposium 2016, Barcelona, Spain.

2. RESULTS

Chapter I

The jellyfish *Rhizostoma luteum* (Quoy and Gaimard, 1827): not such a rare species after all

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ABSTRACT

Rhizostoma luteum was first described in 1827 by Quoy and Gaimard under the name *Orythia lutea* based on nine specimens collected from the Strait of Gibraltar (southern Iberian Peninsula). After 60 years of no scientific records existing for this species, in 2013, a phylogenetic analysis confirmed that *R. luteum* differed from *Rhizostoma pulmo* and *Rhizostoma octopus*. In the present study, we report historical and recent records of living and stranded specimens of *R. luteum* since 1998. We reviewed historical accounts and photographic and videographic materials taken by citizens from the northeastern Atlantic Ocean and the Alboran Sea. Because of its similarity with the closely related Mediterranean *R. pulmo*, *R. luteum* was frequently misidentified in the Alboran Sea and, likewise, with another medusa from the order Rhizostomeae, *Catostylus tagi* in the adjacent Atlantic Ocean coastal regions in the last two decades. The results of this investigation confirm the existence of the scyphomedusa *R. luteum* in the coastal waters of the west and south coasts of the Iberian Peninsula and west and north shores of Africa. Through a citizen science initiative and our own observations, we were able to confirm more than 150 observations of *R. luteum*, over the past 17 years, demonstrating that this medusa is not such a rare species after all.

Keywords: Rhizostomeae, Scyphomedusa, *Rhizostoma pulmo*, *Catostylus tagi*, Citizen science, Alboran Sea

INTRODUCTION

The Scyphozoa constitutes one of the four classes of Cnidaria, containing almost 200 species (Mayer 1910; Kramp 1961; Russell 1970; Arai 1997). This group is found in all oceans and resides in pelagic habitats from the surface to the abyss. Medusae in the order Rhizostomeae are characterised by the absence of marginal tentacles, the structure of the manubrium and its lips branching to form eight oral arms with numerous mouths openings (Mayer 1910; Russel 1970). The systematic division of the genus *Rhizostoma* has been a subject of much discussion in the past and there has been a lot of controversy about the rare species *Rhizostoma luteum*. At present, three species of the genus *Rhizostoma* are recognised (Stiasny 1921; Russell 1970; Holst et al. 2007; Lilley et al. 2009; Fuentes et al. 2011; Prieto et al. 2013): *R. pulmo* (Macri, 1778), *R. octopus* (Linnaeus, 1788) and *R. luteum* (Quoy & Gaimard 1827). *Rhizostoma pulmo* exists in the eastern and western Mediterranean Sea, mainly the Adriatic Sea, the Ionian Sea, the Ligurian Sea, the Tunisian waters and the Black Sea (reviewed in Mariottini and Pane 2010), and the Balearic Sea (Fuentes et al. 2011), while *R. octopus* is established in northwest Europe, being found in shallow coastal hotspots in the Irish Sea (Houghton et al. 2006), in the southern North Sea and the English channel (Lilley et al. 2009) and along the French Atlantic coasts (Lee et al. 2013). *Rhizostoma luteum* is found in the Strait of Gibraltar (Quoy and Gaimard 1827; Grenacher and Noll 1876), off the coasts of southern Portugal (Stiasny 1931, 1936), the west coast of Africa (Ranson 1949; Kramp 1955, 1959), from the Gulf of Cadiz and the Alboran Sea (west and east of the Strait of Gibraltar) and on the Atlantic coast of Morocco (Prieto et al. 2013). Until this present study, *R. luteum* was always referred to as a rare species and only on eight occasions have samples of this jellyfish been collected (Grenacher and Noll 1876; Kramp 1961; Prieto et al. 2013) (see Table 1). In 2013, Prieto et al. (2013) reported the first record of *R. luteum* in the past 60 years. The authors presented reports of live sightings and beached specimens on the Atlantic coast of Morocco and along the south shore of the Iberian Peninsula during summer 2012 and winter 2013 from the Gulf of Cadiz to the Alboran Sea. This was the first account of this species in the southwestern Mediterranean Sea, describing also the genetic signature and confirming the existence of this species.

In the present study, we reviewed the scientific and popular literature (e.g. Underwater Field Guides) for records of *R. luteum*. We also made field observations for

R. luteum between 2013 and 2015. Sightings were compiled focusing mainly on the Alboran Sea and the adjacent Atlantic Ocean coastal regions: Portuguese, Spanish and Moroccan coasts.

MATERIALS AND METHODS

Species identification

To identify the target species in each photograph and video, we used the list of parameters compiled in Table 2, applying morphological characteristics defined in the literature.

Historical scientific literature review

To get a better understanding of where the species has previously been reported, we reviewed all historical references to *R. luteum* by examining the scientific literature since Quoy and Gaimard first described this species in 1827 (see a summary of the historical scientific literature in Table 1).

Table 1 Summary of historical literature review of *Rhizostoma luteum*

Name	**Reference**	**Page Nr.**	**Figure and/or plate**	**Number of specimens collected and location**
Orythia lutea	1827, Quoy & Gaimard	175	IV B, Fig. 1	3 individuals near Ceuta and 6 from the Bay of Algeciras (Strait of Gibraltar)
Rhizostoma lutea	1829, Eschscholtz	51		
R. lutea	1843, Lesson	413		
Stylonectes luteus	1862, Agassiz	151		
Rhizostoma luteum	1876, Grenacher & Noll	160	Fig. XVIII - XX	A few specimens collected near Gibraltar (Strait of Gibraltar)
Stylonectes orithyia	1877, Haeckel			
Pilema orithyia	1878, Haeckel			
P. sylonectes	1879, Haeckel	595		
Rhizostoma luteum	1888, Vanhöffen	43		
R. pulmo var. lutea	1910, Mayer	703		
R. luteum	1921, Stiasny	159		
R. luteum	1931, Stiasny	164	Fig. 4 - 6	1 individual from Cezimbra (Portuguese coast)
R. luteum	1936, Stiasny	1	Fig. 1 - 2	3 individuals from Tejo (Portuguese coast)
R. luteum	1949, Ranson	147		1 individual from the Bay of Tigres, south Angola and 1 from Port Etienne, Mauritania (west coast of Africa)
R. luteum	1955, Kramp	304		1 individual from the Gulf of Guinea (west coast of Africa)
R. luteum	1959, Kramp	26	Fig. 5	5 individuals from different locations on the west coast of Africa
R. luteum	1961, Kramp	378		
R. luteum	1970, Russel	172		
R. luteum	2013, Prieto et al.		Fig. 2	27 individuals from the Gulf of Cadiz, 1 individual from Agadir (west coast of Africa) and 41 individuals in the Alboran Sea

Table 2 List of parameters used to identify target species: *Rhizostoma luteum, Rhizostoma pulmo, Catostylus tagi*

Species	**Colour**	**Shape of mouth arms**	**Terminal appendages**	**References**
Rhizostoma luteum	Umbrella milky bluish-white, with reddish-brown warts. Oral arms bluish with yellowish mouths frills. Terminal appendages deep purple-brown.	The upper arms are longer and more robust than the weaker lower arms and fused throughout, forming a thick manubrium.	Terminal appendages with very long, slender basal stalk and with bean-shape protuberance.	Grenacher and Noll (1876); Mayer (1910); Stiasny (1921); Kramp (1961)
Rhizostoma pulmo	Umbrella milky-yellow, occasionally reddish. Marginal lappets dark violet-blue. Mouth frills orange-yellow to brownish-red or violet. Terminal appendages milky-transparent.	Total length of mouth arms about equal to disc diameter.	Terminal appendages shorter than or equal to upper arm, widest near base, no basal stalk.	Haeckel (1879); Mayer (1910); Stiasny (1921); Kramp (1961)
Catostylus tagi	Umbrella opalescent yellowish or milky bluish-white, sometimes brown. The dendritic ridges of the exumbrellae are reddish or brownish-purple.	The eight oral arms longer than the bell diameter, arm disc wider than bell radius. Terminal end of mouth arms pointed, without appendages.	Without terminal appendages.	Grenacher & Noll (1876); Mayer (1910); Kramp (1961)

Sourcing of photographs of *Rhizostoma luteum*

To acquire both past and present data from the public regarding the presence of *R. luteum,* we launched a citizen science programme. We provided posters with information and contacted professional and amateur photographers and videographers in the study area. They were able to provide opportunistic observations. Additionally, sightings of the three target species (*R. pulmo*, *R. luteum* and *C. tagi*) were compiled from different sources: the media and both national and regional agencies. These included social media sites such as YouTube, Flickr, Facebook and blogs. Also, sightings of these three species were analysed from four open access databases: JellyWatch (http://www.jellywatch.org), PERSEUS (Policy-oriented marine Environmental Redearch in the Southern European Seas; http://www.perseus-net.eu/en/jellyfish_map/index.html), Observadores del Mar (http://www.observadoresdelmar.es/index.php) and iNaturalist (https://www.inaturalist.org). Finally, we examined photographic materials from popular literature referring to the target species present in the Alboran Sea and the northeastern Atlantic Ocean. All sightings were compiled and geo-referenced (see Supplementary Material Appendix A ST1). By having photographic material from each jellyfish, we were able to determine, to the best of our ability, that all sightings close in time and space were actually different specimens. Samples for which there was any doubt were eliminated.

Shoreline surveys for *Rhizostoma luteum*

Over a 2-year period (from October 2013 to October 2015), daily beach surveys and weekly snorkelling surveys (weather permitting) were conducted to record the presence or absence of gelatinous organisms along the beach of La Herradura, the Alboran Sea (36.72111° N, 3.72666° W) and inside the marina (Puerto Deportivo Marina del Este, La Herradura) (see Fig. 1). We measured the bell diameter across the lappets of the collected jellyfish to the nearest cm (oral side facing up), weighed to the nearest 100 g when possible and the terminal appendage length measured when present. To be sure not to recollect the same specimen on the following days, we marked each collected jellyfish with a zip tie attached to its marginal bell.

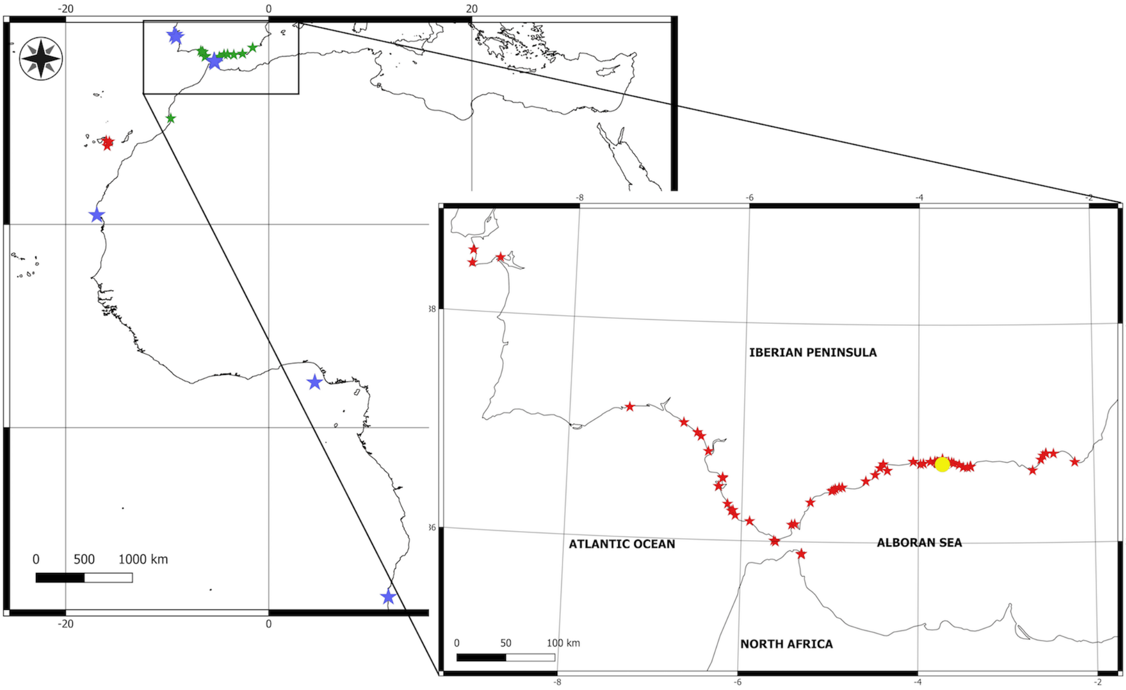

Figure 1 Map of all the observations of *Rhizostoma luteum* compiled in this study: historical scientific data from 1827 to 1955 (blue), Prieto et al. (2013) (green), photographic material 1998–2015 (red) and location of shoreline surveys 2013-2015 (yellow)

RESULTS

Species identification

Rhizostoma luteum (Fig. 2a) differs primarily from its congeners by its distinctive structure of the oral arms, its terminal appendages and its coloration. The umbrella is dome-shaped, milky bluish-white, with small reddish-brown warts. Oral arms are bluish with yellowish mouth frills and the eight terminal appendages are deep purple-brown (Fig. 2b). The bell margin is divided into eight short, oval velar lappets per octant and two smaller pointed rhopalar lappets, having a total of 80 marginal lappets. The proximal portions of the mouth arms are considerably longer and stronger than the distal portion, and throughout most of its length, merge into a thick manubrium. The eight terminal appendages are very long thin stalks and there is a bean-shaped protuberance (Fig. 2c) from the subumbrella wall in each subgenital pit (Grenacher and Noll 1876; Mayer 1910; Stiasny 1921; Kramp 1961).

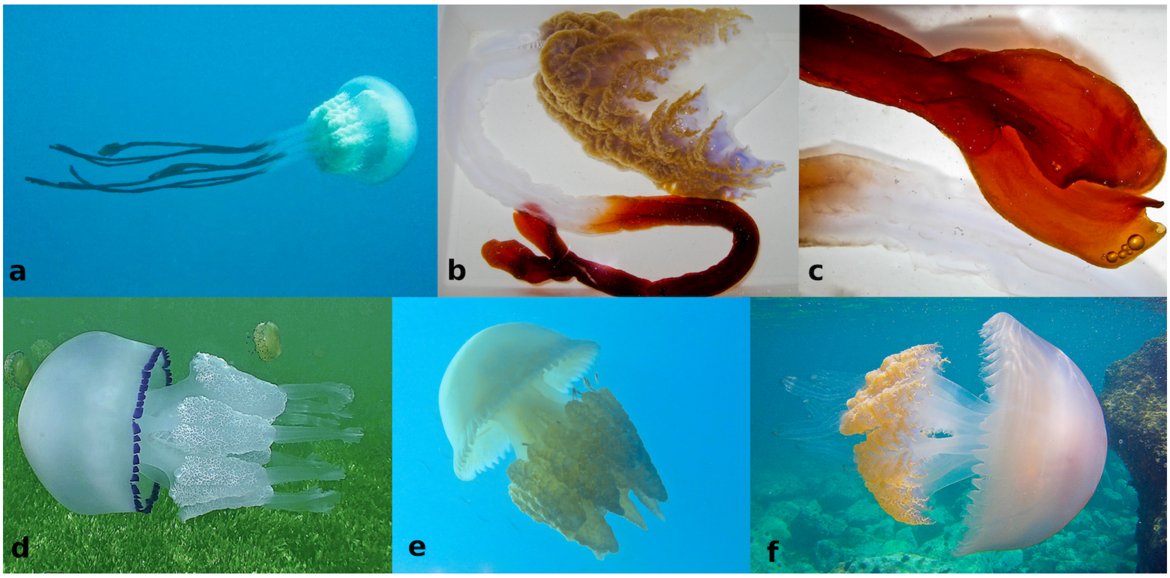

Figure 2 a *Rhizostoma luteum* from La Herradura, northeastern Alboran Sea, photographed on September 23, 2014, bell diameter of 55 cm, longest terminal appendage 165 cm (record number 102, photo by D. Enayati). b A single oral arm of *R. luteum* with its terminal appendage collected on September 18, 2014, total length of the terminal appendage 65 cm (record number 94). c Bean-shaped protuberance of one terminal appendage of *R. luteum* of the same specimen than photograph B. d *Rhizostoma pulmo* from Mar Menor, northwestern Mediterranean, photographed on August 11, 2011, visible are the dark blue marginal lappets and the short terminal appendages (the Mar Menor ecosystem is not included in the compilation of observations performed in this study due to the year-round presence of an isolated population of *R. pulmo*, photo by D. Enayati). e *Catostylus tagi* from Tarifa, Strait of Gibraltar photographed on October 6, 2014, with the characteristically pointy terminal ends of the mouth arms (record number 168, photo by E. Sánchez Castillo). f *Rhizostoma luteum* without any terminal appendages from La Herradura, northeastern Alboran Sea photographed on September 18, 2014 (record number 95, photo by D. Enayati)

The marginal lappets of *R. pulmo* (Fig. 2d) are typically dark cobalt or violet to blue. The umbrella is dome-shaped and milky-yellowish, occasionally even reddish. The surface of the exumbrella is finely granular, being covered with small nettling warts. In each octant, there are eight evenly rounded, velar lappets and two smaller rhopalar lappets, having a total of 80 marginal lappets. The mouth frills are orange-yellow or brownish-red or violet. The total length of the mouth arms are about equal to the disc diameter; the terminal appendages are about one-third of the total length of the arms, triangular in cross-section and widest near their base, and are without a thin basal stalk.

The subgenital papillae are in the form of thickened valves on the marginal edges of the subgenital pits (Haeckel 1879; Mayer 1910; Stiasny 1921; Kramp 1961).

Catostylus tagi (Fig. 2e) differs from the genus *Rhizostoma* by its distinctive structure of the oral arms. The eight oral arms are as long as the bell diameter and the arm disc is slightly wider than the bell radius. The terminal end of the oral arms is pointed and without appendages. The entire medusa is opalescent yellowish or milky bluish-white, sometimes brown, and the dendritic ridges of the exumbrellae are reddish or brownish-purple. The gonads are yellowish. Each octant has four pairs of oval, pointed velar between two small, pointed, ocular lappets, not half as wide or long as the velar lappets, with a total of 80 marginal lappets (Grenacher and Noll 1876; Mayer 1910; Kramp 1961).

Historical scientific literature review

Rhizostoma luteum was described for the first time in 1827 under the name *Orythia lutea* by Quoy and Gaimard. They examined three young medusae captured in the Strait of Gibraltar near Ceuta and 6 from the Bay of Algeciras; the largest specimen was only two thumbs of diameter (taken from the original French description by Quoy and Gaimard and translated by the authors). They described these new species with an umbrella convex, rounded, more than half spherical and very slightly denticulate. Having a thick stalk, and the manubrium divided into four arms, which soon divided to form eight, in which the end of each is slightly forked. In 1862, Agassiz (1862) differentiated this new *Rhizostoma* species from the other ones due to the peculiar shape of the arms; this is the reason why he defined a new genus *Stylonectes*, although he still suggested that *R. luteum* may possibly be a young *R. pulmo*. A few medusae collected from Gibraltar were described and drawn in detail by Grenacher and Noll (1876), leading to an enhanced knowledge about this species. *Rhizostoma luteum* differed primarily from *R. pulmo* and *R. octopus* by its distinctive structure of the oral arms and its terminal appendages. The upper arms are longer and more robust than the weaker lower arms and fused throughout, forming a thick manubrium. Nevertheless, Mayer (1910) recognised only one valid species, *R. pulmo*, referring to the medusae from the Strait of Gibraltar and *R. pulmo* var. *octopus* referring to those of the Atlantic coast of Europe to be local variation. More thorough studies conducted by Stiasny (1921, 1931, 1936) established that all three species of *Rhizostoma* were valid and indicated some real differences (construction of the oral arms, terminal appendages, coloration etc.). Until 1931, this species was only documented from the Strait of Gibraltar; Stiasny (1931) thoroughly described a specimen taken in 1901 near Cezimbra on the Portuguese coast, south of Lisbon, and later on (1936), three specimens were taken in the mouth of the river Tagus (Tejo). Subsequently, in 1949, for the first time, two samples of *R. luteum* were collected from the west coast of Africa. Ranson collected one young specimen from the "Baia dos Tigres", southern Angola, and another one from Mauritania (Port Etienne), similarly concluding that *R. luteum* differs from the other two species. Furthermore, Kramp (1955, 1959) collected various specimens from the west coast of Africa (Gulf of Guinea). He accepted *R. luteum* as a valid species (1961) but recorded *R. octopus* as a local variation. The legitimacy of the three *Rhizostoma* species was finally established by the molecular results performed on the mitochondrial cytochrome c oxidase I (COI) by Prieto et al. (2013), further confirming that morphological identification (Russel 1970) can be considered valid between the three species (for a summary, see Appendix A ST1).

Sourcing of photographs of *Rhizostoma luteum*

This study includes only Rhizostomeae photographs and videos that are clearly identifiable as *R. luteum*, *R. pulmo* or *C. tagi* from the northeast Atlantic Ocean, the Strait of Gibraltar and the Alboran Sea (southwestern Mediterranean Sea). A total of 155 *R. luteum*, six *R. pulmo* and ten *C. tagi* sightings from June 1993 to December 2015 were compiled and geo-referenced (Fig. 1 and see Supplementary Material ST1). One hundred and fifteen photographs were taken from swimming specimens observed by scuba divers and 56 sightings were from beached medusae. Our results include: 106 photographs provided by professional and amateur underwater photographers and videographers, three sightings compiled from the project "Campaña Medusas" by the Spanish Ministry of Environment, 18 found in media reports, 16 photos and videos sourced from searching social media and four observations downloaded from open access databases (see ST1). The first citizen science observation of *R. luteum* occurred in October 1998 (record number 1, see ST1), from the dive spot known as "Peñon de las Caballas", Alboran Sea (36.721111° N, 3.72666° W).

Reviewing the popular literature, referring to the scyphomedusa present in this region, we have been able to confirm the frequent misidentification of *R. luteum* in the past. The following books referred exclusively to *R. pulmo*; however, they were illustrating pictures of *R. luteum*: *Atlas submarino de la Costa del Sol* by Schick (1998), p. 12 (photo p. 13, Fig. 5); *Guía Submarina de Invertebrados No Artrópodos* by Ocaña Martín et al. (2000), p. 77 (photo p. 80); *Fondos Marinos de Murcia* by Calvín (2003), photo p. 168 (photo 410); *Bucear en el último confín de Europa, La Isla de Tarifa* by García - Gómez and Magariño Rubio (2010), photo p. 327 (Figs. 4, 8, 9). Furthermore, Hofrichter (2003) in his book: *Das Mittelmeer* (2003), p. 526, photos *P. pulmo* (p. 512, Fig. 12.27 and p. 528, Fig. 12.42), photo *R. luteum* p. 527 (Fig. 12.41h), displayed pictures of both species, however, referring to them only as *R. pulmo*. To our knowledge, only one guide refers to *R. luteum*, *Fauna Submarina Atlântica* by Saldanha (1997), p. 72, though, without any photographic illustration.

Shoreline surveys for *Rhizostoma luteum*

A total of 15 *R. luteum* were sampled throughout a 2-year period in the region of La Herradura (see ST1): four medusae were beached, two were swimming near the shore, four were caught inside the marina and five were directly measured during snorkel surveys. All free-swimming medusae were actively swimming horizontally between 0 and 8 m, and we observed on, various occasions, that they were swimming against the current. All 15 jellyfish were measured across their widest point as having a diameter between 23 and 58 cm, and a mean diameter of 45.8 cm. There was a tendency of smaller samples during December-January and larger sizes in autumn. The biggest specimen was 58 cm in bell diameter, which was collected on October 12, 2015 (record number 150, Appendix A ST1). Additionally, we weighed eight jellyfish: three beached, four collected inside the marina and one swimming near the shore, with a mass range of 1.25-13 kg (record numbers 84, 85, 86, 94, 101, 102, 122 and 152, see ST1).

All sampled medusae had a dome-shaped, milky bluish-white umbrella with small reddish-brown warts. They had eight oval, pointed marginal lappets per octant and one smaller lappet on each side of the rhopalar. The terminal appendages were very long and had the shape of thin stalks with a bean-shaped protuberance (Fig. 2b, c), to be noted that most jellyfish were missing or had partly dismembered appendages. The longest terminal appendage measured was 210 cm, with a well-developed terminal protuberance; its bell diameter was 47 cm and was sampled on September 5, 2014, during a snorkel survey (record number 91, see ST1). We believe that, since the terminal appendages are very long in adult animals, they might sever easily, as seen in Fig. 2f.

Additionally, on two occasions, January 19 and August 14, 2012, stranded individuals of *R. pulmo* (record numbers 160 and 161, see ST1) were collected during seashore surveys in the Doñana National Park (the Gulf of Cadiz).

Seasonal variation

Rhizostoma luteum

Observations of *R. luteum* between 1998 and 2015 indicate that there was a higher abundance of *R. luteum* during the years, 2015 and 2014, with 39 and 33 observations, respectively (see Appendix ST1). To study the seasonality of this species in the different geographic areas, all the observations have been combined per month and are represented in a single plot where the accumulative number of sightings is relative to the size of the symbol (Fig. 3). During these two decades of recordings, the data indicate a higher seasonal occurrence during autumn for the coast of Portugal, the Gulf of Cadiz and the Strait of Gibraltar (Fig. 3). Meanwhile, all sightings took place during spring for the northwest coast of Africa. In the case of the Alboran Sea, sightings occurred between April and January, covering almost the whole year around (Fig. 3). Moreover, most observations (total number 123) occurred in the geographic area of the Alboran Sea.

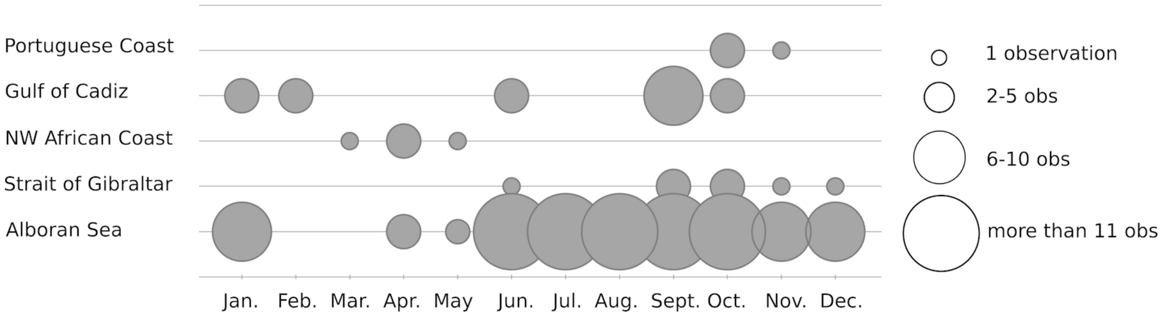

Figure 3 Seasonal occurrence of *R. luteum* from 1998 to 2015 in the different geographic areas. All the observations have been combined per month. The accumulative number of sightings is relative to the size of the bubble: small bubble, one observation; medium bubble, 2-5 observations, big bubble 6-10 observations and large bubble, more than 11 observations

Rhizostoma pulmo and *Catostylus tagi*

The reviewed data revealed only four confirmed observations of *R. pulmo* by scuba divers between 1993 and 2015; all sightings were from the NE coast of the Alboran Sea during summer (see record numbers from 156 to 159, Supplementary Material ST1). There were more spottings in the 1990s, however, without photographic records (personal communication). Our field sampling revealed one sighting in summer and another in winter in the Gulf of Cadiz (record numbers 160 and 161, see ST1). *Catostylus tagi* was observed all year around (record numbers 162-171, see ST1), mostly in the northeast Atlantic Ocean (the Portuguese coast and the Strait of Gibraltar) and only once inside the Mediterranean Sea in summer (August 2009, number 164).

DISCUSSION

The results of this investigation confirm that the existence of the scyphomedusa *R. luteum* is not that rare in the coastal waters of the west and south coasts of the Iberian Peninsula and the northwest shore of Africa. Over the past two decades, we were able to confirm 155 observations, through photographic and videographic identification and field sampling. However, many additional reports were received in which we were not always able to make conclusive identifications; these sightings were not included in this study. Therefore, *R. luteum* may have been even more frequently present over the last two decades. Moreover, the absence of observations during four years (2002-2005) might simply reflect a lack of photographic material, rather than an absence of the jellyfish. On the other hand, the increase of jellyfish sightings over the last five years could be due to the fact that, nowadays, cheaper underwater cameras are available to the public and the participation in citizen science projects is growing, which increase the opportunities of reported observations. Using citizen science is optimal to seek large volumes of field data over a wide geographical area (Silvertown 2009). Moreover, our results show that citizen science is a useful tool to document the past and present presence of gelatinous macroplankton. Due to their relatively large size and fragile body, traditional methods of sampling often fails, as, often, jellyfish caught by conventional plankton nets are strained through the mesh and lost or damaged beyond recognition, or even some are fast swimmer and can usually avoid the nets (Hamner et al. 1975). There have been some very successful citizen science programmes in the past, like the CIESM JellyWatch (2009-2013), the results of which let to the records of new species from the Mediterranean Sea and to a better evaluation of the distribution of native species (Boero 2013). However, citizen science is, in most cases, limited to coastal areas (beach or near shore observation), except for the rare occasions of information provided by the boat owners. To overcome this, observation from cruise boats or aircraft (or nowadays even drones) may be used as a complementary method of observation. As in the Irish Sea, the use of visual surveys from ships of opportunity or aircraft has provided novel insight on *R. octopus* (Doyle et al. 2007; Houghton et al. 2006). Such techniques could provide a rapid way for assessing the broad-scale distribution of *R. luteum*, perhaps identifying coastal and open sea hotspots.

There has been a reappearance of another large scyphomedusa, *Drymonema dalmatinum* (Bayha and Dawson 2010; Malej et al. 2014), from the Adriatic Sea and eastern Mediterranean Sea, which was similarly described as a rare species; however, in the last 15 years, observations of *D. dalmatinum* have been more frequent. The authors speculate that the increase may be due to the proliferation of their prey, *Aurelia* sp. in the last decade (Kogovšek et al. 2010). Maybe there has also been a comparable increase in the prey of *R. luteum* in the last two decades in the western Mediterranean Sea. However, nothing is known about the diet of *R. luteum*.

In comparison to the other two species studied in the present work (*R. pulmo* and *C. tagi*), the number of sightings has only increased in the case of *R. luteum*. It must be noted that most of the observations of *R. luteum* were made in the region of La Herradura, the northeastern Alboran Sea. This region may have an actual greater abundance or it could be that it has a higher occurrence of sightings because of the year-round presence of scuba divers. The limited data obtained in our survey on the occurrence of *R. pulmo* shows that this species was present during 2012 in the Gulf of Cadiz and seemed to have been more abundant in the 1990s in the Alboran Sea. Our results indicate that, at least in the last several decades, the abundance of *R. luteum* was greater than that of *R. pulmo* in the Gulf of Cadiz and the Alboran Sea, although this issue needs further investigation.

Our data show the almost year-round presence of adult *R. luteum* in the Alboran Sea. Comparing our observation of *R. luteum* with that of *R. pulmo* by Fuentes et al. (2011), they observed juvenile *R. pulmo* along the Catalan coast at the beginning of May and adults disappearing at the end of August/September. However, in the Mar

Menor (northwestern Mediterranean), ephyrae appeared at the end of May/June and adult medusae normally disappeared during January to March.

We believe that, that because of its similarity with the closely related Mediterranean *R. pulmo*, *R. luteum* was frequently misidentified in the past and possible the same misidentification of *R. luteum* occurred in the adjacent Atlantic Ocean coastal regions with the very similar *C. tagi*, as this Rhizostomeae is very abundant during summer on the shores of Portugal and, furthermore, can be found on the west coast of Africa and in the Strait of Gibraltar (Grenacher and Noll 1876; Mayer 1910; Kramp 1955, 1961). Nonetheless, the past misidentification of *R. luteum* with *R. pulmo* could explain the lack of scientific records citing *R. luteum* in the southwestern Mediterranean Sea up until 2012 (Prieto et al. 2013). Stiasny, in 1936, previously mentioned this possibility of confusion in the region of the Mediterranean Sea. He mentioned the unexpected slow northern movement of the species, expanding its habitat from the Strait of Gibraltar to the Portuguese coast (Stiasny 1931, 1936), and pointed out the lack of records from the Alboran Sea.

To conclude, this study provides an extended revision of the scientific literature, popular literature and field data confirming the more frequent appearance of the jellyfish *R. luteum* over the past two decades in the Alboran Sea and the adjacent Atlantic Ocean coastal regions. Furthermore, in this framework, citizen science campaigns are an effective tool to study the past occurrence of gelatinous macroplankton.

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REFERENCES

Agassiz L (1862) Contributions to the natural history of the United States of America. IV. Little, Brown & Company, Boston, pp 151

Arai MN (1997) A functional biology of Scyphozoa. Chapman and Hall, London, pp 316

Bayha KM, Dawson MN (2010) New family of allomorphic jellyfishes, Drymonematidae (Scyphozoa, Discomedusae), emphasizes evolution in the functional morphology and trophic ecology of gelatinous zooplankton. Biol Bull 219:249-267

Boero F (2013) Review of Jellyfish Blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 92. FAO, Rome, p 53

Calvín JC (2003) Fondos Marinos de Murcia. Tipos, paisajes, flora y fauna, estado de conservación y mejores inmersiones. Calvín, Murcia, pp 168

Doyle TK, Houghton JDR, Buckley SM, Hays GC, Davenport J (2007) The broad-scale distribution of five jellyfish species across a temperate coastal environment. Hydrobiologia, *579*(1), 29–39 doi:10.1007/s10750-006-0362-2

Eschscholtz F (1829) System der Acalephen. Eine ausführliche Beschreibung aller Medusenartigen Strahltiere. Ferdinand Dümmler, Berlin, pp 51

Fuentes V, Straehler-Pohl I, Atienza D, Franco I, Tilves U, Gentile M, Acevedo M, Olariaga A, Gili JM (2011) Life cycle of the jellyfish *Rhizostoma pulmo* (Scyphozoa: Rhizostomeae) and its distribution, seasonality and inter-annual variability along the Catalan coast and the Mar Menor (Spain, NW Mediterranean). Mar Biol 158 (10):2247-2266

García Gómez JC, Magariño Rubio S (2010) Bucear en el último confín de Europa, La Isla de Tarifa. Instituto de Estudios Campogibraltareños, p 327

Grenacher H, Noll FC (1876) Beiträge zur Anatomie und Systematik der Rhizostomeen. Abhandlungen der Senckenbergischen Naturforschung Gesellschaft, 10. Frankfurt am Main

Haeckel E (1877) Prodrom. System. Med. Nr. 498

Haeckel E (1878) In litteris Mus. Zool. Jenens

Haeckel E (1879) Das System der Medusen: Erster Theil einer Monographie der Medusen. G. Fischer, Jena, pp 672

Hamner WM, Madin LP, Alldredge AL, Gilmer RW, Hamner PP (1975) Underwater observations of gelatinous zooplankton : sampling problems, feeding biology, and behavior. Limnology and Oceanography 20(6):907–917

Hofrichter R (2003) Das Mittelmeer II. Teil 1, Fauna, Flora, Ökologie. OMEGA, Heidelberg, pp 512-528

Holst S, Sötje I, Tiemann H, Jarms G (2007) Life cycle of the Rhizostome jellyfish *Rhizostoma octopus* (L.) (Scyphozoa, Rhizostomeae), with studies on cnidocysts and statoliths. Mar Biol 151:1695–1710

Houghton JDR, Doyle TK, Davenport J, Hays GC (2006) Developing a simple, rapid method for identifying and monitoring jellyfish aggregations from the air. Mar Ecol Prog Ser 314: 159–170

Kogovšek T, Bogunović B, Malej A (2010) Recurrence of bloom-forming scyphomedusae: wavelet analysis of a 200-year time series. Hydrobiologia 645:81-96

Kramp PL (1955) The medusae of the tropical west coast of Africa. Atl Rep 3:239-324

Kramp PL (1959) Medusae mainly from the west coast of Africa. Mém Inst Sci Nat Belg 3:1-33

Kramp PL (1961) Synopsis of the medusae of the world. J Mar Biol Assoc UK 40:7-382

Lee PLM, Dawson MN, Neill SP, Robins PE, Houghton JDR, Doyle TK, Hays GC (2013) Identification of genetically and oceanographically distinct blooms of jellyfish. J R Soc Interface 10: 20120920. doi:10.1098/rsif.2012.0920

Lesson RP (1843) Histoire Naturelle des Zoophytes. Acalèphes. Librairie Encyclopédique de Roret, Paris, p 51

Lilley MKS, Houghton JDR, Hays GC (2009) Distribution, extent of inter-annual variability and diet of the bloom-forming jellyfish *Rhizostoma* in European waters. J Mar Biol Assoc UK 89 (1):39–48

Malej A, Vodopivec M, Lucic D, Onofri I, Pestorić B, (2014) The lesser-known medusa *Drymonema dalmatinum* Haeckel 1880 (Scyphozoa, Discomedusae) in the Adriatic sea. Annales Ser Hist Nat 24:79-86

Mariottini GL, Pane L (2010) Mediterranean jellyfish venoms: a review on scyphomedusae. Mar Drugs 8:1122–1152

Mayer AG (1910) Medusae of the world. III. The Scyphomedusae. Carnegie Institution of Washington, Washington

Ocaña Matín A, Sánchez Tocino L, López González S, Viciana Martín JF (2000) Guía Submarina de Invertebrados No Artrópodos. 2nd Ed. Editorial Comares, Granada, pp 77 - 80

Prieto L, Armani A, Macías D (2013) Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. Mar Biol 160:3241-3247

Quoy JRC, Gaimard JP (1827) Observations zoologiques faites à bord de l'Astrolabe en mai 1826, dans le Détroit de Gibraltar. Ann Sci Nat Paris 10:175-237

Ranson G (1949) Résultats scientifiques des croisières du navire école belge "Mercator" IV. II Méduses. Mém Inst Sci Nat Belg 2 (33):121-158

Russell FS (1970) The medusae of the British Isles II. Pelagic Scyphozoa with a Supplement to the First Volume on Hydromedusae. Cambridge University Press, Cambridge, pp 284

Saldanha L (1997) Fauna submarina Atlântica: Portugal continental, Açores, Madeira. Publicações Europa-América, p 72

Schick KL (1998) Atlas submarino de la Costa del Sol. Schick, Marbella, pp 12-13

Silvertown J (2009) A new dawn for citizen science. Trends Ecol Evol 24: 467–471

Stiasny G (1921) Studien über Rhizostomeen mit besonderer berücksichtigung der Fauna des Malaiischen Archipels nebst einer Revision des Systems. In Capita Zoologica, E. D. van Oort, ed. Martinus Njhoff, Gravenhage

Stiasny G (1931) Die Rhizostomeen-Sammlung des British Museum (Natural History) in London. Zoöl Meded 14:137-178

Stiasny G (1936) *Rhizostoma luteum* (Quoy und Gaimard), im Tejo vor Lissabon nachgewiesen. Arch Mus Bocage 7:1-6

Vanhöffen E (1888) Untersuchungen über Semaeostome und Rhizostome Medusen. Bibliotheca Zoologica, Cassel, pp 43

Chapter II

An update of the geographic distribution of the large jellyfish *Rhizostoma luteum* (Cnidaria: Scyphozoa) from the Northeast Atlantic and the Mediterranean Sea

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ABSTRACT

In ever-changing environment records of species across broad spatial scales are essential for decision-making processes in biodiversity conservation. Hence, fundamental knowledge of the geographic range, interannual and seasonality of many jellyfish (Cnidaria: Scyphozoa) are missing.

The aim of the present paper is to update the geographic range of the large jellyfish *Rhizostoma luteum* from the northeast Atlantic and the Mediterranean Sea and to provide information about its interannual and seasonality occurrence. We used citizen science data, own observations, records from scientific literature, grey literature, and compiled photographic and video images from various online sources (social media and open access platforms). The present work updates the available data set of *R. luteum* up to 2019, as more than 350 observations were compiled since 1987 and expand the range of distribution of this medusa to the Bay of Biscay (NE Atlantic Ocean) and Balearic Basin (NW Mediterranean Sea). Finally, this works validate the use of citizen science data as a tool to study the distribution of macro-gelatinous zooplankton and its interannual and seasonality occurrence.

Keywords: Citizen science, public engagement, *Rhizostoma pulmo*, *Rhizostoma octopus*, *Catostylus tagi,* interannual, seasonality

INTRODUCTION

The geographical range limits of species are dynamic and fluctuate over time, however, climate change is driving a universal redistribution of life on earth. Around the southern Iberian Peninsula, rising ocean temperatures (Shaltout and Omstedt 2014; IPCC 2014) and the decrease in rainfall (Guerreiro et al. 2017) has been documented in recent decades. Therefore, in an ever-changing environment, a precise knowledge of the spatial distribution of taxa is essential for decision-making processes in biodiversity conservation, both for present and under future global changes scenarios. Various studies have revealed that warming oceans might increase the population of jellyfish and shift its distribution, and/or change the timing and length of their seasons (Purcell 2005; Lynam et al. 2011; Astorga et al. 2012; reviewed in Purcell et al. 2012).

Because of the lack of previous data in some regions, the absence of prior records on the species may be misinterpreted as a complete absence of the species over time. Condon and al. (2012) stated that the increase in the number of gelatinous organisms blooms might be a case of “shifting baseline syndrome”. This syndrome was first proposed for the fisheries-related studies by Pauly (1995) and consists in that each generation of marine scientists accept as a baseline the stock-size and species compositions that occurred at the beginning of their career, and used this to evaluate changes. Resulting in a gradual shift of the baseline perception, therefore, using inappropriate reference points. Scientists are increasingly using non-traditional methods to assist with defining biological baselines and for monitoring environmental changes. Citizen science turned to be a powerful tool to study the past/present occurrence and geographic distribution of gelatinous macrozooplankton (Boero 2013; Malej et al. 2014; Pires et al. 2017; Kienberger and Prieto, 2018a, 2018b), and are considered to be a potentially valuable source of information on changes in species distribution and biodiversity (Gledhill et al. 2014). Due to their relatively large size and fragile body, traditional methods of sampling regularly fails, as often, jellyfish caught by conventional plankton nets are strained through the mesh and lost or damaged beyond recognition, or even some are fast swimmers and can usually avoid the nets (Hamner et al. 1975).

The first mention of the large Scyphozoa *Rhizostoma luteum* was by the French naturalists Quoy and Gaimard in 1827 under the name *Ortythia lutea* during a cruise on the exploration ship of the French Navy Astrolabe in May 1826. It was based on nine specimens collected from the Strait of Gibraltar (southern Iberian Peninsula). Until 1959 only on 7 occasions a few (<20) individuals were collected (see review in Kienberger and Prieto 2018a), and was reported from a large geographical range. The most southern report was from southern Angola (west coast of Africa) and the most northern one from the mouth of the river Tagus near Lisbon (Portuguese Coast). During June-July 2012 and January-February 2013 sightings of living and stranded specimens were reported on 15 occasions from the Atlantic coast of Morocco and along the south shore of the Iberian Peninsula and for the first time east of the Strait of Gibraltar (Prieto et al. 2013). In a previous study (Kienberger and Prieto, 2018a), we were able to confirm its recurrent occurrence in the last decades from the NE Atlantic Ocean and the Alboran Sea (SW Mediterranean Sea). *Rhizostoma luteum* was misidentified with it congener *Rhizostoma pulmo* (Macri 1778) from the Mediterranean Sea and with another Rhizostomeae *Catostylus tagi* (Haeckel, 1869), which is common from the Bay of Biscay, Portuguese coast, the Strait of Gibraltar and eastern Central Atlantic Ocean (Kramp 1961). In a review about the distribution of the genus *Rhizostoma* in Europe waters (Lilley et al. 2009), the authors did not find any records of *R. luteum*, and because of the ambiguity between *R. pulmo* and *R. octopus* they stayed at the genus level. Hence, our knowledge of *R. luteum* geographic distribution and seasonality is far from complete. Information on the known geographic distribution of *R. luteum* has mainly been based upon published scientific literature from the last century.

Citizen science data represent a significant and growing source of knowledge that was unachievable otherwise. In spite of the weakness that the obvious disadvantage is the lack of homogeneous data across space and time (Boero 2013; Harvey et al. 2018), its advantage is that jellyfish are often very striking and easy to spot. The aim of the present work is to update the data set of observations of *R. luteum* by extending the previous one to 2019. The newly available information will allow us to update its range for its northern and western boundaries and to provide information about seasonality trends along the northeast and eastern central Atlantic and southwestern Mediterranean coast.

MATERIALS AND METHODS

We analysed photographic and video images obtained from an ongoing citizen science initiative launched in 2013 which provided opportunistic observations, own observations, and reviewed records from the scientific literature, and compiled from different online sources: media (both national and regional agencies), and social media sites such as YouTube, Flickr, Insta Stalker, Facebook and blogs etc. Observations from various open access platform using citizen science data were included: iNaturalist (https://www.inaturalist.org), JellyWatch (http://www.jellywatch.org), Observadores del Mar (http://www. observadoresdelmar.es/index.php), PERSEUS (Policy- References oriented marine Environmental Research in the Southern European Seas; (http://www.perseus-net.eu/en/jellyfish_map/index.html), Red de Observadores del Medio Marino en Canarias (http://www.redpromar.com).

We used morphological characters defined in Kienberger and Prieto (2018a) to identify the target species. All sightings were compiled by date, geo-referenced, mention of swimming/beached individual, and the approximate diameter of the umbrella (see Appendix B ST1). Only records were considered when a clear image was available or a personal confirmation by the observer. To the best of our ability, we were able to determine, that all sightings close in time and space were different specimens by having photographic material for most observations (Fig. 1). Samples for which there was any doubt were eliminated. Many reports were received, though, because of the lack of photographic material or poor condition of specimens conclusive identification was not always possible. These sightings were not included in this study. Consequently, *R. luteum* might have been even more frequently present over the last three decades around the Iberian Peninsula.

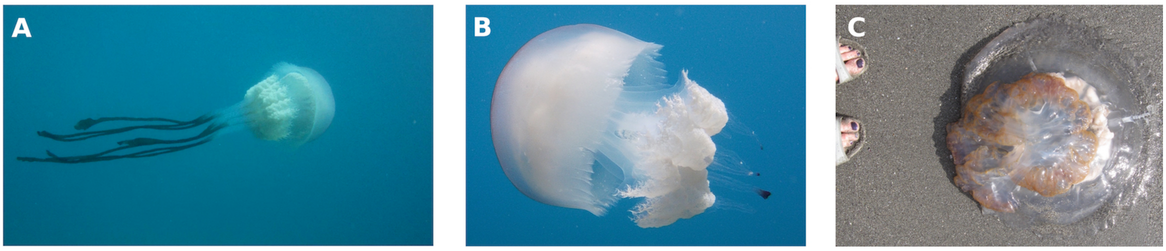

Figure 1 *Rhizostoma luteum* (Quoy & Gaimard 1827). A *Rhizostoma luteum* with eight deep purple-brown terminal appendages with a bean-shaped protuberance (longest appendage 165 cm), bell diameter of 55 cm (September 23, 2014, photo by D. Enayati). B *Rhizostoma luteum* with severed terminal appendage, bell diameter 51 cm (March 30, 2016, photo by K. Kienberger). C *Rhizostoma luteum* beached specimen, bell diameter 52.2 cm, wet weight 5.35 kg without terminal appendages (May 3, 2015, photo by K. Kienberger)

RESULTS AND DISCUSSION

A total of 213 confirmed observations around the Iberian Peninsula and Canaries waters were added in this present study, updating the confirmed sightings of *R. luteum* to 368 observations since 1987 until 2019 (March) see Fig. 2 and listed in Appendix B ST1.

Bay of Biscay and western English Channel (northeast Atlantic Ocean)

A total of 28 observations were reported since 1987 from Pontevedra (NW Spain) to Brittany (NW France). Aguirrezabalaga et al. (1988) reported various specimens seen during a dive in September 1987 from the Bahia de Higuer, (Fuenterrabia, Basque Coast) that were reported as *Rhizostoma* sp. Examining the original photographic material, we were able to determine that all specimens were *R. luteum.* In the local media on various occasions, beached jellyfish were reported as *R. octopus*, *R. pulmo* or *Rhizostoma* sp.

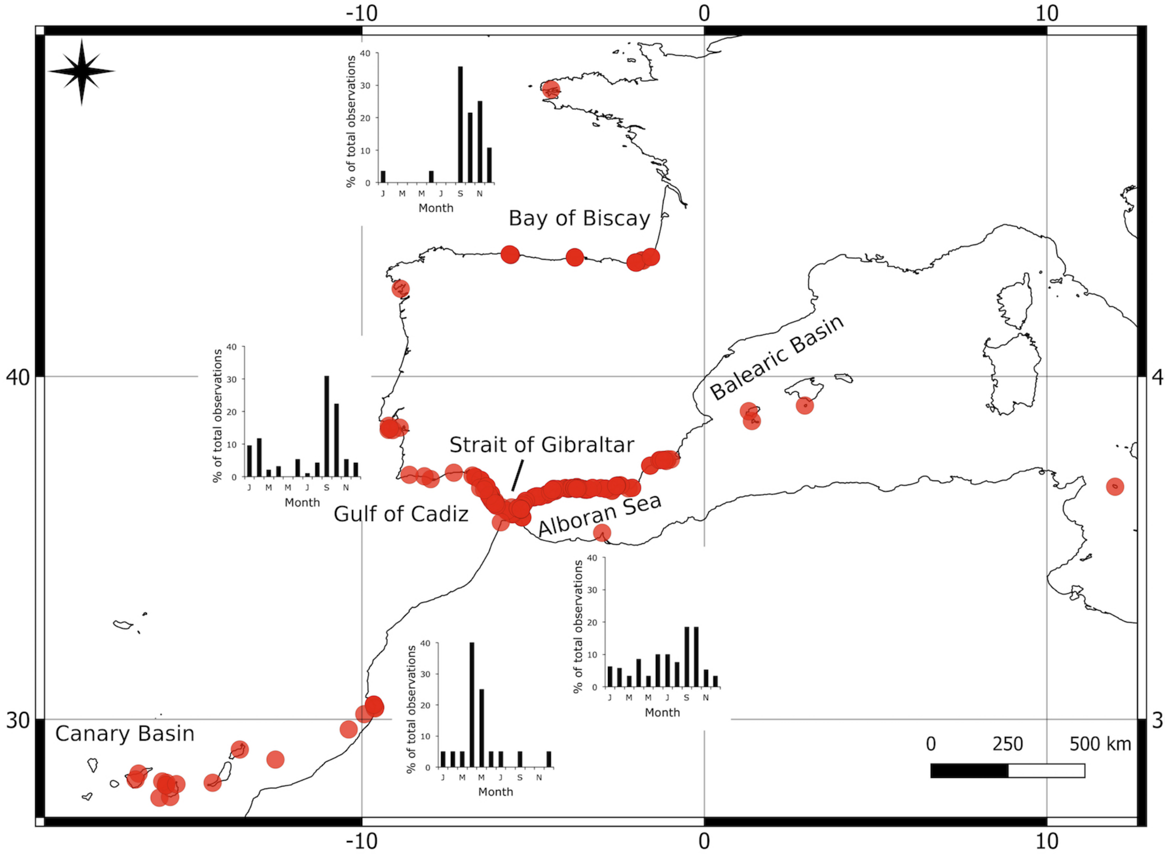

Figure 2 Distribution of *Rhizostoma luteum* from the northeast and eastern central Atlantic, and the Mediterranean Sea

Isolated medusae have been observed from time to time principally during summer-autumn (A. Altuna, personal communication). However, no photographic materials were available. These observations match our results, as most reports were from September to December in the Bay of Biscay. All sightings are from adult medusae (>30 cm). It seems that they were often in poor conditions, this might explain, why until now they have been misidentified with its congener *Rhizostoma octopus* and *Catostylus tagi,* which overlap distribution in the Bay of Biscay (Kramp, 1961).

Our results suggest that the northern range of *R. luteum* might be expanded to the Bay of Biscay. As from the western English Channel only one observation has been reported (Brest, Brittany, Nicolas Djeghri, personal communication), further studies are needed, to be able to determine if this was an occasional sighting or *R. luteum* is actually more common on the coast of western France.

Southern Portugal, Gulf of Cadiz and Strait of Gibraltar (northeast Atlantic Ocean)

A total of 94 sightings of *R. luteum* have been reported from the southwestern Iberian coastal water since 2006. Since 2010 it has been recorded interannual. Observations have been made year-round with a tendency of a higher seasonal occurrence during September-February. Young specimens (bell diameter < 30 cm) were collected from a beach in Doñana National Park on the June 12, 2012 (Prieto et al., 2013). In the same study, at the beginning of both periods of stranding events, strong westerly winds were present.

Eastern Central Atlantic Ocean

Twenty (20) sightings of *R. luteum* were reported since 2007 from the NW Coast of Africa and Canary Basin, with almost annually records since 2011. Most observations occurred in April and May, with sporadic sightings during the other months. All medusae had a diameter over 30 cm. From Canary Island, it is not clear if *R. luteum* low observations were only due to the lack of reports or to its real sporadic occurrence.

Alboran Sea (Southwest Mediterranean Sea)

Since 1998 more than 200 observations of *R. luteum* were reported from the Alboran Sea and has been annually present since 2007. In the Alboran Sea, most observations have been made during 2016 (28%) and a decline of observations has been registered (2017, 2018) in the last two years of our data set (Fig. 3). This decline might be due to a real decline of the abundance of *R. luteum* in the Alboran Sea, or due to a lack of reports. Large specimens were present year-round, with a tendency of a higher amount of sightings from September to October. Young medusae (bell diameter between 9.8-19.3 cm) were collected during February see details in Kienberger et al. 2018.

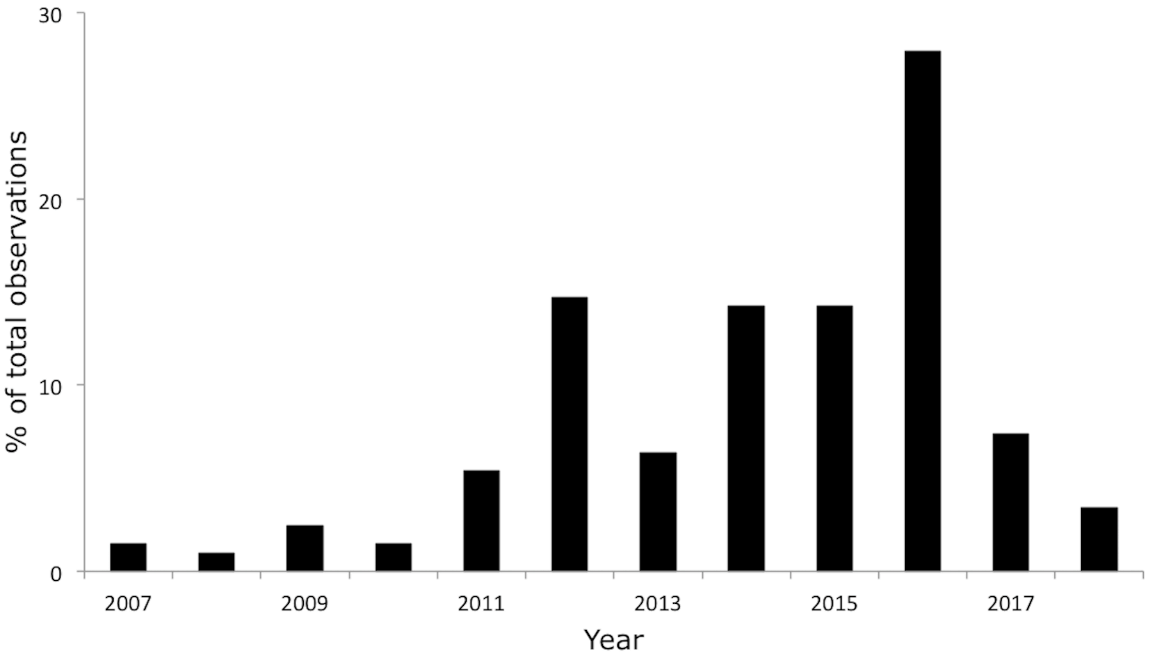

Figure 3 Interannual variability in abundance of *Rhizostoma luteum* in the Alboran Sea (SW Mediterranean Sea) from 2007 to 2018

Coast of Murcia and Balearic Basin (northwest Mediterranean Sea)

Since 2011 a total of 14 specimens of *R. luteum* were reported from the coast of Murcia (Prieto et al. 2013; present study) and the Balearic Basin. From the Balearic Basin, three sightings of *R. luteum* have been registered from Cabrera (March 2016), Formentera (September 2017) and Ibiza (October 2017) thus, these represent the first record of *R. luteum* in the Balearic Basin. Because of its ongoing confusion with *R. pulmo* in this region, its occurrence might have been greater over the last decades.

Central Mediterranean Sea

Boero (2011) reported on one medusa seen from the Island of Pantelleria (Strait of Sicily, southern Italy) on June 2010, which was identified as *Catostylus tagi*. At that time, being the most evident identification, as since 1959 there was no record of *R. luteum*. It was believed that *R. luteum* most distinctive feature was the 8 long, dark purple-brown terminal appendages. However, they sever easily and many medusae are observed with several shorter appendages or even all missing (Fig. 1, present study and see Fig. 2 in Kienberger and Prieto 2018), therefore, the lack of appendages cannot be used as a morphological character. Nonetheless, we were able to ratify this sighting to

R. luteum using the following criteria (Mayer 1910; Grenacher and Noll 1876; Kramp 1961): 1) Structure of oral arms. *Rhizostoma luteum* has eight pairs of scapulets or epaulettes upon the outer sides of the eight mouth arms. The upper oral arms are longer and more robust than the weaker lower arms and fused throughout, forming a thick manubrium. *Catostylus tagi* mouth arms do not bear scapulets and the oral arms are longer than the bell diameter with terminal end pointed without clubs, filaments nor appendages. 2) Morphology of the velar lappets. In *R. luteum* the bell margin is divided into eight short, oval velar lappets per octant and two smaller pointed rhopalar lappets. They are the same colour as the umbrella, which is milkish-white. *Catostylus tagi* has four pairs of large, triangular velar lappets and two small pointed ocular lappets in each octant. The dendritic ridges of the exumbrellae are reddish or brownish-purple (umbrella colouration opalescent yellowish or milky bluish-white, sometimes brown). As stated by Boero this adult jellyfish was evidently transported by strong Westerlies, as winter 2009-2010 featured an anomalous of intensive westerly winds, transporting as well various colonies of the siphonophore *Physalia physalis* (Portuguese man-of-war) originating from the Atlantic Ocean to the eastern Mediterranean basins (Prieto et al. 2015). Nonetheless, our results show that elevated temperature and salinity in this region should not permit the asexual reproduction of the benthic polyp phase in the eastern basin of the Mediterranean Sea (Chapter IV of this Doctoral Thesis).

CONCLUSION

Based on photographic and video images, we updated hereby, *Rhizostoma luteum* geographic range to the Bay of Biscay (NE Atlantic Ocean) and the Balearic Basin (NW Mediterranean Sea). We report the year-round presence of this large *Rhizostoma* from the Gulf of Cadiz, Strait of Gibraltar and the Alboran Sea, with a greater abundance from late summer to winter. This present study supports the clear potential of citizen science in helping to map the geographic dispersion of macro-gelatinous organisms.

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REFERENCES

Aguirrezabalaga F., Altuna A., Miner A., Peña J., Romero A., San Juan R., San Vicente C., Serrano A. & Ibañez M. 1988. Contribucion al conocimiento de la fauna marina en la costa vasca. VI. *Lurralde*, 11: 217-265

Astorga D., Ruiz J. & Prieto L. 2012. Ecological aspects of early life stages of *Cotylorhiza tuberculata* (Scyphozoa: Rhizostomae) affecting its pelagic population success. *Hydrobiologia,* 690(1):141-155. doi: 10.1007/s10750-012-1036-x

Boero F. 2011. New species are welcome, but … what about the old ones? In: *Italian Journal of Zoology*: 1–2. doi: 10.1080/11250003.2011.562708

Boero F. 2013. Review of Jellyfish Blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean 92, FAO, Rome, pp 54

Condon R.H., Graham W.M., Duarte C.M., Pitt K.A., Lucas C.H. & et al. 2012. Questioning the Rise of Gelatinous Zooplankton in the World's Oceans. *American Institute of Biological Sciences*, 62(2),160-169. doi: 10.1525/bio.2012.62.2.9

Gledhill Daniel C., Hobday Alistair J., Welch David J., Sutton Stephen G., Lansdell Matthew J., Koopman Mathew, Jeloudev Adrian, Smith Adam, Last Peter R. (2014) Collaborative approaches to accessing and utilising historical citizen science data: a case-study with spearfishers from eastern Australia. *Marine and Freshwater Research* 66: 195-201. doi.org/10.1071/MF14071

Grenacher H., Noll F.C. 1876. Beiträge zur Anatomie und Systematik der Rhizostomeen. Abhandlungen der Senckenbergischen Naturforschung Gesellschaft, 10. Frankfurt am Main

Guerreiro S.B., Birkinshaw S., Kilsby C., Fowler H.J., Lewis E. 2017. Dry getting drier - The future of transnational river basins in Iberia. *J Hydrol Reg Stud* 12:238-252. doi: 10.1016/j.ejrh.2017.05.009

Hamner W.M., Madin L.P., Alldredge A.L., Gilmer R.W. & Hamner P.P. 1975. Underwater observations of gelatinous zooplankton: Sampling problems, feeding biology, and behavior. *Limnology and Oceanography*, 20(6), 907-917. doi: 10.4319/lo.1975.20.6.0907

Harvey G.K.A., Nelson T.A., Paquet P.C., Ferster C.J. & Fox C.H. 2018. Comparing citizen science reports and systematic surveys of marine mammal distributions and densities. *Biological Conservation*, 226:92-100. doi: 10.1016/j.biocon.2018.07.024

IPCC, 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, RK Pachauri and LA Meyer (eds.)]. IPCC, Geneva, Switzerland. doi: 10.1017/CBO9781107415324

Kienberger K. & Prieto L. 2018a. The jellyfish *Rhizostoma luteum* (Quoy & Gaimard, 1827): not such a rare species after all. *Marine Biodiversity*, 48(3):1455-1462. doi: 10.1007/s12526-017-0637-z

Kienberger K. & Prieto L. L 2018b. The first record of Drymonema dalmatinum from the northern Alboran Sea (western Mediterranean). *Marine Biodiversity*, 48(3):1281-1282. https://doi.org/10.1007/s12526-016-0620-0

Kienberger K., Riera-Buch M., Schönemann A.M., Barsch V., Halbauer R., Prieto L. 2018. First description of the life cycle of the jellyfish *Rhizostoma luteum* (Scyphozoa: Rhizostomeae). *PLoS ONE* 13(8). doi: 10.1371/journal.pone.0202093

Kramp P.L. 1961. Synopsis of the medusae of the world. *J Mar Biol Assoc UK* 40:7–382

Lilley M.K.S, Houghton J.D.R. & Hays GC 2009. Distribution, extent of inter-annual variability and diet of the bloom-forming jellyfish *Rhizostoma* in European waters. *Journal of the Marine Biological Association of the United Kingdom* 89(1): 39–48. doi: 10.1017/S0025315408002439

Lynam C.P., Lilley M.K.S., Bastian T., Doyle T.K., Beggs S.E. & Hays G.C. 2011. Have jellyfish in the Irish Sea benefited from climate change and overfishing? *Global Change Biology*, 17(2):767-782. doi: 10.1111/j.1365-2486.2010.02352.x

Malej A., Vodopivec M., Lucic D., Onofri I. & Pestorić B 2014. The lesser- known medusa *Drymonema dalmatinum* Haeckel 1880 (Scyphozoa, Discomedusae) in the Adriatic sea. Annales *Ser Hist Nat* 9:79-86

Mayer A.G. 1910. Medusae of the world. III. The Scyphomedusae. Carnegie Institution of Washington, Washington

Pauly D. 1995. Anecdotes and the shifting baseline syndrome of fisheries. *Trends in Ecology & Evolution*, 10(10): 430. doi: 10.1016/S0169-5347(00)89171-5

Pires RFT., et al. (2017) Untangling *Velella velella* (Cnidaria: Anthoathecatae) transport: a citizen science and oceanographic approach. *Marine Ecology Progess Series*, 591: 241–251.

Prieto L., Macías D., Peliz A. & Ruiz J. 2015) 'Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: A permanent invasion or a casual appearance?. *Scientific Reports*, 5: 11545. doi: 10.1038/srep11545

Prieto L., Armani A. & Macías D. 2013. Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. Marine Biol ogy, 160(12):3241-3247. doi: 10.1007/s00227-013-2293-6

Purcell J.E. 2005. Climate effects on formation of jellyfish and ctenophore blooms: a review. J Mar Biol Assoc UK 85(3):461-476. doi: 10.1017/S0025315405011409

Purcell J.E., Atienza D., Fuentes V., Olariaga A., Tilves U., Colahan C. & Gili J. 2012. Temperature effects on asexual reproduction rates of scyphozoan species from the northwest Mediterranean Sea. *Hydrobiologia,* 690: 169-180. doi: 10.1007/s10750-012-1047-7

Quoy J.R.C, Gaimard J.P. 1827. Observations zoologiques faites à bord de l'Astrolabe, en mai 1826, dans le Détroit de Gibraltar. *Ann Sci Nat Paris,* 10:175–237

Shaltout M., Omstedt A. 2014. Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. *Oceanologia,* 56(3):411-443. doi: 10.5697/oc.56-3.411

Chapter III

First description of the life cycle of the jellyfish *Rhizostoma luteum* (Scyphozoa: Rhizostomeae)

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ABSTRACT

Jellyfish blooms are a significant environmental problem that is increasing and may be influenced by anthropocentric practices such as overfishing, pollution, eutrophication, translocation, climate change, and ocean acidification. Many jellyfish have unknown life cycles leading to these blooms. We describe for the first time, the life cycle of scyphozoan jellyfish *Rhizostoma luteum* from the planula to the young medusa stages, based on laboratory observations. We also provide a preliminary assessment of temperature related to life stages. Comparisons were made with early life history stages of its sibling species *Rhizostoma pulmo* and *Rhizostoma octopus*. The life cycle of *R. luteum* follows the general pattern of metagenesis of scyphozoans. Scyphistoma culture was maintained in filtered seawater at 17-17.5 °C, salinity 37 and light photoperiod (12:12 h light:dark). Scyphistomae were exposed to an experimental temperature descent for two days to test their survival capacity under severe winter conditions. Only one asexual reproduction mode was observed, which is employed for propagation, consisting of podocyst formation with excystment, subsequent development of scyphistoma, strobilation and liberation of viable ephyra. The development of the ephyra to metaephyra was photodocumented, reaching the metaephyra stage in approximately 21-25 days. Young medusae grow rapidly and maturity was reached after a 3-month post-liberation period with a mean bell diameter of 13.27 ± 2.26 cm and wet weight of 181.53 ± 53 g. The life cycle of *R. luteum* resembles that of its congeners, with the distinction that it has the unique features of being a brooding species (internal fertilisation with subsequent release of planulae) and under the conditions tested, the predominantly strobilation type observed was monodisc, and not polydisc as with the other two species in the genus *Rhizostoma*. As *R. luteum* shows sufficient requisite to form blooms if environmental circumstances change, it is important to understand its life cycle.

Keywords: blooming species; brooding species; ephyrae; monodisc; planulae; podocysts; polyps; scyphistomae

INTRODUCTION

In recent decades, blooms (sudden outbreaks of one species which comes to dominate the plankton for a period) of jellyfish in coastal waters appear to be increasing in both frequency and intensity, producing negative ecological, social, and economic impacts. Jellyfish blooms are a significant environmental problem and may be influenced by anthropocentric practices/stressors such as overfishing of predators and competitors, accidental translocations, eutrophication of coastal waters, pollution, aquaculture, artificial structures, changes in freshwater flows, ocean acidification, and climate change [1-5].

The scyphozoan medusa *Rhizostoma luteum* (Quoy & Gaimard 1827) is an eastern Atlantic species with a distribution range from Lisbon (39°N) to Angola (16°S), entering the Alboran Sea (southwestern Mediterranean Sea) through the Strait of Gibraltar with its most eastern range being 2°E (Aguilas, Cartagena) [6, 7 and references therein]. The northern Alboran Sea includes the world-renowned Costa del Sol, which counts for 42% of all tourism in Andalusia (southern Spain). The most important sector for Andalusia is the service sector (sea-based economies), with nearly 30 million annual visitors and with revenues from tourism of €13,830 million for 2017 [8]. Jellyfish stings are among the most common reasons for requesting medical assistance at first aid stations in the summer months. Negative media reports may arise if the closing of beaches persists because of the presence of jellyfish, and could drive tourists to seek alternative destinations. *Rhizostoma luteum* is moderately venomous in a similar manner to *R. pulmo* (Kienberger, pers. observ.). However, because of its larger size (about 70 cm in diameter [6, 7]), the presence of *Rhizostoma luteum* near the coasts can have a negative impact on the image of touristic areas, as aforementioned, which are very sensitive to such issues.

Rhizostomeae are characterised by the absence of marginal tentacles and the manubrium forms eight oral arms with numerous mouth openings [9, 10]. The genus *Rhizostoma* contains currently three species recognised as valid [6, 9, 10]: *Rhizostoma pulmo* (Macri 1778) from the Mediterranean Sea and the Black Sea; and *Rhizostoma octopus* (Linnaeus 1788) appearing in northwest European coastal waters. In 2013, Prieto et al. reported the first record of *R. luteum* in the past 60 years and confirmed the validation of this species in the genus using molecular analysis that was performed on the mitochondrial cytochrome c oxidase I (COI) [6]. Until the study in 2017 [7], it was believed that this jellyfish had a sporadic occurrence, however, the authors demonstrated that this *Rhizostoma* was not as rare as was previously assumed. *Rhizostoma* was merely misidentified in the past along with its congener *R. pulmo* and another Rhizostomeae, *Catostylus tagi*, since there is an overlapping of their distributions with *R. luteum* in some regions.

Several previous authors have provided a description of adult specimens of *R. luteum* [6, 7, 9-14], however, the stages of the life history have never been reported until the present study. Meanwhile, the life stages of its congeners are better studied by various authors. Because of the increased interest in jellyfish for both environmental and socio-economic reasons, it is important to study scyphozoan life cycle and population dynamics, as these can provide important information with which to understand inter-annual fluctuations and which may also offer clues towards a better understanding of their role in marine ecosystems. However, the complete life cycle has been described for only a few Rhizostomeae [reviewed in 15], and the knowledge of the sessile stages is still scant. Scyphistomae for the vast majority of species have never been found in their natural environment on account of their small size. Considering that the varieties of modes of asexual reproduction are more diverse than previously assumed, their ecological consequences have probably been underestimated [16, 17]. It is necessary to understand all stages of the life cycle, and not just the free-swimming medusa stage. Furthermore, as the ephyra stage of most species is very similar, it is important to include a detailed identification key when describing a new life cycle, which can be used to identify ephyrae, thus enabling the early detection of harmful jellyfish blooms in plankton samples. A study in 2010 established in the pattern of the gastric system, the only character that was constant in newly detached ephyra which was, therefore, the most reliable characteristic for identification [18].

Because of its broad distribution, the minimum sea surface temperature varies greatly in winter from 14 °C for Portugal, Malaga and Cartagena, to 25 °C for the Gulf of Guinea [19]. Therefore, all or some life stages might be exposed to substantial temperature changes whereby, on the one hand, there is exposure to seasonal temperature fluctuation and, on the other hand, there are oscillations due to strong regional coastal upwelling [20-22]. Many studies have shown that temperature changes (warming or cooling) affect the asexual reproduction and strobilation rates of the scyphistomae [reviewed in 23].

The aim of the present paper is to describe for the first time the stages of the life cycle from planulae to young medusae of *R. luteum*, based on observations made on laboratory cultures and data collected *in situ*. Comparisons are made of its life cycle with its sibling species *R. pulmo* and *R. octopus.* Additionally, we conducted a preliminary assessment of the effects of temperature on the early life stages.

MATERIALS AND METHODS

Ethics statement

The jellyfish *Rhizostoma luteum* is not an endangered or protected species. No specific permissions were required for the location and activities of our field studies since it is a public dive location.

Planulae

Planulae were collected on October 12, 2015, during weekly snorkel surveys and records were kept of the presence or absence of gelatinous organisms in the dive location, known as Marina del Este, La Herradura, southern Spain (36.720278° N, 3.728333° W). Planulae were gathered directly from the gonad of a free-swimming female medusa (Fig. 1) by puncturing the epidermis, using small scissors and a Pasteur pipette under the water. The medusa was swimming at a depth of 4 m; the bell diameter was 58 cm (measured during the collection of the planulae). The sea temperature and salinity 21.06°C and 37.46, respectively, were obtained by using a conductivity-temperature-depth instrument (CTD, NKE Instrumentation). Directly after collecting the planulae (during less than one hour), their identity was confirmed using a microscope and they were allocated to 220 ml (diameter 6.5 cm; depth 5 cm) glass jars filled with non-filtered seawater. The following day, they were transported for subsequent development of the life cycle to the Institute of Marine Sciences of Andalusia (Spanish acronym is ICMAN-CSIC) in Cadiz, southern Spain, and to the Vienna Zoo, Austria.

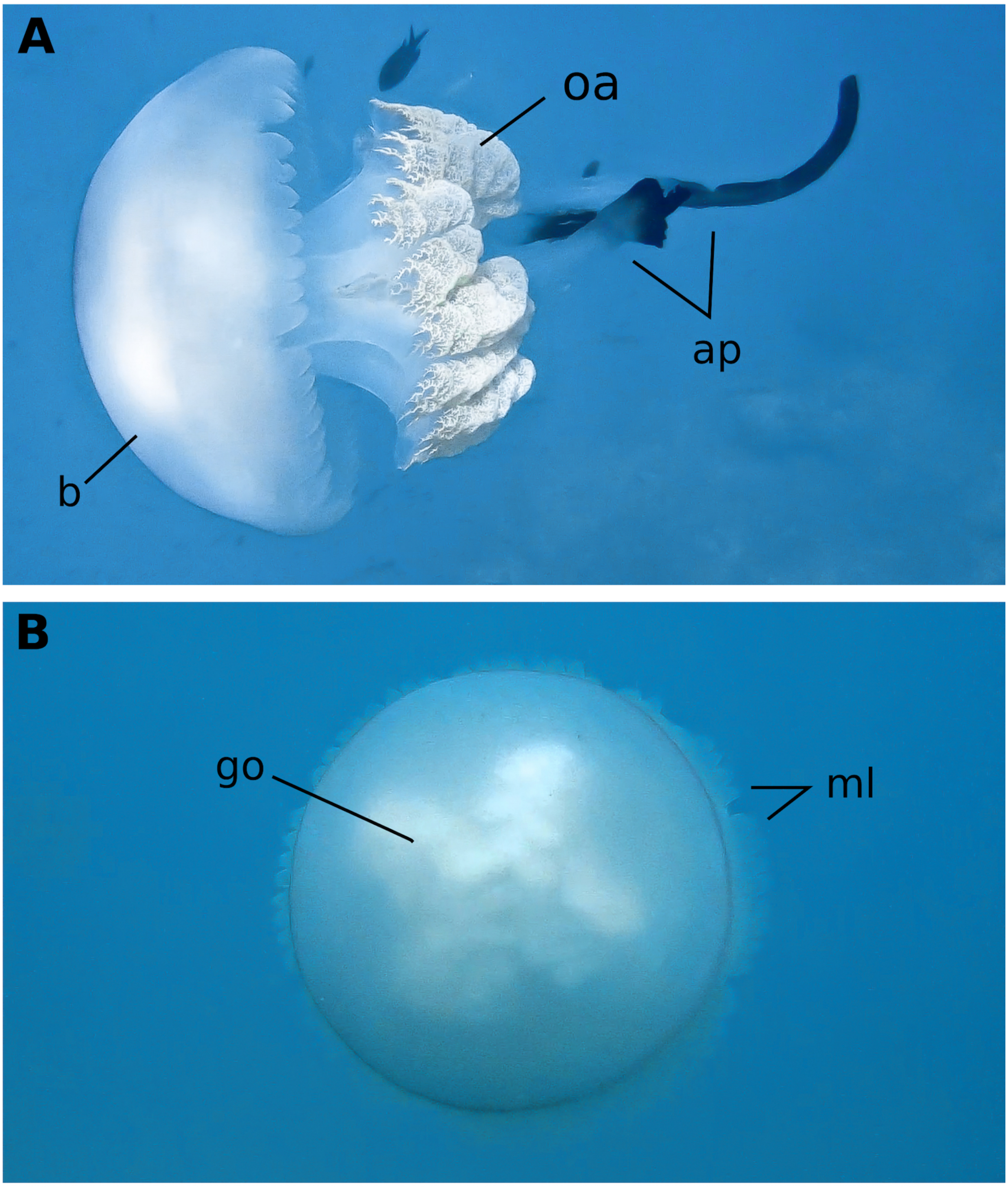


Figure 1. Adult *Rhizostoma luteum*. Female adult medusa swimming in the coastal water of La Herradura, NE Alboran Sea, photographed on 12th October 2015, bell diameter of 58 cm; (A) Side view; (B) Aboral view. *ap* appendages, *b* bell, *go* gonad, *ml* marginal lappet, *oa* oral arms. Photos by D. Enayati

Scyphistomae

A glass slide (7.5 x 2.5 cm) was used as a substrate for the planulae to attach themselves to in each flask. The scyphistomae culture was maintained with filtered seawater without circulation in an incubator (IBERCEX F-4) with light photoperiod (12:12 h light:dark) and fed once a week with rotifers *at libitum* in the dark. Two hours after feeding, the rearing medium was exchanged with previously aerated seawater. The conditions of the seawater were 17-17.5 °C, salinity 37. Light intensity was 360 mmol quanta m^{-2} s^{-1}. The light source consisted of four Philips MASTER TL-D 18W/840 fluorescent tubes. The light intensity was measured using a calibrated Biospherical Instrument sensor (QSL2100, San Diego, CA, USA).

Since *R. luteum* is common to the southwestern Mediterranean Sea, it is plausible that some medusae could eventually enter the coastal lagoon of the Mar Menor (southeast Spain). Due to the dimensions of the lagoon, the temperature can drop to 4 °C in severe winter conditions, which have been demonstrated to affect scyphistomae survival of other species [24]. In order to check the effect of a drop in temperature for this species, the experiment consisted of two treatments. Scyphistomae in the first treatment (4 replicates) were exposed to a temperature of 4 °C for two days, and the control group (3 replicates) stayed at a constant temperature. The average number on individuals per replicate was 6 (S2 Dataset, Appendix C). The drop in temperature was obtained by decreasing at a rate of 1 °C per hour. After two days at 4 ºC, the scyphistomae were again maintained at the previously described culture conditions. The number of podocysts produced, podocysts developing into scyphistomae, and ephyrae liberated under constant condition were monitored weekly for 16 weeks. All stages were examined and photographed under a binocular dissecting microscope.

Ephyrae

Ephyrae were cultured in 200-ml glass flasks at constant temperature and salinity, 23 °C and 33, respectively, and with light photoperiod (12:12) at the Aquarium House of the Vienna Zoo, Austria. Water baths maintained stable temperatures (± 0.5 °C) and artificial seawater was made by dissolving a commercially prepared sea salt mix (Instant Ocean® Synthetic Sea Salts) in reverse-osmosis water waiting, 24 hours for the salt to dissolve properly. Ephyrae were fed daily with newly hatched *Artemia salina* nauplii, enriched with Selco$^{®}$ S.presso *ad libitum*. After 4 hours of feeding, ephyrae were transferred daily, using a small container, to new flasks with fresh, previously aerated seawater.

Metaephyrae were reared to young medusae in laboratories at the Aquarium House of the Vienna Zoo, Austria in jellyfish kreisel tanks (hereinafter, kreisel) having volumes of 8, 15, 300 and 600 l. The 8 and 15 l kreisels were aerated but not connected to a Life Support System, hence 100% water changes were performed daily while the metaephyrae were fed twice daily with enriched *Artemia* nauplii. The kreisels were surrounded by a water bath, which was connected to an aquarium chiller (Aqua Medic Titan 1500). The temperature and salinity was at a constant, 24 ± 0.5 °C and 34 ± 0.5, respectively. Young medusae were transferred to kreisels with 300 and 600 l volume. Every kreisel was connected to a Life Support system consisting of a bio filter (10 l Sera Siporax), a protein skimmer (H&S 300-2xF5000) and an aquarium chiller (Aqua Medic Titan 1500). Freshly hatched enriched *Artemia salina* nauplii were given to the young medusae ad *libitum* twice daily. Water changes (100%) and basic cleaning of the kreisels were undertaken every 3 days.

Daily photographing of 10 ephyrae using a Nikon® SMZ800N stereomicroscope, and the image-processing NIS-Elements software, was undertaken in order to follow the development of the gastric system, manubrium and marginal lappets until reaching the metaephyra state (Stage 7). Each ephyra was placed in a petri dish with the manubrium facing upside. Magnesium was not used to achieve a slowed pulsation of the ephyra. Instead, the surrounding water was removed using a plastic pipette, leaving only a small sheet of water over the flattened ephyra. To avoid stress, the photo was taken as quickly as possible, when the ephyra was fully expanded. Afterwards, the medusa was transferred back into the culturing flask. The following standard measurements for scyphistomae [25] and ephyrae [18] were used: total body length (TBL), hypostome length (HL), calyx length (CL), stalk length (StL) and mouth disc diameter (MDD), total body diameter (TBD), central disc diameter (CDD), total marginal lappet length (TMLL), lappet stem length (LStL), rhopalial lappet length (RLL) and manubrium length (ML). We used the following proportions (%) to compare body proportions of scyphistomae compared to body length: CL/TBL x 100, HL/TBL x 100, StL /TBL x 100, MDD/TBL x 100. Moreover, ephyrae measurements compared to body diameter: RLL/TBD x 100, LStL/TBD x 100, CDD/TBD x 100 and lappet length:

RLL/TMLL x 100, LStL/TMLL x 100. A total of 10 scyphistomae and 10 ephyrae from 10 strobilae were measured. Data are presented as mean ± 0.5 mm standard deviation.

Young medusae

Twelve (12) young medusae reared in the laboratory were measured after 56 and 106 days post-liberation (n =5 and n=7, respectively) and compared to 7 young jellyfish that had been collected in their natural environment inside the marina (Puerto Deportivo Marina del Este, La Herradura, Southern Spain) in February 2016. During the sampling days, the sea temperature and salinity were obtained by using a conductivity-temperature-depth instrument (CTD, NKE Instrumentation) and had an average of 15.07 °C and 37.13, respectively. Individual bell diameter across the lappets was measured to the nearest 0.1 cm by placing the jellyfish flat onto a plastic ruler (oral side facing up), and it was weighed to the nearest 1 g. After amputating the manubrium at its base, morphometric measures were taken to the nearest 0.1 cm. Sex and gonadal maturity state were determined using a microscope.

Statistics

Statistical analyses of the data were performed using R (v3.4.1) software. An ANOVA assumption was tested on a dataset prior to the evaluation of variance. If data failed normality, a non-parametric Kruskal-Wallis analysis of variance was used. To compare the data before and after the drop in temperature, a Welch Two Sample t-Test was conducted. The significance of which was judged at the 0.05 level. Linear regression was used to test the bell size variability in relation to the wet weight of a young medusa.

RESULTS

Planulae

The gonads are visible from the aboral view in the adult jellyfish (Fig. 1B). *Rhizostoma luteum* has separate sexes, and the planulae are brooded by the female medusae inside the gonadal cavity. Planulae had a two-layered structure, white in colour, oval and elongate (Fig. 2A), the sizes varied from 126 to 139 µm in length and 73 to 106 µm in width (n=4; 132 ± 5 µm lengths, 84 ± 15 µm widths). Planulae placed in glass jars immediately moved through the water column while rotating around their own axis, but then they were negatively buoyant, and accumulated near the bottom of the jar.

Settlement of planulae was observed after 3 to 5 days; however, 15 days later some free-swimming planulae were still observed. There was no significant preference of settlement on the bottom of the glass flasks (BOTT), glass slide (GS) and sides of glass flasks (LAT) (Kruskal-Wallis test, P=0.23; n=20), see Fig. 3. Notwithstanding, no settlement was observed at the air-water interface. The raw data file has been included in the Appendix C (S1 Dataset).

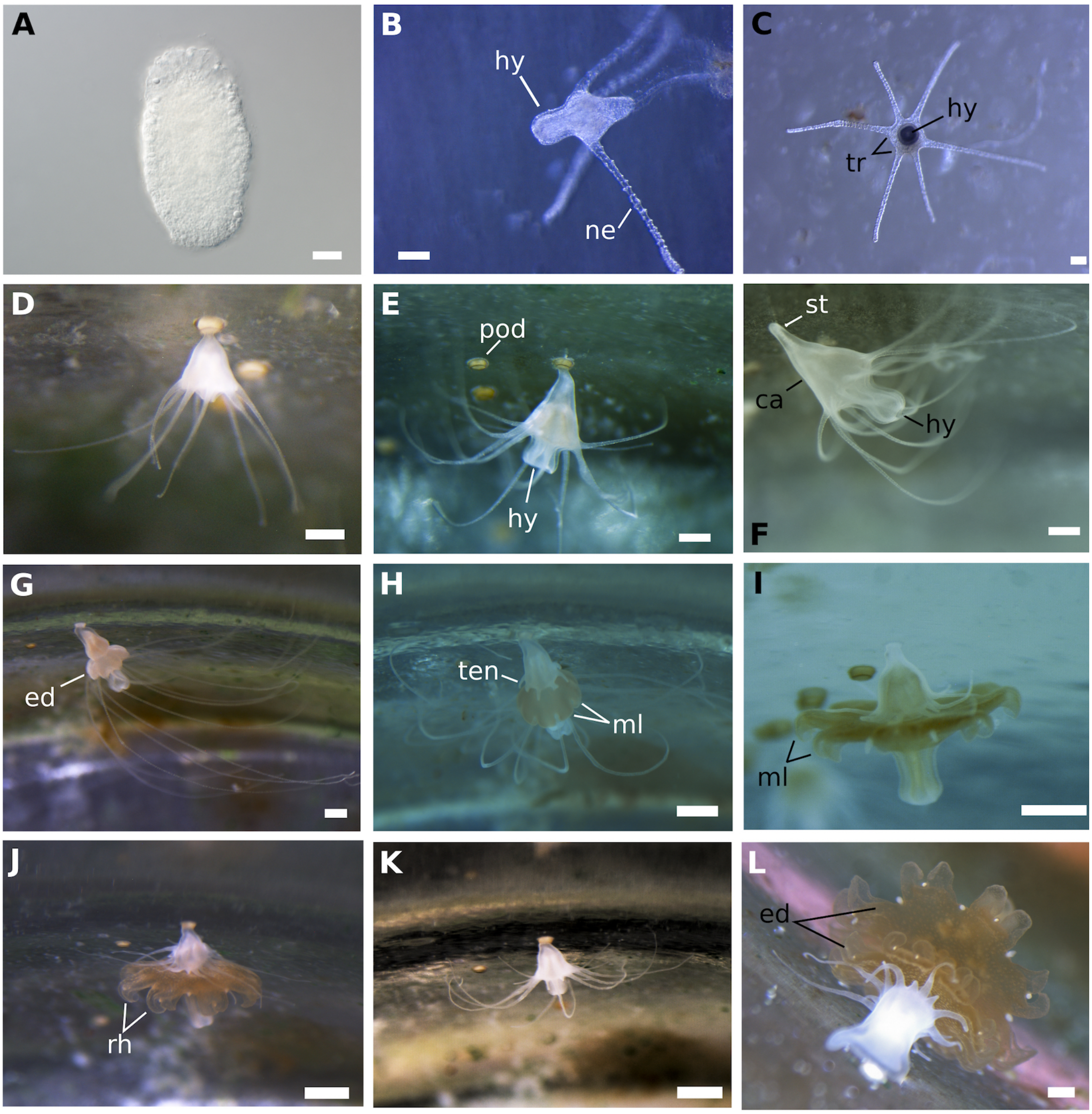


Figure 2. Various stages of the life cycle of *Rhizostoma luteum*. (A) Planula larva with a two-layered structure; (B) Newly metamorphosed 4-tentacled scyphistoma; (C) Top view of a 6-tentacled scyphistoma; (D) Young scyphistoma with 8 filiform tentacles and prominent hypostome; (E) Intermediate 12-tentacled scyphistoma; (F) Fully developed scyphistoma with 16 filiform tentacles; (G-J) Strobilation stages of the same scyphistoma during laboratory monitoring; (G) Early strobila with developing segments; (H) Basal scyphistoma regenerating new tentacles; (I) Mid-strobila with regressing tentacles and developing ephyral segment; (J) 4-day-old strobila about to liberate one ephyra; (K) Residuum after releasing ephyra; (L) Strobila with two ephyrae. *ca* calyx, *ed* ephyra disc, *hy* hypostome, *ml* marginal lappets, *ne* nematocysts batteries, *pod* podocyst, *rh* rhopalium, *st* stalk, *ten* tentacle, *tr* tentacular ring. Scale bar 1 mm for all except (A) 20 µm and (B, C) 100 µm. Photos by T. Schwaha: A; K. Kienberger: B, C; M. Riera-Buch and A.M. Schönemann: D-L

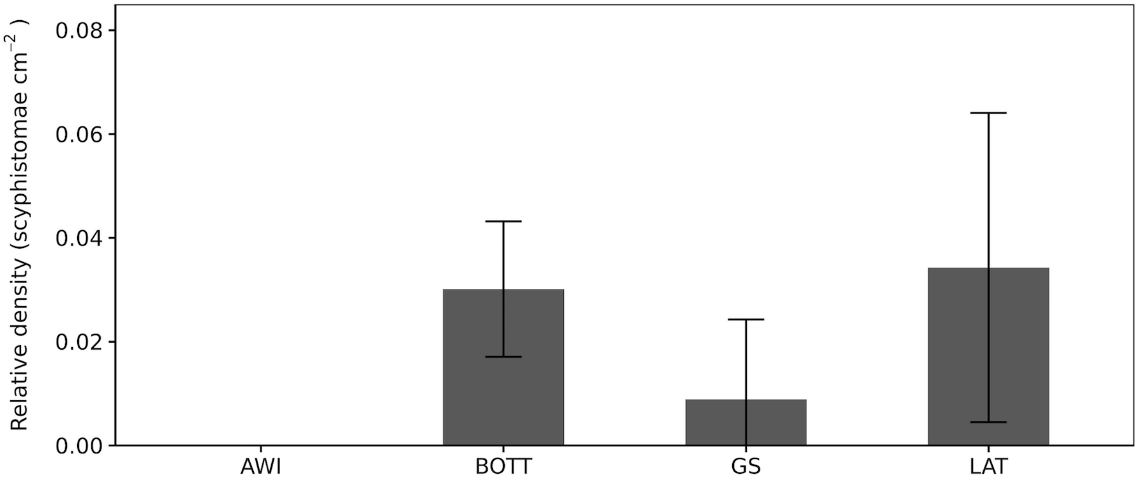
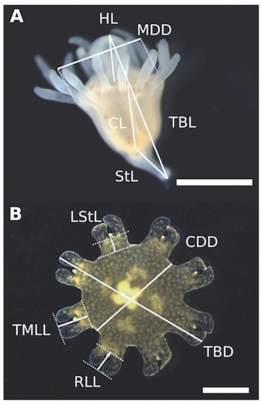


Figure 3 Settlement preferences of *Rhizostoma luteum* planulae. Mean density of scyphistomae per substrate. *AWI* air-water interface; *BOTT* bottom of the glass flasks: *GS* glass slides; *LAT* sides of glass flasks. Error bars are the standard deviations of the 4 replicates

Scyphistomae

Approximately 72 hours after the planulae had settled, translucent-white scyphistomae with four primary tentacles developed. Nematocysts batteries were visible on the tentacles (Fig. 2B). Cup-shaped scyphistoma with 8 and 12 tentacles with a prominent club-shaped hypostome developed after approx. 2 - 3 weeks (Fig 2D,E). Around 5 weeks after the planulae had settled, fully developed (16-tentacles) scyphistomae matured (Fig. 2F) and had a conical to broad funnel-like shape. The total body length (TBL) varied from 1.26 to 2.89 mm (n=10; 1.79 ± 0.48 mm) and had a mean mouth disc diameter (MDD) width of 1.02 mm (see Fig. 4 for body proportions). The number of tentacles was highly variable, up to 21, but typically scyphistomae had 16 filiform tentacles. A single whorl of tentacles surrounded the peristome and the conspicuous, four-lipped and club-shaped hypostome, about 35% of the TBL. The scyphistomae colour ranged from opaque to white and became light rose-orange after ingestion of nauplii. The base of the scyphistomae was attached to the substrate by a thin, short stalk (about 20% of TBL). Over the 16 weeks of monitoring after the decrease in temperature, ratios were calculated from the initial number of scyphistoma before the change in temperature, maintaining the ratio over time for the treatment with the temperature shock, and with a slight increase in the number of scyphistoma for the control (Fig 5). An independent-sample t-test was conducted to compare the total number of scyphistoma with and without a temperature shock. There was no significant difference in the mean number of scyphistoma with and without a temperature shock (see Table 1). The raw data file has been included in the Appendix C (S2 Dataset).



	SCYPHISTOMA	
Anatomy:	**Stalk and calyx region:**	**Body proportions:**
• 16 filiform tentacles • Hypostome: conspicuous, four-lipped and club-shaped • Widest body diameter is the mouth disc	• Thin, short stalk • Calyx shape: cone to broad funnel-like shape	• HL ≈ 35% of the TBL • CL ≈ 45% of the TBL • StL ≈ 20% of the TBL • MDD ≈ 57% of TBL
	EPHYRA	
Anatomy:	**Gastric system:**	**Body proportions:**
• 8 lappet stems • 16 bread knife-like rhopalial lappets • 3-7 gastric filaments per quadrant • Long manubrium	• Velar canals are rhombical • Rhopalial canals are slightly forked: the sharp tips end at the base of the rhopalium	• RLL ≈ 12% of the TBD • LStL ≈ 12% of the TBD • CDD ≈ 60% of the TBD • ML ≈ 45% of the CDD • RLL ≈ 50% of the TMLL • LStL ≈ 51% of the TMLL

Figure 4 Morphology and body proportions of *Rhizostoma luteum* scyphistoma and ephyra. (A) Measuring points and measurements defined for scyphistomae: *CL* calyx length, *HL* hypostome length, *MDD* mouth disc diameter, *StL* stalk length, *TBL* total body length; (B) Measuring points and measurements for newly released ephyra: *CDD* central disc diameter, *LStL* stalk length, *ML* manubrium length, *RLL* rhopalial lappet length, *TBD* total body diameter, *TMLL* total marginal lappet length. Scale bar 1 mm. Photos by K. Kienberger

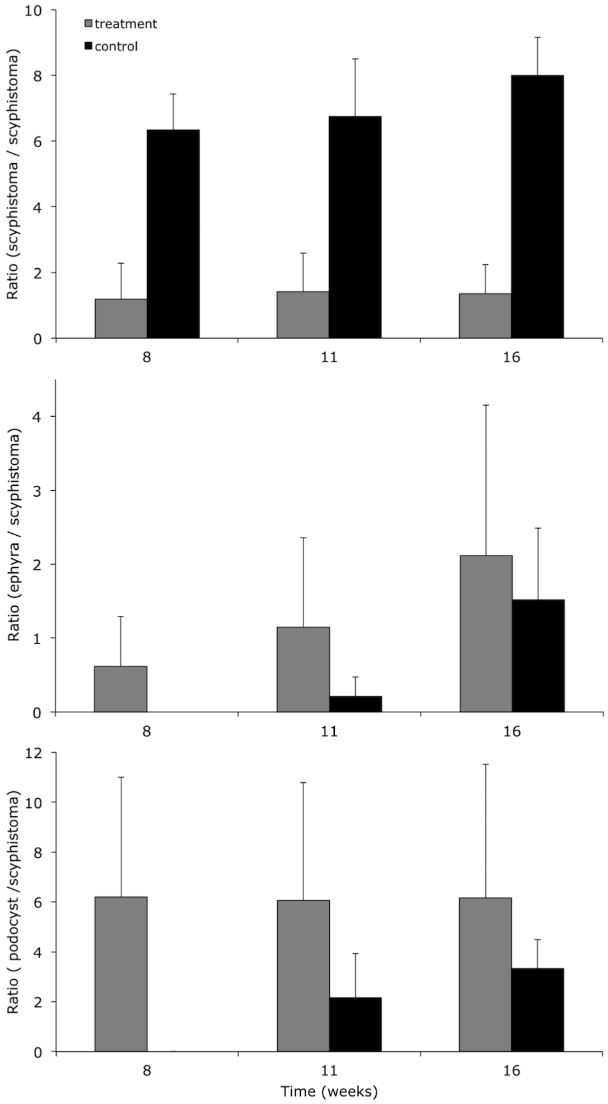


Figure 5 Production of Rhizostoma luteum scyphistoma, podocyst and ephyra. Average ratio per initial scyphistoma (n=39) of the production of new scyphistoma, podocyst and ephyra after the scyphistomae were exposed to a drop in temperature and then a return to the regular temperature conditions (grey) and control (black). Error bars are the standard deviations of the 3-4 replicates

Table 1 *Rhizostoma luteum* statistical results. Results of the Welch Two Sample t-Test, testing for differences of production of scyphistoma, podocyst and ephyra among treatments with and without (control) a temperature drop, $P < 0.05$ is considered significantly different

	Time (week)	t	df	P value
Scyphistoma	8	-0.366	4.931	NS
	11	-0.447	4.655	NS
	16	-0.832	3.139	NS
Podocyst	8	-2.875	3.000	P=0.032
	11	-3.662	3.281	P=0.015
	16	-2.839	2.000	NS
Ephyra	8	-1.502	3.000	NS
	11	-1.427	3.037	NS
	16	-1.105	3.017	NS

NS Not significant

Strobilation

The calyx elongated at the first phase of strobilation and the new ephyra formed immediately below the tentacle crown (Fig. 2G). The developing ephyra acquired mustard to dark gold colourations, tentacular lobes on the distal segment became more pronounced and the basal scyphistoma regenerated new tentacles (Fig. 2H). The ephyra lappets elongated, rhopalia appeared and the manubrium lengthened. Scyphistoma tentacles on the ephyra began to undergo regression, contracting and expanding periodically, until they were resorbed completely (Figs. 2I, 2J). After liberation, the residuum TBL was equal as pre-strobilation and had already redeveloped 16 filiform tentacles (Fig. 2K). At a constant temperature of 17-17.5 °C, the predominant type of strobilation monitored was monodisc (one ephyra per strobila), and during the entire experiment only once was a polydisc strobilation observed (Fig. 2L). The duration of the strobilation process was 5-6 days and the mortality rate of scyphistomae after strobilation was nil. There was no significant difference in the total number of ephyra liberated between the two treatments (see Table 1). Both treatments increased ratio over time (see Fig. 5). The raw data file has been included in the Appendix C (S2 Dataset).

Asexual reproduction of scyphistomae

At the base of the stalk of fully developed scyphistomae (16-tentacles), podocyst formation (Fig. 6) was observed by way of stolon formation. The podocyst were typically yellowish brown in colour (Figs. 6A-C), roundish discs with a concavity on top, the diameter ranged between 326 and 496 µm (n = 9; 430 ± 52 µm). Finger-shaped stolon developed from the lower stalk and attached to the substrate (Figs. 6D-F). It is noteworthy that this stolon never realised buds nor did it develop tentacles, as expected for lateral budding. Scyphistomae produce earlier podocyst in greater amounts and in the treatment using temperature shock (week 8) than without using temperature shock (week 11), see Fig. 5. Mean podocyst production differed significantly by treatment according to an independent t-test only in week 8 and 11 (Table 1). The raw data file has been included in the Appendix C (S2 Dataset). Furthermore, new scyphistomae excysted from the podocysts from the scyphistomae after 7 days with a temperature drop. Production increased over time, in the control treatment, but none of the scyphistomae excysted from the podocysts.

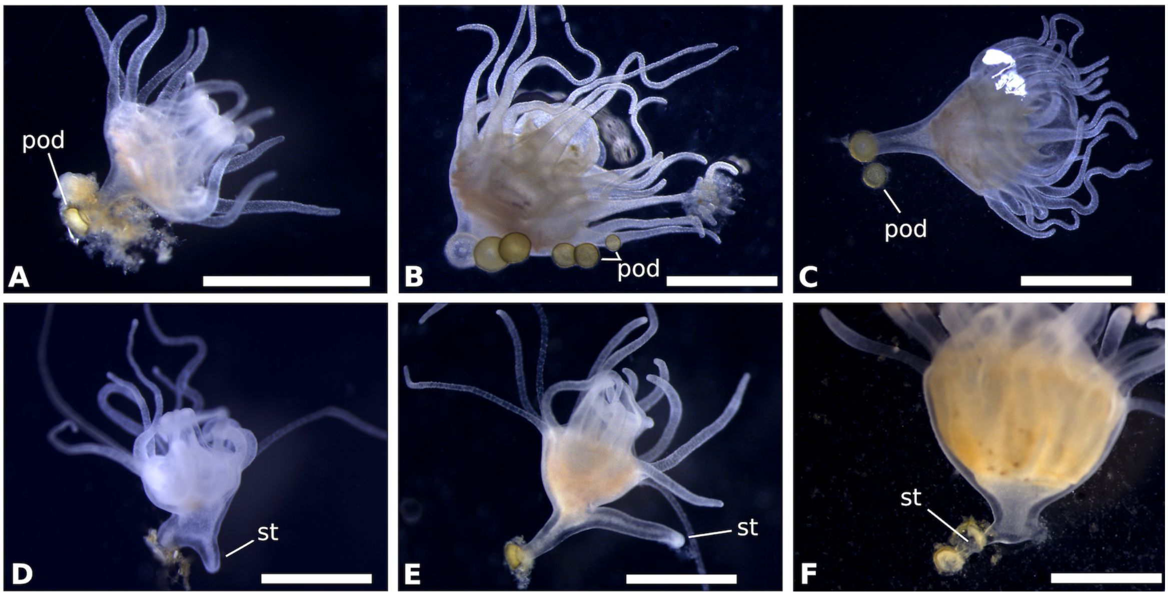


Figure 6 Asexual reproduction of *Rhizostoma luteum*: Podocyst formation by means of stolon. (A) Podocyst lateral view; (B,C) Formation of podocyst at the base of scyphistoma stalk; (D-F) Consecutive stolon development on the stalk. *pod* podocyst, *st* stolon. Scale bar 1 mm. Photos by K. Kienberger

Ephyrae

Stage 0: Newly-released ephyra from the strobila (Figs. 7A-D)

Newly liberated ephyrae reached between 3.41 to 4.52 mm width from lappet tip to lappet tip when extended (TBD; n=10; 4.01 ± 0.35 mm) with a mean central disc diameter (CDD) of 2.39 ± 0.22 mm, about 60% of the TBD (see Fig. 4 for body proportions and Table 2 for mean values). Typical ephyrae had 8 marginal lappets (Figs. 7A-C), about 17% were irregular ephyrae and mostly with 9 marginal lappets. Using the identification key for young ephyrae defined in [18], each arm contained a pair of bread knife-like lappets (rounded tips) and a single rhopalium, situated between the two lappets. Ephyrae were coloured from light mustard to dark gold with darker nematocyst warts scattered over the exumbrella. The four-lipped (cross-shaped) manubrium was long (Figs. 7C, 7D), approx. 45% of the CDD. The gastric system consists of a central stomach and 16 gastric pouches. The 8 rhopalial canals were slightly forked, the tips of which end at the base of the rhopalium, with forked sharp tips (Fig. 7A), and 8 velar (adradial) canals were flat rhombical (defined in [18]) and their tips end at the umbrella rim. Each gastric cavity per quadrant had three to seven gastric filaments (Figs. 7 A, 7B).

Stage 1: 48h post-liberation (Figs. 7E-H)

TBD and ML increased approx. 21% and the marginal lappet elongated 30%. Tiny oral tentacles developed on the distal ends of the manubrium. Between the marginal lappets, the apices of the velar canals expanded outwards and a there were velar lappet bud outgrowths from the gastric canal.

Stage 2: 3-4 days post-liberation (Figs. 7I-L)

The manubrium continued to lengthen (approx. 40% since stage 1). Pairs of small velar lappets developed from the velar lappet buds situated between the marginal lappets. The velar canals fused with the rhopalial canals forming a primary ring canal.

Stage 3: 5-6 days post-liberation (Figs. 7M-P)

The velar lappet pairs widened and expanded outwards. The manubrium commenced to split into 4 oral arms (approx. length 61% increment since Stage 2). Some connections of the primary ring canal between the velar and rhopalial canals were beginning to separate.

Stage 4: 7-8 days post-liberation (Figs. 8A-D)

After one week, the TBD doubled in size and the ML tripled in size. However, the marginal lappet reduced its total length, as the CDD expanded. All connections of the primary ring canal were closed and eliminated.

Stage 5: 9-15 days post-liberation (Figs. 8E-H)

The velar lappet extremities extended almost until the rhopalial lappet tips, completing the umbrella. The four oral arms divided, forming eight. Above the oral arms, eight epaulettes buds started to develop on the manubrium. The disconnected side branches of the velar canals grew to a T-like shape.

Stage 6: about 16-20 days post-liberation (Figs. 8I-L)

TBD tripled since the liberation of the ephyra and the CDD was 94% of the TBD. The oral arms changed to become J-shaped in lateral view. Small oral tentacles developed on the epaulettes. The velar canal formed a secondary ring canal and fused with the rhopalial canals forming a third ring canal.

Stage 7: About 21-25 days post-liberation (Figs. 8M-P)

3-4 weeks after the ephyra released from the strobila, the TBD and CDD increased by about 4.8 and 7.6 times, respectively. The velar canals developed a fine-meshed network. The mesogloea thickened and the flat exumbrella of the young ephyra transformed into a typically bell-shaped medusa (metaephyra). The bell of the metaephyra was entirely of a dark golden yellow colour and the oral arms were slightly lighter in colour. During the first seven stages the ephyra displayed a linear grow. TBD had a mean increase of 0.68 mm per day (n = 126, TBD=0.68 day + 3.16, R^2 =0.90, $p < 0.0001$). Similarly, the CDD and ML increased linearly with growth (n = 126, CDD=0.76 day + 0.75, R^2 =0.90, $p < 0.0001$; n = 64, ML=0.27 day + 0.37, R^2 =0.905, $p < 0.0001$). The raw data file has been included in Appendix C (S3 Dataset and S4 Figure).

Table 2 Morphometric measures of *Rhizostoma luteum* ephyra

Stage/days	***n***	**TBD**	**CDD**	**TMLL**	**ML**
0 / 0	10	4.01 ± 0.35	2.39 ± 0.22	0.93 ± 0.10	0.71 ± 0.22
1 / 1	7	4.86 ± 0.96	2.71 ± 0.51	1.21 ± 0.23	0.86 ± 0.25
2 / 3-4	7	5.81 ± 0.85	3.69 ± 0.58	1.19 ± 0.19	1.20 ± 0.28
3 / 5-6	7	7.02 ± 0.91	4.80 ± 0.68	1.27 ± 0.16	1.93 ± 0.41
4 / 7-8	7	7.78 ± 1.04	5.91 ± 1.01	1.18 ± 0.21	2.42 ± 0.33
5 / 9-15	7	11.10 ± 2.19	9.38 ± 2.42	1.22 ± 0.16	3.41 ± 0.41
6 / 16-20	6	15.37 ± 2.15	14.41 ± 2.52		
7 / 21-25	6	19.40 ± 2.56	18.30 ± 2.54		

Mean value (mm) and standard deviation of morphometric measures analysed in the 7 ephyra stages of *Rhizostoma luteum* reared in the laboratory. *n* number of individuals measured, *TBD* total body diameter, *CDD* central disc diameter, *TMLL* total marginal lappet length, *ML* manubrium length

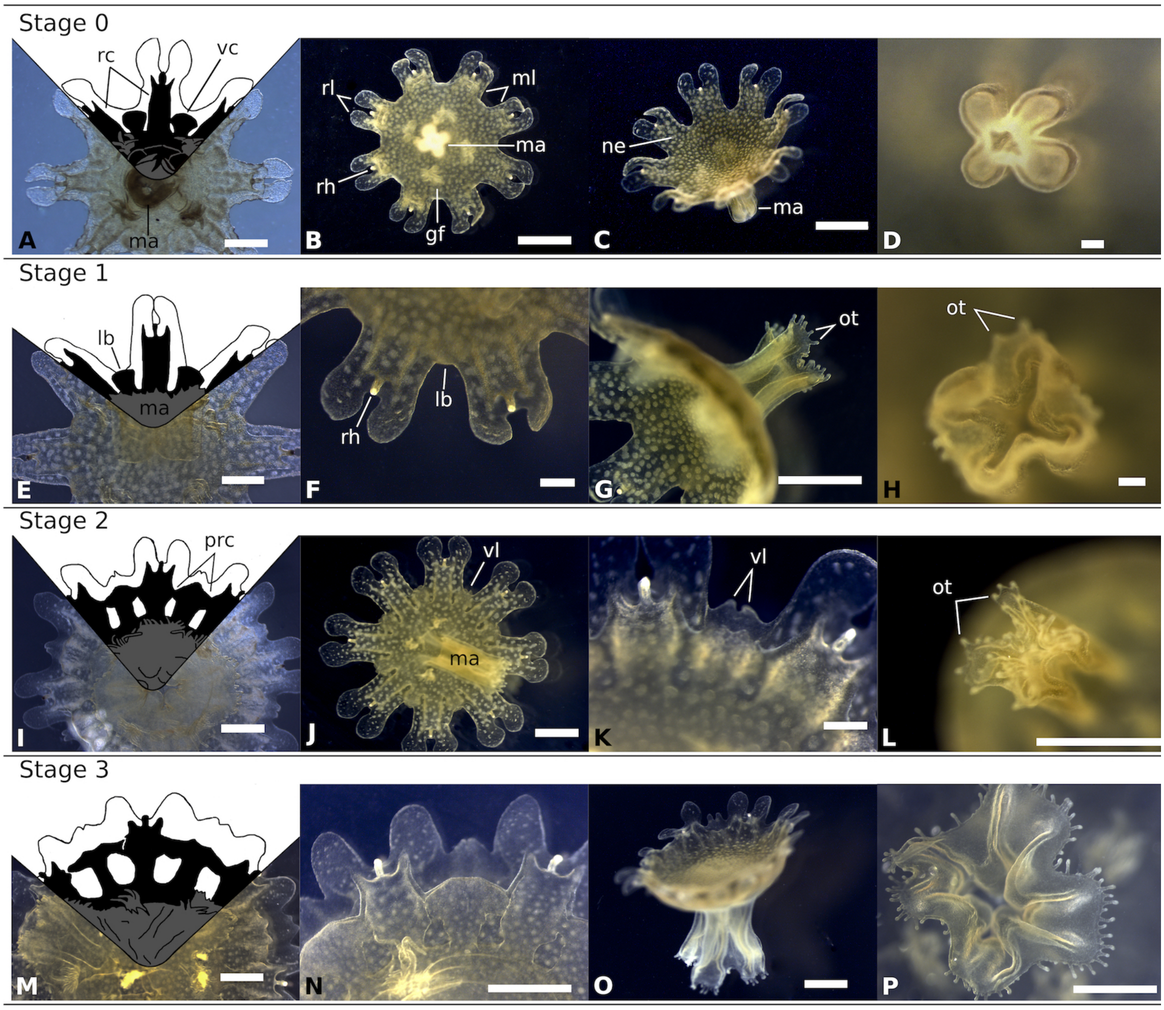


Figure 7 Typical stages in the development of *Rhizostoma luteum* ephyra. Stage 0 to 3. Stage 0 (A-D): Newly released ephyra; (A) Drawing of gastric system; (B) Oral view; (C) Aboral view; (D) Cross-shaped mouth without oral tentacles; Stage 1 (E-H): 48 h post-liberation; (E) Drawing of gastric system; (F) Enlargement of marginal lappets; (G) Side view; (H) Cross-shaped mouth with oral tentacles; Stage 2 (I-L): 3-4 days post-liberation; (I) Drawing of gastric system; (J) Oral view; (K) Enlargement of marginal lappets; (L) Cross-shaped mouth with oral tentacles; Stage 3 (M-P): 5-6 days post-liberation; (M) Drawing of gastric system; (N) Enlargement of marginal lappets; (O) Side view; (P) Enlargement of manubrium. *Black* gastric system, *gf* gastric filaments, *lb* lappet bud, *ma* manubrium, *ml* marginal lappets, *ne* nematocyst, *ot* oral tentacles, *prc* primary ring canal, *rc* rhopalar canal, *rh* rhopalium, *rl* rhopalar lappets, *vc* velar canal, *vl* velar lappets. Scale bar 1 mm for all except (D, F, H) 100 µm. Drawings by R. Halbauer. Photos by K. Kienberger

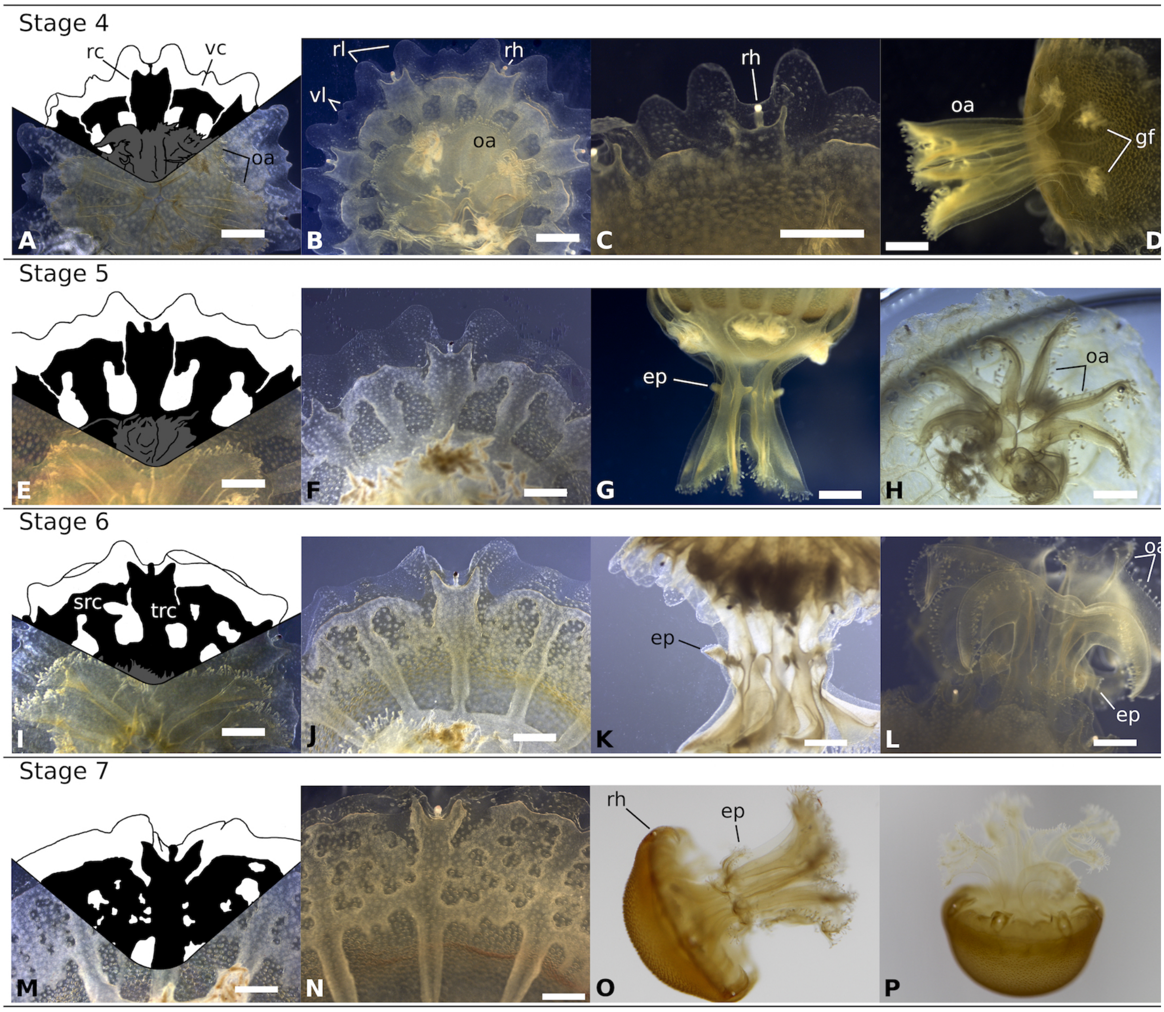


Figure 8 Typical stages in the development of *Rhizostoma luteum* ephyra. Stages 4 to 7. Stage 4 (A-D): 7-8 days post-liberation; (A) Drawing of gastric system; (B) Oral view; (C) Enlargement of marginal lappets; (D) Side view; Stage 5 (E-H): 9-15 days post-liberation; (E) Drawing of gastric system; (F) Oral view; (G) Side view; (H) Enlargement of oral arms; Stage 6 (I-L): 16-20 days post-liberation; (I) Drawing of gastric system; (J) Oral view; (K) Side view; (L) Enlargement of oral arms; Stage 7 (M-P): 21-25 days post-liberation; (M) Drawing of gastric system; (N) Oral view; (O,P) Side view; *Black* gastric system, *ep* epaulette*, gf* gastric filaments, *oa* oral arm, *rc* rhopalar canal, *rh* rhopalium, *rl* rhopalar lappets, *src* secondary ring canal, *trc* third ring canal, *vc* velar canal, *vl* velar lappets. Scale bar 1 mm. Drawings by R. Halbauer. Photos by K. Kienberger: A-N; A.M. Schönemann: O,P.

Young medusae

Two months after ephyra release, the young medusa reached a bell diameter of 8.1 to 9.9 cm (n=5; 8.66 ± 0.72 cm) and a wet weight between 35 to 59 g (n=5; 44 ± 10 g). The gastrovascular canal system was well developed (Fig. 9A). The 16 radial canals were connected to each neighbour, tracing a circular fine-meshed network (anastomosing canals). The anastomosing canals outgrow until the umbrella margin, and being approx. 1/3 of the length of the area between the umbrella margins and the central stomach. The rhopalar lappets were narrow and pointed. Terminal appendages began to develop (Fig. 9D). The mean values of morphometric measures that were analysed are summarised in Table 3 and illustrated in Figs. 9B-C. All young medusae were immature. The raw data file has been included in the Appendix C (S5 Dataset).

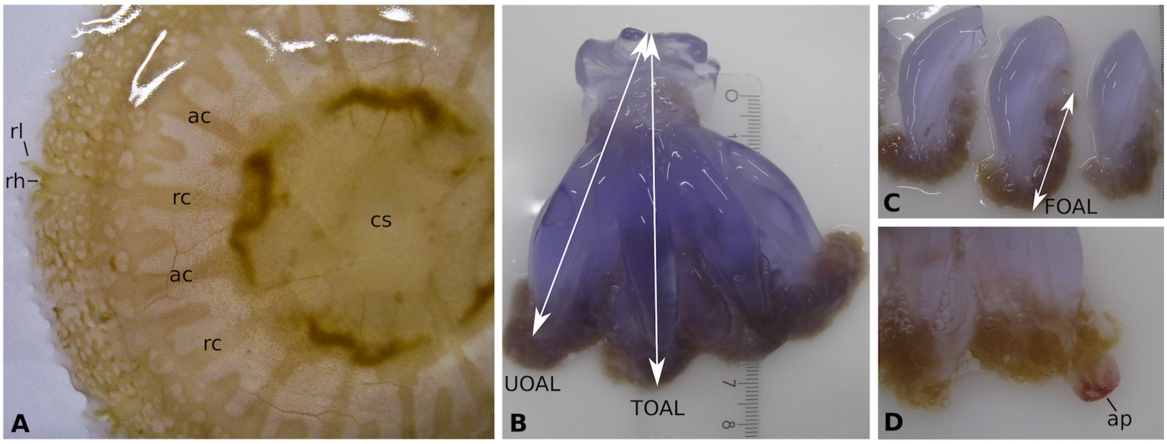


Figure 9 Young medusa *Rhizostoma luteum*: 2-month post-liberation. (A) Aboral view, bell diameter 5.8 cm; (B) Amputated manubrium; (C) Amputated oral arms; (D) Oral arm with one terminal appendage, length 0.2 cm; *ac* adradial canal; *ap* appendage, *cs* central stomach; *rc* rhopalar canal; *rl* rhopalar lappets; *rh* rhopalium; *FOAL* frilled oral arms length, *TOAL* total oral arm length, *UOAL* unfrilled oral arm length. Photo by K. Kienberger

Table 3 Morphometric measures of young medusa of *Rhizostoma luteum*

Time after release	n	Bell size (cm)	Wet weight (g)	TOAL (mm)	UOAL (mm)	FOAL (mm)
2 months	5	8.66 ± 0.72	44 ± 10	3.4 ± 0.6	2.4 ± 0.6	1.3 ± 0.2
3 months	7	13.27 ± 2.26	181 ± 53	7.5 ± 0.6	5.9 ± 0.7	3.6 ± 0.6

Mean values and standard deviation of morphometric measures of young medusa of *Rhizostoma luteum* reared in the laboratory. *n* number of individual measured, *TOAL* total oral arm length, *UOAL* unfrilled oral arms length, *FOAL* frilled oral arms length

Three-month post-liberation (Fig. 10), the bell diameter reached a mean bell diameter of 13.27 ± 2.26 cm (n=7) and wet weight of 181 ± 53 g, the largest exemplar having a diameter of 16.4 cm and wet weight of 242 g (see Table 3). The umbrella had a very thick mesogloea, and it was coloured light to dark violet with small reddish-brown warts. Oral arms were also coloured violet with mustard mouth frills. Appendages were light purple-brown when present, the longest measured was 5.5 cm. Less than 2% of the juvenile medusae developed 1 to 3 short terminal appendages, but never developed all 8. In analysing the gonads under a microscope, 4 contained male spermatozoa, and 3 contained oocytes in different development stages and planulae. There was no visible sexual dimorphism in the colour of the gonads. Bell diameter was positively correlated with wet weight (linear regression: n=12, bell size = 26.904 x - 181.1, R^2 =0.95, $p < 0.001$; see Fig 11). The raw data file has been included in the Appendix C (S4 Dataset).

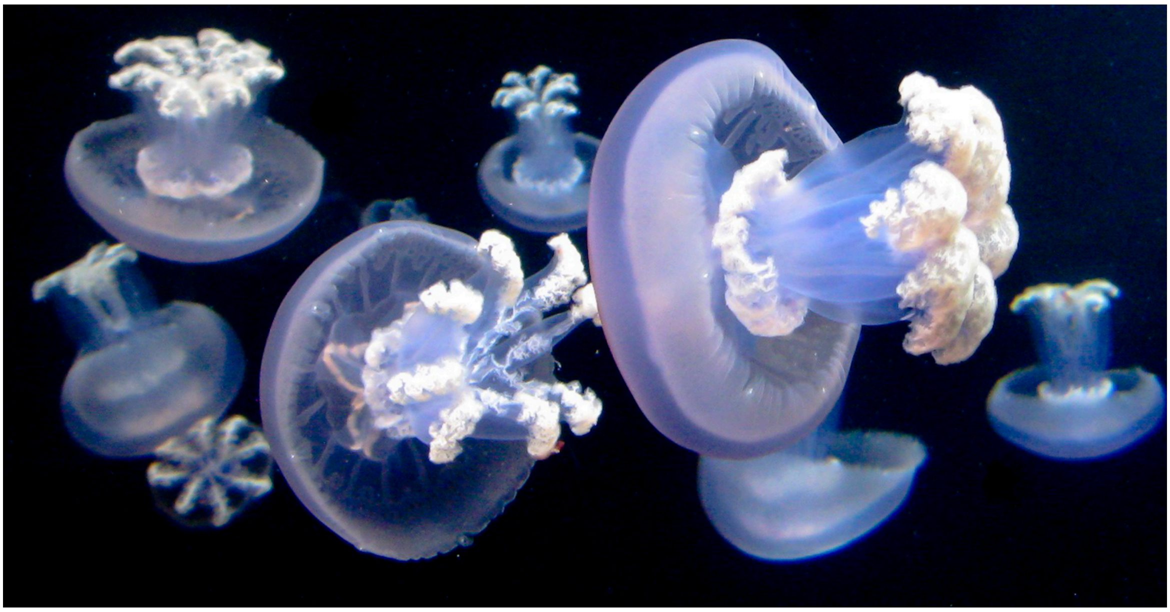


Figure 10 Young medusa of *Rhizostoma luteum* reared in the laboratory. Approximately three- month post-liberation. Photo by K. Kienberger

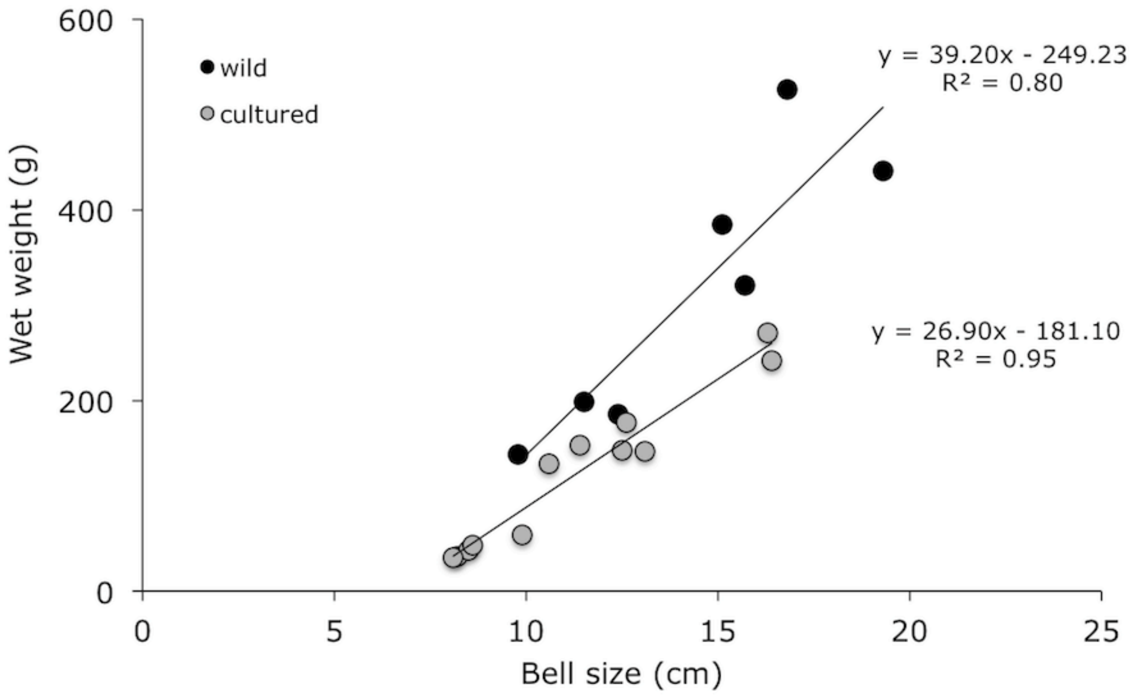


Figure 11 Positive linear relationship between bell diameter and wet weight of young medusa of *Rhizostoma luteum*. Data were compiled from specimens reared in the laboratory (grey dots) and collected in the wild (black dots). Each data point represents a different medusa. Regression lines and their equations are represented

The young medusae collected in the wild (Table 4) had a bell diameter of 9.8 to 19.3 cm (n =7; 14.37 ± 3.30 cm) and a wet weight between 143 to 526 g (n =7; 314 ± 144 g). Having already the typical colouration seen in larger *R. luteum*; the umbrella was milky bluish-white with small reddish-brown warts and the oral arms were bluish with mustard mouth frills and with deep purple-brown appendages. 4 specimens had short appendages, but none had all 8 appendages completely developed. The largest appendage was 9.8 cm (bell diameter 16.8 cm). None of the collected jellyfish were sexually mature, as no fertilized eggs, embryos or planulae were present in the gonads. Further, there was a good positive correlation between the bell diameter and wet weight (n=7, bell size = 39.20 x - 249.23, R^2 =0.80, p < 00617; see Fig. 11).

Table 4 Measurements of young medusa of *Rhizostoma luteum* collected in the wild

Collecting dates (2016)	Bell size (cm)	Wet weight (g)	Number of appendages/ mean length (cm)
2 February	15.7	321	2 / 4.8 ± 0.42
4 February	11.5	198	7 / 4.0 ± 0.70
4 February	16.8	526	7 / 8.17 ± 2.18
4 February	12.4	186	1 / 2.2
4 February	19.3	441	Not present
8 February	9.8	143	Not present
8 February	15.1	384	Not present

DISCUSSION

Rhizostoma luteum has a typical metagenetic scyphozoan life cycle in which benthic scyphopolyps asexually strobilate ephyrae that grow into sexually reproducing medusae. In general, its life cycle is similar to its congeners, with the distinction that *R. luteum* is a brooding species and the strobilation type is predominantly monodisc. Brooding is common among the Rhizostomeae medusae [15, 26]. However, to our knowledge, this character is described for the first time in the genus *Rhizostoma*, as the other two species *R. pulmo* [27, 28] and *R. octopus* [15, 29-31] were defined as non-brooding medusae (see review Table 5).

Table 5 Summary of the characters of the planula, scyphistoma, strobilation process and ephyra of the genus *Rhizostoma* described by different authors

Species	Planulae brooded	Culture conditions (°C)/ salinity/ light cycle	Planula size length/ width (µm)	Settl-ment (days)	Scyphistoma fully developed size range (mm)	Number of tentacles	Scyphistoma hypostome	Asexual reproduction	Strobilation rate (ephyrae per strobila; strobilation type)	Strobilation duration (days)/ Strobilation temperature	Ephyra size after release (mm in diameter)	No. of marginal lappets	Shape of rhopalial lappet	Colour of ephyra	Reference
R. luteum	✓	17-17.5°C/ 37/ 12:12	126-139; mean 132/ 73-106; mean 84	3-5	1.34-2.5; mean: 1.74	14–16 filiform tentacles	Conspicuous and flexible in all stages	Podocysts	1; monodisc	5-6/ at 17.5°C	3.41-4.52; mean: 4.01	Typical 8, up to 11	Bread knife shaped	Light yellow to light brown	Present study
R. pulmo	✗	n.d.	500/n.d.	n.d.	12	32	Long and flexible	Polyp buds, swimming buds, podocysts	12-18 segments (no detachment of ephyrae)	No development of ephyrae/ temperature changes has no effect	No development of ephyrae	No develop-ment of ephyrae	No develop-ment of ephyrae	No develop-ment of ephyrae	[27]
R. pulmo	n.d.	10-15°C/ 36/ without light	n.d.	n.d.	1.45	16	Long, club shaped	Podocysts, lateral polyp buds	8; polydisc	n.d.	3.19-3.34; mean: 3.27	8	Spade like	Milk trans-parent	[18,32]
R. pulmo	n.d.	21°C/ 37-38/ 12:12	n.d.	n.d.	0.96-2.15; mean: 1.69	14-18 filiform tentacles, mean: 16	Long	Lateral polyp buds, stolonial polyp buds, podocysts, pedalocysts	8; polydisc	n.d.	2.28-3.93; mean: 3.17	Typical 8, range 5-9	Spade to lancet-shaped	Opaque white	[28]
R. pulmo	n.d.	14°, 21°, 28°C/ 38-39/ 12:12	n.d.	n.d.	n.d.	n.d.	n.d.	Buds	13.5 at 14°C; 6.8 at 21°C; 8.5 at 28°C; polydisc	40.5/ at 14°C; 12.2/ at 21°C; 13.5/ at 28°C	n.d.	n.d.	n.d.	n.d.	[33]
R. pulmo	n.d.	10°, 15°, 20°C/34/dark	n.d.	n.d.	n.d.	n.d.	n.d.	Podocysts	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	[34]
R. octopus	n.d.	n.d.	100-110	3-5	0.4	10-16	n.d.	Podocysts	n.d.	n.d.	n.d.	8	n.d.	n.d.	[35]
R. octopus	✗	5-20°C/35/ n.d.	110-150/ 80-90	1-5	2.3	Up to 24	Large and flexible	Podocysts, polyp buds (rare)	1-5; polydisc	18-31/ change from 15-10°, 5-10°or 10-15°	2.7-5.8; mean 4.5	8	n.d.	n.d.	[15,30]
R. octopus	n.d.	5-10°C/ 36/ without light	n.d.	n.d.	1.9	16-20	Conspicuous and flexible in all stages	Podocysts, lateral polyp buds	5-12; polydisc	n.d.	3.30-5.96; mean: 4.81	8	Bread knife shaped	Milky trans-parent	[12,32]
R. octopus	✗	10°C/ 35/n.d.	107-148; mean 127/ 76-105; mean 85	2	n.d.	n.d.	Conspicuous and flexible in all stages	Laterally polyp buds, podocysts, longitudinal fission	1-5; polydisc	7-34/ 10°C constant	2.7–5.8; mean: 4.5	8	n.d.	Light yellow to light brown	[31]

n.d. not documented

The order Rhizostomeae has been divided into two suborders Kolpophorae and Dactyliophorae (including the genus *Rhizostoma*), based on the difference in the development of their gastrovascular network [10]. The development of the canal system in *R. luteum* was typical of the suborder Dactyliophorae, forming outwards from the ring canals and having 16 canals connecting to the stomach (see Figs. 7-9). Dactyliophorae are mostly non-brooding and they are typically polydisc under optimal conditions (Table 6), except for *Catostylus mosaicus* and *Rhopilema verrilli*, which retain their planulae and usually have monodisc strobilation [reviewed in 15, 26], identical to *R. luteum* (present study). On the other hand, all Kolpophorae species with known life cycles are brooding species and their strobilation type is monodisc. Within Scyphozoa evolution, polydisc strobilation appears to be an ancestral character [36] with monodisc strobilation having arisen in Kolpophorae, *Sanderia malayensis* [reviewed in 37] and in *R. luteum* (present study). Moreover, all Dactyliophorae produce podocysts ([15], *R. luteum* present study), they are chitin-covered cysts, which form beneath the pedal discs of the scyphistoma, containing stored nutritional reserves and they can stay dormant for a prolonged period of time (i.e. *Aurelia aurita* cysts survived for 3.2 years [38]). In 2009 Arai wrote, “The earliest literature speculated that podocysts were produced during poor conditions, and that they provided protection against predation or a limited food supply. More recent papers show that podocysts may indeed protect against predation, but the rate of their production is usually positively correlated with the availability of food in otherwise good conditions” [39]. The suggestion by Arai was confirmed by recent studies, showing that the rate of podocyst production is affected by environmental conditions such as temperature, food supply [34], salinity, and dissolved oxygen concentration [38].

Table 6 Summary of brooding behaviour and ephyrae per strobila of the suborder Dactyliophorae (Rhizostomeae) as described by different authors

Family	**Species**	**Brooding behaviour**	**Ephyrae per strobila**	**References**
Catostylidae	*Catostylus mosaicus*	✓	Typically monodisc; up to 5	[40]
Lychnorhizidae	*Lychnorhiza lucerna*	✗	Polydisc; 3	[26,41]
Rhizostomatidae	*Rhizostoma pulmo*	✗	Polydisc; 12-18	[32]
	Rhizostoma octopus	✗	Polydisc; 1-5	[18,29]
	Rhizostoma luteum	✓	Monodisc	Present study
	Rhopilema esculentum	✗	Polydisc; up to 17	[42]
	Rhopilema nomandica	✗	Polydisc; 5-6	[43]
	Rhopilema verrilli	✓	Typically monodisc; up to 3	[44,45]
Stomolophidae	*Nemopilema nomurai*	✗	Polydisc; 3-7	[46]
	Stomolophus meleagris	✗	Polydisc; 1-3 typically 2	[47]

In the present study, the production of podocysts was significantly higher only in week 8 and 11 between the treatments with and without a drop in temperature. After the temperature was set back to 17-17.5 ºC, the podocysts were able to rapidly regenerate new scyphistomae and, subsequently, they strobilated and produced ephyrae. Fewer, and only from week 11 onwards, podocysts were produced in the control; no excysting was monitored (see Fig. 5). Furthermore, formations of finger-shaped stolons were observed on the stalk (Figs. 6D-F) of fully-grown scyphistomae (16 tentacles). These stolons were merely part of podocyst formation, as the release of a bud-like formation or the formation of tentacles were not observed. Further, in a previous study the rhizostome propagation was defined mostly by a reduced number of asexual scyphistoma-to-scyphistoma modes (mainly podocysts: Type 5 [34, 17]). *Rhizostoma pulmo* asexual reproduction occurred by lateral budding, by means of stolons, and podocysts (Type 1, 3, 5 [28, 17]). Podocyst production was more frequent and occurred without changes in temperature (21 °C temperature, 37-38 salinity, and 12:12 light cycle). The rate of asexual reproduction in *R. octopus* was low at all cultivation temperatures, nevertheless, the most frequent mode was the production of podocysts (Type 5 [15, 17]) at a constant temperature of 20 °C (salinity 35). Actually, fewer were formed at a lower temperature. The rate of lateral budding by means of stolons (Type 3 [15, 17]) was low at all cultivation temperatures (less than 1% during a 2-year observation period).

As with other species of the genus, the fully developed scyphistomae of *R. luteum* had a conspicuous hypostome and filiform tentacles (Fig. 2F). The body proportion ratios of *R. luteum* (calyx ~ 45%, stalk ~ 20%, hypostome ~ 35%) correspond to the group of Rhizostomida, as described in [48]. Scyphistoma never lost its filiform tentacles (Figs. 2G-J) during the various phases of strobilation. Furthermore, the large hypostome could be a useful character with which to identify scyphistomae in the field, as the only other Rhizostomeae abundant in the western Mediterranean Sea is *Cotylorhiza tuberculata*, which does not have a significant hypostome [24]. All of the *R. luteum* scyphistomae in the present study always survived and were able to feed after the liberation of ephyra. On the other hand, *Cotylorhiza tuberculata* has a higher mortality rate of scyphistoma after strobilation (up to 92% [24]). Under the tested conditions, the predominant type of strobilation observed in *R. luteum* was monodisc, being therefore a distinctive feature of the species compared to its sibling species, which usually have polydisc strobilation (Table 4) under optimal conditions. *R. luteum* scyphistomae strobilated repeatedly in both treatments and produced viable ephyrae. *R. pulmo* scyphistomae strobilated spontaneously also under constant temperature in springtime (21 °C temperature, 37-38 salinity and 12:12 light cycle [28]). However, *R. octopus* did not produce ephyrae without a temperature change (15 to 10 °C; 5 to 10 °C; 10 to 15 °C [15]). Ephyrae of *R. luteum* generally developed into an 8-rayed type typical of scyphozoan taxa with 16 bread knife-like rhopalial lappets, like *R. octopus*, though, *R. pulmo* has round spatula-like rhopalial lappets [18]. It took the ephyra approximately 21 days to develop until stage 7 (TBD; n=6; 19.40 ± 2.54 cm), increasing 4.8 times its total body diameter since its liberation, and having a daily growth rate of 0.68 mm; in comparison *Cotylorhiza tuberculata* had a grow rate of 0.08 mm per day [49]. In general, the development of the ephyra was very similar to its congeners, apart from the important size difference that was already visible in the first week after liberation of the ephyra (*R. luteum* (mm): 1w: 7.78 ±1.04, 2w: 11.10 ± 2.19, 3w: 19.4 ± 2.56 (present study); *R. octopus* (mm): 1w: 5.8 ± 0.8; 2w: 7.7 ± 0.9; 3w: 9.5 ± 1.3 [15]). At three-months post-liberation, the medusae become mature and they were 5.3 times larger than *R. octopus* (2-3 cm [15]; *R. luteum*: mean TBD 13.27 cm, present study). However, as soon as the *R. luteum* in the present reached the mature state, they did not grow larger. They even started to decrease in size and decomposed completely. It is the belief of the present authors that the significant size difference is due to the consecutive large kreisels that were used to rear the jellyfish. Nonetheless, none of the young medusae showed any sexual dimorphism in the colour of the gonads. Mature *R. octopus* collected in the wild, had a sexual dimorphism in the colour of the gonads visible through the bell; female gonads were brown-coloured and contained brown, ripe eggs, and the male gonads were whitish-blue [15]. Moreover, as all of the young medusae that were collected *in situ* had a very similar bell diameter; it can be assumed that they belong to one cohort. This seems to agree with the fact that in temperate coastal and shelf ecosystems, the majority of jellyfish populations consist of single cohorts growing and maturing synchronously, with sexually mature females with planula larvae that are present for between 1 and 5 months, reviewed in [50]. Notwithstanding, in the present study on three occasions mature medusa were collected in the wild, which was in April, May and October (Kienberger, unpublished data). It is interesting to note that there was a significant colour difference between the young medusae that were reared in laboratory conditions and the wild medusae (see Figs. 1 and 10). Cultivated medusae having a violet umbrella and oral arms are different to those collected in the wild, which are milky bluish-white in colour. Colouration differences have been seen in the order Rhizostomeae as reared in the laboratory (e.g. *Mastigias papua, Phyllorhiza punctata* and *Cotylorhiza tuberculata*; Bartsch and Halbauer, pers. observ.).

Our results suggest that a temperature shock does not have an effect on the mean amount of scyphistoma. However, scyphistomae strobilated and developed podocysts earlier in the treatment with a temperature shock (week 8) than in the control (week 11), see Fig. 5. Since the scyphistoma phase of *R. luteum* seems highly resistant to fast falls in temperature, a strong episode of upwelling (decreasing sea surface temperature), a usual feature of some regions of the east Atlantic where these jellyfish have been reported, would most likely trigger the production of podocyst and might start the strobilation process. Furthermore, in the hypothetical case, adult medusae would enter the lagoon of the Mar Menor, the scyphistomae phase could survive a severe winter condition (with temperatures as low as that tested in the present study) while, on the contrary, other species such as *Cotylorhiza tuberculata* scyphistomae will not survive [24]. To our knowledge, no data are available for *R. pulmo*, which is the other dominant species in the lagoon.

It is suggested in [51] that there are taxonomically and phylogenetically characteristics predisposing some medusozoans to occur *en masse*. These include character states and character complexes related to podocyst formation, strobilation, extensive canal systems, large size, rapid growth rate, elevated Reynolds number, oral arms, lack of photosymbiosis and shallow-water habitat. As all these adaptations are present in *R. luteum* and it may predispose it to occur *en masse*. However, to our knowledge, *R. luteum* seems does not occur *en masse* at the present, as most of the reported sightings in the last two decades are of individuals or on occasions a few individuals. We know of only one beaching episode on 12 June 2012, where a total of 24 individuals were stranded on a single beach in Doñana National Park (SW Iberian Peninsula, Spain), and that report is the sole example in our dataset [6, 7, Kienberger, unpublished data], which had more than 10 individuals. On the other hand, *R. pulmo* and *R. octopus*, reviewed in [52], have been reported to be important blooming coastal species in recent years, showing important inter-annual fluctuations in their population density. Comparing the synapomorphic characteristics related to the ability to form blooms, we believe that *R. luteum* may have sufficient pre-requisites to form blooms, when environmental circumstances change, all supposing that *R. luteum* would be able to take advantage of those new conditions. Changes in environmental conditions can be driven by a broad variety of diverse causes of different temporal and spatial scales of variability, independently of their sources (natural or antropogenic). The next step for further research would be to evaluate the *R. luteum* capacity to form blooms and also to disentangle its response to environmental conditions.

CONCLUSION

As expected, the life cycle of *Rhizostoma luteum* in the present study was similar to that described for its congeners, with the exception of two features: first, the female *R. luteum* is a brooding species; second, under the conditions that were tested, the predominant type of strobilation observed was monodisc. As polydisc strobilation is likely an ancestral condition, monodisc strobilation in *R. luteum* may have arisen for the first time in the genus *Rhizostoma*. Nevertheless, further studies are needed in order to check whether, under different environmental conditions, this species is able to turn to the polydisc strobilation type as with its congeners. Furthermore, being evolutionarily predisposed to form problematic blooms if environmental conditions permit, it is important to disentangle the environmental control, such as the tolerance limits and optimal conditions for survival and asexual reproduction of early life stages of this jellyfish.

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REFERENCES

1. Purcell JE. Climate effects on formation of jellyfish and ctenophore blooms: a review. J Mar Biol Assoc U.K. 2005; 85(3): 461–476. doi: 10.1017/S0025315405011409
2. Purcell JE, Uye S, Lo W. Anthropogenic causes of jellyfish blooms and their direct consequences for humans : a review. Mar Ecol Prog Ser. 2007; 350: 153–174. doi: 10.3354/meps07093
3. Lynam CP, Gibbons MJ, Axelsen BE, Sparks CAJ, Coetzee J, Heywood BG, Brierley AS. Jellyfish overtake fish in a heavily fished ecosystem. Curr Biol. 2006; 16: R492–R493.
4. Duarte CM, Pitt KA, Lucas CH, Purcell JE, Uye S, Robinson K, et al. Is global ocean sprawl a cause of jellyfish blooms? Front Ecol Environ. 2012; 11(2): 91-97.
5. Boero F. Review of jellyfish blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean. No. 92. Rome, FAO 2013. 53 p.
6. Prieto L, Armani A, Macías D. Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. Mar Biol. 2013;160(12): 3241-7. doi: 10.1007/s00227-013-2293-6
7. Kienberger K, Prieto L. The jellyfish *Rhizostoma luteum* (Quoy & Gaimard, 1827): not such a rare species after all. Mar Biodiv. 2017. doi: 10.1007/s12526-017-0637-z

8. Turismo y Planificación Costa del Sol. Balances y Proyectos, Costa del Sol, Málaga. 2018. [cited 03 July 2018]. Available from: https://www.costadelsolmalaga.org/base/descargas/290746/dossier-prensa-2018
9. Mayer AG. Medusae of the world. III. The Scyphomedusae. Washington: Carnegie Institution of Washington; 1910.
10. Russell FS. The medusae of the British Isles II. Pelagic Scyphozoa with a Supplement to the First Volume on Hydromedusae. Cambridge: Cambridge University Press; 1970. pp. 284.
11. Grenacher H, Noll FC. Beiträge zur Anatomie und Systematik der Rhizostomeen. Abhandlungen der Senckenbergischen Naturforschung Gesellschaft, 10. Frankfurt am Main; 1876.
12. Stiasny G. Die Rhizostomeen-Sammlung des British Museum (Natural History) in London. Zoöl Meded; 1931: 14. pp 137-178.
13. Stiasny G. *Rhizostoma luteum* (Quoy und Gaimard), im Tejo vor Lissabon nachgewiesen. Arch Mus Bocage; 1936: 7. pp 1-6.
14. Kramp PL. Synopsis of the medusae of the world. J Mar Biol Assoc U.K. 1961;4: 7-469.
15. Holst S, Sötje I, Tiemann H, Jarms G. Life cycle of the rhizostome jellyfish Rhizostoma octopus (L.) (Scyphozoa, Rhizostomeae), with studies on cnidocysts and statoliths. Mar Biol. 2007;151(5): 1695–710. doi: 10.1007/s00227-006-0594-8
16. Fautin DG. Reproduction of Cnidaria. Can J Zool. 2002; 1735–1754. doi: 10.1139/Z02-133
17. Adler L, Jarms G. New insights into reproductive traits of scyphozoans: Special methods of propagation in *Sanderia malayensis* Goette, 1886 (Pelagiidae, Semaeostomeae) enable establishing a new classification of asexual reproduction in the class Scyphozoa. Mar Biol. 2009;156(7): 1411-20.
18. Straehler-Pohl I, Jarms G. Identification key for young ephyrae: a first step for early detection of jellyfish blooms. Hydrobiologia. 2010; 645:3–21. doi: 10.1007/s10750-010-0226-7
19. NOAA National Oceanic and Atmospheric Administration, CoastWatch SST. [cited 19 July 2017]. Available from: http://www.ospo.noaa.gov/Products/ocean/sst.html
20. Lutjeharms JRE, Meeuwis JM. The extent and variability of South-East Atlantic upwelling. S Afr J Mar Sci. 1987;5(1): 51-62. doi: 10.2989/025776187784522621
21. Sarahn T, Lafuente JG, Vargas M, Vargas JM, Plaza F. Upwelling mechanisms in the northwestern Alboran Sea. J Mar Syst. 2000;23: 317-331.
22. Vargas JM, Garca-Lafuente J, Delgado J, Criado F. Seasonal and wind-induced variability of Sea Surface Temperature patterns in the Gulf of Cadiz. J Mar Syst. 2002;38(3): 205-219. doi: 10.1016/S0924-7963(02)00240-3
23. Arai MN. A Functional Biology of the Scyphozoa. Chapman & Hall. London; 1997. pp. 169.
24. Prieto L, Astorga D, Navarro G, Ruiz J. Environmental control of phase transition and polyp survival of a massive-outbreaker jellyfish. PLOS ONE. 2010;5(11): e13793. doi: 10.1371/journal.pone.0013793
25. Straehler-Pohl I, Widmer CL, Morandini AC. Characterizations of juvenile stages of some semaeostome Scyphozoa (Cnidaria), with recognition of a new family (Phacellophoridae). Zootaxa. 2011;2741: 1-37.
26. Schiariti A, Christiansen E, Morandini AC, da Silveira FL, Giberto DA, Mianzan HW. Reproductive biology of *Lychnorhiza lucerna* (Cnidaria:

Scyphozoa: Rhizostomeae): Individual traits related to sexual reproduction. Mar Biol Res. 2012;8(3): 255-64. doi: 10.1080/17451000.2011.616897

27. Paspaleff GW. Über die Entwicklung von *Rhizostoma pulmo* Agass. Arb Biol Meeresst Varna; 1938;7. pp 1-17.
28. Fuentes V, Straehler-Pohl I, Atienza D, Franco I, Tilves U, Gentile M, et al. Life cycle of the jellyfish *Rhizostoma pulmo* (Scyphozoa: Rhizostomeae) and its distribution, seasonality and inter-annual variability along the Catalan coast and the Mar Menor (Spain, NW Mediterranean). Mar Biol. 2011;158(10): 2247-66
29. Holst S, Jarms G. Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. Mar Biol. 2007;151: 863-71. doi:10.1007/s00227-006-0530-y
30. Holst S. Grundlagen der Populationsentwicklung verschiedener Scyphozoa (Cnidaria) der Deutschen Bucht. PhD Thesis, Universität Hamburg. 2008. Available from: http://ediss.sub.uni-hamburg.de/volltexte/2008/3635/pdf/Dissertation_Sabine_Holst_2008.pdf
31. Holst S. Morphology and development of benthic and pelagic life stages of North Sea jellyfish (Scyphozoa, Cnidaria) with special emphasis on the identification of ephyra stages. Mar Biol. 2012;159: 2707-22. doi:10.1007/s00227-012-2028-0
32. Straehler-Pohl I. Die Phylogenie der Rhopaliophora (Scyphozoa und Cubozoa) und die Paraphylie der 'Rhizostomeae'. PhD Thesis, Universität Hamburg. 2009. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.427.7719&rep=rep1&type=pdf
33. Purcell JE, Atienza D, Fuentes V, Olariaga A, Tilves U, Colahan C, et al. Temperature effects on asexual reproduction rates of scyphozoan species from the northwest Mediterranean Sea. Hydrobiologia. 2012;690: 169-80. doi: 10.1007/s10750-012-1047-7
34. Schiariti A, Morandini AC, Jarms G, Von Glehn Paes R, Franke S, Mianzan H. Asexual reproduction strategies and blooming potential in Scyphozoa. Mar Ecol Prog Ser. 2014;510: 241-53. doi: 10.3354/meps10798
35. Kühl H. Hydrography and biology of the Elbe estuary. Oceanogr Mar Biol Ann Rev. 1972;10: 225-309.
36. Collins AG. Phylogeny of Medusozoa and the evolution of cnidarian life cycles. J Evol Biol. 2002;15: 418–432.
37. Helm RR. Evolution and development of scyphozoan jellyfish. Biol Rev. 2017. doi: 10.1111/brv.12393
38. Thein H, Ikeda H, Uye S. The potential role of podocysts in perpetuation of the common jellyfish Aurelia aurita s.l. (Cnidaria: Scyphozoa) in anthropogenically perturbed coastal waters. Hydrobiologia. 2012;690(1): 157–67. doi: 10.1007/s10750-012-1045-9
39. Arai MN. The potential importance of podocysts to the formation of scyphozoan blooms: a review. Hydrobiologia. 2009;616: 241–6. doi: 10.1007/s10750-008-9588-5
40. Pitt KA. Life history and settlement preferences of the edible jellyfish Catostylus mosaicus (Scyphozoa : Rhizostomeae). Mar Biol. 2000;136: 269-79.
41. Schiariti A, Kawahara M, Uye S, Mianzan HW. Life cycle of the jellyfish *Lychnorhiza lucerna* (Scyphozoa: Rhizostomeae). Mar Biol. 2008;156(1): 1-12. doi: 10.1007/s00227-008-1050-8

42. Ding G, Chen J. The life history of Rhopilema esculenta Kishinouye. J Fish China. 1981;5: 93-104.
43. Lotan A, Ben-Hillel R, Loya Y. Life cycle of Rhopilema nomadica: a new immigrant scyphomedusan in the Mediterranean. Mar Biol. 1992;112: 237–242. doi: 10.1007/BF00702467
44. Cargo DG. The sessile stages of a Scyphozoan identified as Rhopilema verrilli. Tulane Stud Zool Bot. 1971;17: 31-34.
45. Calder DR. Laboratory observations on the life history of Rhopilema verrilli (Scyphozoa: Rhizostomeae). Mar Biol. 1973;21: 109-114. doi: 10.1007/BF00354606
46. Kawahara M, Uye S, Ohtsu K, Iizumi H. Unusual population explosion of the giant jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae) in East Asian waters. Mar Ecol Prog Ser. 2006;307: 161-173. doi: 10.3354/meps307161
47. Calder DR. Life history of the Cannonball jellyfish, *Stomolophus meleagris* L. Agassiz, 1860 (Scyphozoa, Rhizostomida). Biol Bull. 1982;162: 149-162. doi:10.2307/1540810
48. Straehler-Pohl I. Cubozoa and Scyphozoa: The results of 20 years of scyphozoan life cycle research with new results on cubozoan life cycles to suggest a new nomenclature referring to both classes. In: Frontiers in ecological studies of jellyfish, editors. Toyokawa M, Miyake H, Nishikawa J. 2017. pp 17-29.
49. Astorga D, Ruiz J, Prieto L. Ecological aspects of early life stages of *Cotylorhiza tuberculata* (Scyphozoa: Rhizostomae) affecting its pelagic population success. Hydrobiologia. 2012;690(1): 141-55. doi: 10.1007/s10750-012-1036-x
50. Lucas CH, Graham WM, Widmer C. Jellyfish life histories: role of polyps in forming and maintaining scyphomedusa populations. Adv Mar Biol. 2012; 63:133-196.
51. Dawson MN, Hamner AE. A character-based analysis of the evolution of jellyfish blooms: Adaptation and exaptation. Hydrobiologia. 2009; 616(1): 193–215. doi: 10.1007/s10750-008-9591-x
52. Lilley MKS, Houghton JDR, Hays GC. Distribution, extent of inter-annual variability and diet of the bloom-forming jellyfish Rhizostoma in European waters. J Mar Biol Assoc U.K. 2009; 89(1): 39–48. doi: 10.1017/S0025315408002439

Chapter IV

Environmental forcing in early life stages of the scyphozoan jellyfish *Rhizostoma luteum* (Quoy and Gaimard 1827)

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ABSTRACT

Understanding the role of early life stages in the formation of jellyfish outbreaks, whether they are cyclic or in response to climate change, is important and has been neglected until recently. The aim of this study was to determine the response to environmental forcing in the early life stages of the large jellyfish *Rhizostoma luteum.* Laboratory experiments were carried out to examine the effects of different temperature and salinity conditions on planula settlement, scyphistoma development, podocyst production, ephyra liberation and development until the metaephyra stage. The results show that the planula larvae were highly resilient to a wide thermal and salinity window and they settled faster in warmer incubating conditions (≥23 °C). However, the benthic scyphistoma stage showed physiological stress in the warmest (28 °C) and saltiest (38) treatments. Asexual propagation was greater at mid-temperature and the metaephyra stage was achieved faster at 23 °C and in hyposaline conditions. The ongoing ocean warming and the decrease in rainfall might explain the increase in population, observed in recent decades, in the southern Iberian Peninsula. Nonetheless, the elevated T-S condition in the NW Mediterranean Sea might be a natural barrier for *R. luteum* early life stages. Their ability to grow in hyposaline conditions raises the possibility that some or all reproductive stages may occur in estuaries or other brackish environments. As in the wild, the sessile and ephyra phases have never been detected, and the present study may present a clue regarding where to start searching for these vital stages.

Keywords: Scyphistomae; asexual reproduction; growth; planulae settlement; polyps; podocysts; temperature; salinity

INTRODUCTION

The majority of ordinary people associate a ‘jellyfish’ with the free-swimming bell-shaped medusa, which is the stage that is perceived by the tourist, fishery and aquaculture industries as a nuisance. However, this final life-cycle stage is far removed from the predominant stage. Generally, scyphomedusae have a bipartite life cycle that includes a benthic asexual stage (scyphistoma or most commonly termed as ‘polyp’) while being attached to hard surfaces, and a pelagic sexual stage (medusa). Scyphozoan life cycles are among the most complex of life styles, with several stages, inhabiting different ecosystems and varying in size by orders of magnitude. The population size of the adult medusae is determined by the abundance and survival of each of the life stages of planulae, scyphistomae, production of ephyrae (strobilation), and young medusae in the water column (Purcell et al. 1999; Purcell 2005; Arai 2005). The overall knowledge of the ecology and distribution of the early life stages is meagre. It must be taken into consideration that for most species the scyphistomae have never been identified in the field, due to their minute size, preference of colonizing the undersides of substrates, and cryptic character (Lucas et al. 2012). This complex life history makes it complicated to determine which phase of the life cycle is the most affected by altering environmental conditions. Some studies have been conducted in the laboratory to determine the influence of environmental factors, such as temperature, salinity, light and food supply on the early life stages (reviewed in Purcell 2005; Holst and Jarms 2010; Prieto et al. 2010; Astorga et al. 2012; Purcell et al. 2012; Schiariti et al. 2014; Feng et al. 2018). It is assumed that the variety of modes of asexual reproduction are more diverse than previously presumed, hence, its ecological consequence has probably been underestimated (Fautin 2002; Adler and Jarms 2009; Schiariti et al. 2014). As a result of the complexity of the early life stages in many scyphozoa species, the influence of environmental factors is still not fully understood.

Various medusa species, particularly those inhabiting coastal and shelf ecosystems, are tolerant to a wide range of environmental conditions of temperature, salinity, food availability, oxygen concentration and pH (Arai 1997; Lucas 2001; Purcell 2005). Warming ocean temperatures might permit some scyphozoan species to broaden or shift their distributions, prolong their seasonal occurrence or even survive as an adult medusa during the colder winter months (reviewed in Purcell 2012; Purcell et al. 2018). Furthermore, high temperatures have been reported to positively influence asexual reproduction, thus achieving a larger population (Lucas et al. 2012; Purcell et al. 2012; Schiariti et al. 2014). Several species of scyphozoan jellyfish occur in estuaries and brackish waters, however, for many species it is still unknown whether establishment and reproduction of the benthic stage occur in those low-salinity waters (reviewed in Holst and Jarms 2010). As all life stages of scyphozoans are considered osmoconformers, apart from the podocyst stage (reviewed in Arai 1997). Therefore, the benthic stage is limited by the inability of planulae to survive and settle, or the incapability of scyphistomae to develop and strobilate in hyposaline conditions.

The scyphozoan jellyfish *Rhizostoma luteum* (Quoy and Gaimard 1827) belongs to the order Rhizostomeae and is a temperate coastal species with a distribution range from the west to the south coasts of the Iberian Peninsula and west and north-mid shores of Africa (see map in Fig. 1). In 2017, Kienberger and Prieto (2018) were able to demonstrate that this medusa is common along the southern Iberian coastal area. The life cycle of *R. luteum* has been described by Kienberger et al. (2018) and it has a typical metagenetic life cycle (polypoid/medusoid). *Rhizostoma luteum* has separate sexes, and the planulae are brooded (internal fertilisation with subsequent release of planulae) by the female medusae. Brooding serves to protect embryos from stressful external conditions by retaining progeny inside the female body, effectively reducing the risk of pelagic stages being exposed to predation or other environmental stressors. After the release of the planulae into the water column, they settle onto hard substrates and metamorphose into scyphistomae. The fully developed scyphistoma, typically, has a conspicuous club-shaped hypostome and 16 filiform tentacles and these characteristics are useful for identifying the benthic state of the group of Rhizostomida (Straehler-Pohl 2017). The pelagic medusa population increases through strobilation, and a single scyphistoma produces and releases one planktonic ephyra (monodisc strobilation) at a time, which grows into a sexually reproducing medusa (Kienberger et al. 2018). After releasing the ephyra, the scyphistoma may continually repeat this process. Apart from strobilation, the only form of asexual reproduction that has been observed in *R. luteum* is the formation of chitin-covered podocysts. The purpose of the podocysts is the propagation and persistence of the benthic populations. In general, the life cycle resembles that of its sibling species *Rhizostoma pulmo* and *Rhizostoma octopus* with the exception of two features: the female *R. luteum* is a brooding species, and the strobilation type is predominantly monodisc, and not polydisc, as with the other two species in the genus.

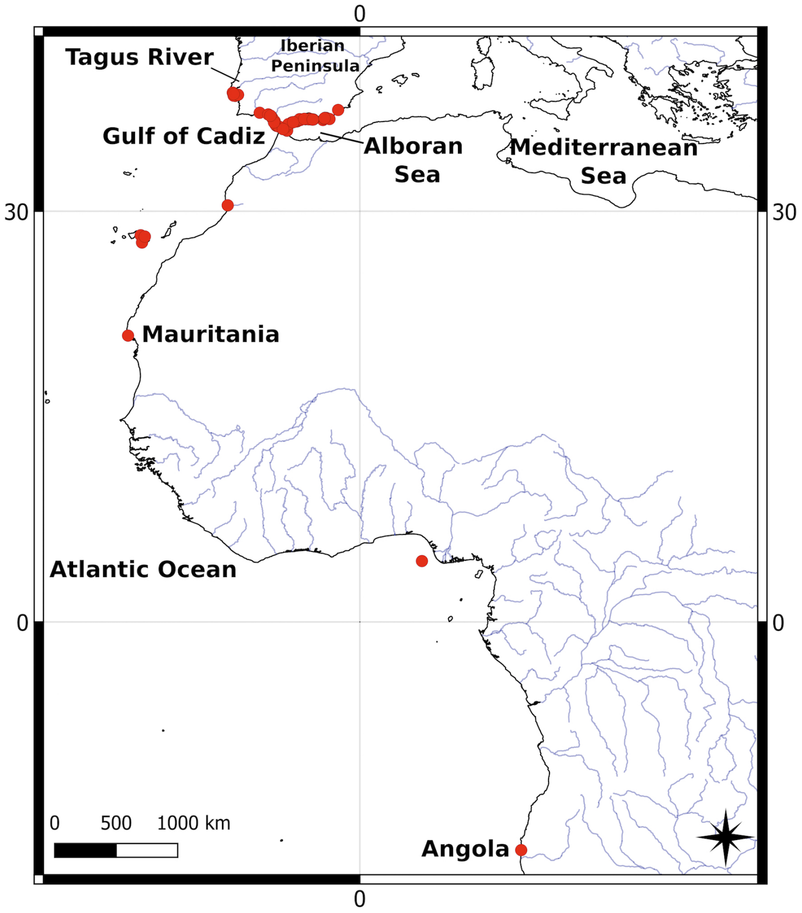

Figure 1 Range of spatial distribution of the jellyfish *Rhizostoma luteum* (Figure redrawn from Kienberger and Prieto, 2018)

A recent study revealed that *R. luteum* might be exploited as a sustainable resource to be used as a potential biomass for several beneficial human usages (Prieto et al. 2018), therefore, knowing the environmental factors controlling the early life stages is fundamental. On the other hand, because of its imposing size (about 70 cm), its presence near the coast can have a negative impact on the image of coastal area with high tourist development. Especially as this medusa seems to be evolutionarily predisposed to form problematic blooms if environmental conditions permit (Kienberger et al. 2018). Consequently, it is important to understand the role of early life stages in the formation of jellyfish outbreaks, whether they are cyclic or in response to climate change. The purpose of the present study was to conduct laboratory experiments to test the tolerance to combined effects of temperature and salinity on the early life stages of *R. luteum* and the consequences for the adult medusa population. Thus, we hypothesise that the resistance of *R. luteum* early life stages to the range of environmental conditions in the latitudes where it is usually observed is the key characteristic of its ecological success. In the wild the sessile and ephyra phases have never been detected, hence, the present study may present a clue regarding where to start searching for these early life stages.

MATERIALS AND METHODS

Planula settlement, scyphistoma development, podocyst production, and ephyra liberation and development were evaluated in laboratories at the Aquarium House of the Vienna Zoo, Austria. Treatments were chosen to test responses to the local environmental conditions (temperature and salinity) where adults have been observed (see map in Fig. 1). The temperature range that was tested covers the extremes values from winter and summer in natural locations of *Rhizostoma luteum*. In the Gulf of Cadiz (NE Atlantic Ocean) and Alboran Sea (SW Mediterranean Sea), temperatures oscillate between 14 and 23 °C, and for the most southern observation in Angola (SE Atlantic Ocean) the sea surface temperature might reach 28 °C. In the brackish waters of the mouth of the Tagus River (Lisbon), salinities could be 24, while in Angola/Mauritania, Gulf of Cádiz and Alboran Sea the usual salinities are 30, 36.5 and 38, respectively (NOAA 2019).

Experimental set-up

Rhizostoma luteum used in this study originated from cultures obtained from one mature jellyfish in southern Spain in October 2015 (details in Kienberger et al. 2018). In the laboratory, various medusae were reared at constant temperature and salinity, 24±0.5°C and 34±0.5, respectively, until reaching maturity from ephyra cultures liberated on 11 June 2017 (rearing conditions described in Kienberger et al. 2018). For all experiments described in the present study, stock solutions of artificial seawater were made by dissolving commercially prepared sea salt mix (Instant Ocean® Synthetic Sea Salts) in reverse-osmosis water, waiting 24 hours for the salt to dissolve properly. Salinities were measured with a portable pH-temperature-salinity meter (HACH® HQ40D). Flasks were placed in corresponding water baths, to maintain stable temperatures (± 0.5 °C), that were connected to an aquarium chiller (Aqua Medic Titan 1500), under 12L-12D photoperiod with light intensity of 23.1 lux. All stages were monitored and photographed using a Nikon® SMZ800N stereomicroscope and the NIS-Elements image-processing software.

Experimental design

Experiment A: Planulae settlement

As *R. luteum* is a brooding species and planulae were collected from one female medusa using a Pasteur pipette (64 days post-liberation, bell diameter of 14.7 cm). Sub-samples of equal volume (0.5 ml) containing approximately 80 planulae were obtained from an agitated container with the collected planulae and were transferred immediately to the experimental treatments without acclimation period. Sixteen (16) treatments were performed (Table 1) consisting of four temperatures (14, 18, 23, 28 °C) and four salinities (24, 30, 36.5, 38). Each cylindrical glass flask (6.0 cm diameter) was filled with 150 ml of the corresponding artificial seawater, covered by a plastic lid and kept without aeration. A glass slide of 7.5 x 2.5 cm was placed diagonally as a substrate for the planulae to attach themselves to within the flask (Schiariti et al. 2008; Astorga et al. 2012). Different numbers of replicates (6, 12, 9 and 9 replicates at 14, 18, 23 and 28 °C for each salinity tested, respectively) were employed, as Experiment A was the starting point for further experiments detailed in the next subsections. Though for Experiment A we monitored and considered only 3 replicates for each treatment. We checked daily various subsamples of each flask using a Nikon® SMZ800N stereomicroscope and determined whether planulae were swimming or not. During Experiment A the cultures were not fed and the water was not changed. Experiment A was running for 8 weeks.

Table 1 Summary of the different experiments performed on the early life stages of Rhizostoma luteum

Stages and process of the life cycle	Treatments (Temperature/Salinity)	Replicates per treatment
Experiment A		
Planulae settlement	14°C /24, 30, 36.5, 38	3
	18°C /24, 30, 36.5, 38	3
	23°C /24, 30, 36.5, 38	3
	28°C /24, 30, 36.5, 38	3
Experiment B		
Scyphistoma development, asexual reproduction and strobilation	14°C /24, 30, 36.5, 38	6
	18°C /24, 30, 36.5, 38	12
	23°C /24, 30, 36.5, 38	9
	28°C /24, 30, 36.5, 38	9
Experiment C		
Growth rate ephyrae	14°C/24, 33, 38	5
	18°C/33, 38	5
	23°C/24, 33	5
	28°C/24, 33, 38	5

Experiment B: Scyphistoma development, asexual reproduction and strobilation

For Experiment B (Table 1) we used the settled planulae from Experiment A in the same laboratory conditions. After settlement of the planula and development of the first tentacles scyphistomae were fed (initial number of scyphistomae). The scyphistoma culture was fed every 3-4 days with newly hatched *Artemia salina* nauplii enriched with Selco® S.presso *ad libitum*. Three (3) hours after feeding, the rearing medium was exchanged with new, aerated artificial seawater. All the replicates were monitored at intervals of 1 to 4 days. To quantify the increase or mortality rate in each treatment scyphistomae were counted, and their asexual reproductive strategies (podocysts and ephyrae production) were monitored for 6 weeks. The total body diameter (TBD) of each newly released ephyra was measured and photographed using a Nikon® SMZ800N stereomicroscope, and then each sample was removed from the experiment. On the last day of the experiment, the total body length (TBL) of fully-grown scyphistoma (16-tentacles) was measured.

Experiment C: Growth of early medusa stages

This experiment was performed to test the hypothesis that the development and survival of the new released ephyra may be affected as they move with the currents to different temperatures and salinities, while taking into consideration that the main stock of scyphistoma colonies (based upon several years of our working experience in the laboratory) probably develop better under conditions that are typical of brackish waters. Laboratory experiments were conducted to establish the growth of 50 ephyrae (*n* = 5 per treatment, see Table 1) over the range of four temperatures (14, 18, 23 and 28 °C) and three salinities (24, 33 and 38). Ephyrae used in Experiment C all originated from the same stock culture at constant temperature and salinity, 23±0.5 °C and 24±0.5, respectively. The newly released ephyrae had a mean total body diameter (TBD) of 3.99 ± 0.49 mm (n=50). As soon as they were liberated, they were individually transferred into 200-ml glass flasks without any acclimation period, covered by a plastic lid and kept without aeration. All flasks were maintained at constant T-S in a water bath as described in the above. Ephyrae were fed daily with newly hatched *Artemia salina* nauplii enriched with Selco® S.presso *ad libitum*. After 4 hours of feeding, ephyrae were transferred, using a small container, to new flasks with fresh seawater that was previously aerated. The total body diameter (TBD) of each ephyra was measured daily using a Nikon® SMZ800N stereomicroscope and the image-processing NIS-Elements software. This was undertaken in order to follow the development of the gastric system, manubrium and marginal lappets which would enable identification of the development stage. Each ephyra was placed in a petri dish with the manubrium facing upside. We compared the TBD and the development stage (as defined by Kienberger et al. (2018) for *R. luteum*) 7, 14 and 21 days post-liberation, and the time period (days) required to reach the metaephyra stage in each treatment (maximum duration of 38 days). This state is easily distinguished as the mesogloea thickens and the flat exumbrella of the ephyra transforms into a typical bell-shaped young medusa for *R. luteum*, as described in Kienberger et al. (2018).

Statistical analysis

All data were tested for normality (Shapiro-Wilks test) and homoscedasticity of variances was evaluated using the Barlett test and the Levene test; data with normal distribution were tested by two-way analysis of variance (ANOVA). Significant ANOVA results were tested using Turkey’s pair-wise comparisons. Data that were not normally distributed, and even after applying various transformations, were tested by a non-parametric analogue (Kruskal-Wallis ANOVA on rank) and using Dunn’s pair-wise comparisons in post hoc tests. A significant level of α = 0.05 was chosen for all tests that were performed in this study. All statistical analyses of the data were performed using R (v3.4.1) software and results have been included in the Appendix D (ESM Tables 1, 2 and 3). Data are presented as mean ± 0.01 standard deviation (SD).

RESULTS

Transition from pelagic to benthic phase

Experiment A: Planulae settlement

The planula larvae mean settlement time was significantly affected by temperature (ANOVA, $F_{3,32}$ =129.9, P<0.001), salinity ($F_{3,32}$ =23.7, P<0.001), and their interaction ($F_{9,32}$ =2.26, P=0.043; all statistical results are given in Appendix D ESM Table 1). In the two warmest incubating conditions (23 and 28 °C), planulae started to settle within the first week (Fig. 2) and the majority settled within 2 weeks, which was significantly faster than in the colder treatments (5 weeks). Larvae in the saltiest conditions (38) swam for a significantly longer period of time (4 weeks), than at lower salinities.

Sixty-three percent (63%) of planulae settled on the bottom (BOTT) of the flasks, and 29 and 8% developed on the glass slide (GS) and on the side of the flasks (LAT), respectively. In the intermediate temperature groups (18 and 23 °C), planulae settled on all 3 locations, except in the highest salinity condition (38). The amount of scyphistomae per location was significantly affected by temperature (Kruskal-Wallis test, H_3 =13.10, P=0.004), salinity (H_3 =18.47, P<0.001); and location (H_2 =8.44, P=0.015; ESM Table 2).

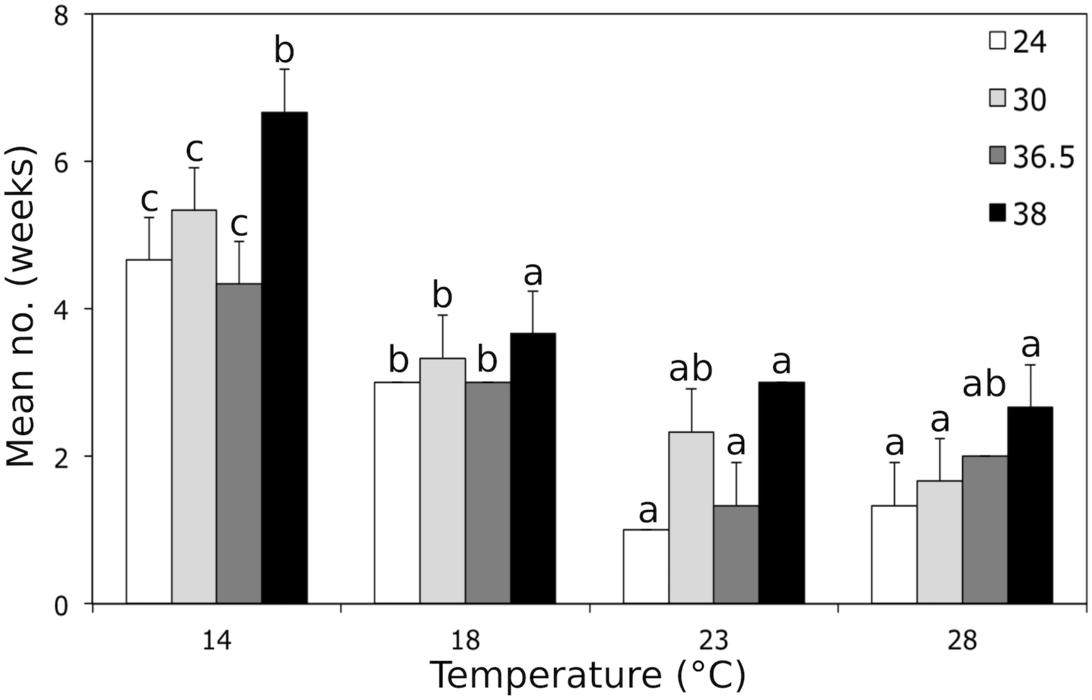

Figure 2 *Rhizostoma luteum* planulae settlement through time. Mean number of weeks that planulae are swimming in each treatment under different T-S combinations. Colours stand for different salinities tested. Error bars are the upper standard deviation of the three replicates. Means with different letters were significantly different as determined by Tukey's HSD tests at α=0.05

Benthic phase

Experiment B: Scyphistomae development

The scyphistoma population were significantly affected by temperature (ANOVA, $F_{3,128}$ =5.33, P=0.002), salinity ($F_{3,128}$ =9.59, P<0.001) and their interaction ($F_{9,128}$=2.18, P=0.027; ESM Table 1). Scyphistoma survival was positive between 14 - 23 °C and in salinity ranges of 24 - 36.5. Significantly more scyphistomae developed at salinity 24 (Fig. 3) in all temperature groups. Survival rate was negative in the warmest temperature group (28 °C) and saltiest treatment (38). Mortality rate ranged from 17 to 83% with a 100% mortality at 28 °C-28.

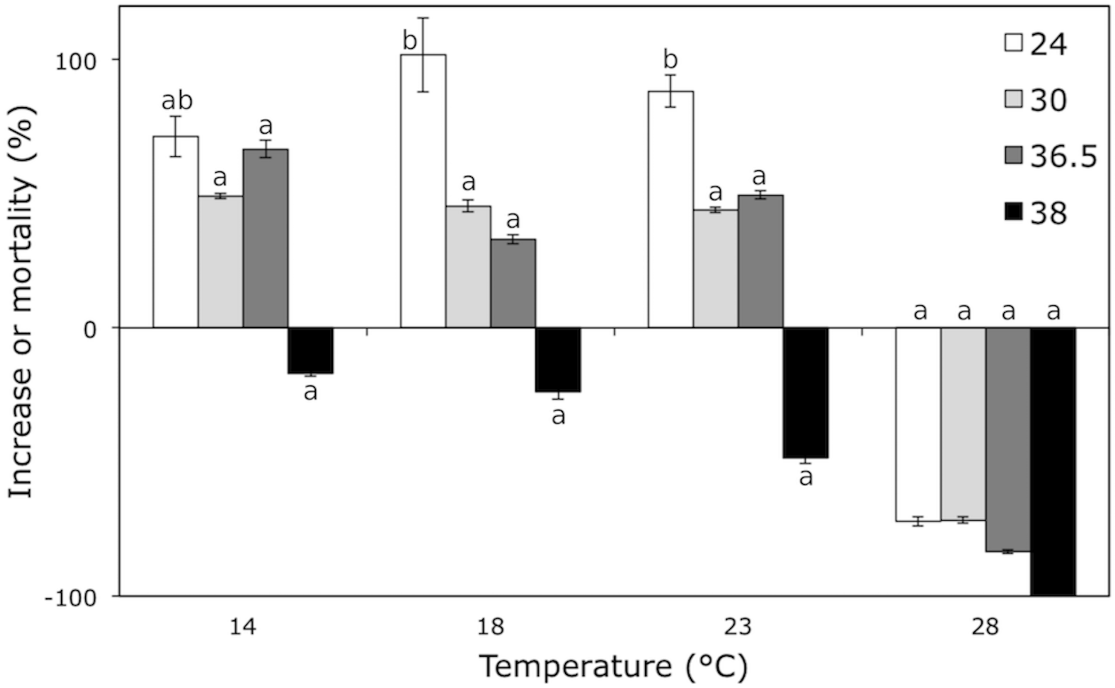

Figure 3 *Rhizostoma luteum* percentage of scyphistoma increase and mortality under different T-S combinations during 6 weeks. Colours stand for different salinities tested. Error bars are the upper and lower standard deviation of the 6, 12, 9 and 9 replicates at 14, 18, 23 and 28°C, respectively. Means with different letters were significantly different as determined by Tukey's HSD tests at α=0.05

Scyphistomae at 23 °C had a greater total body length (TBL, Kruskal-Wallis test, H_3=18.77, P<0.001; Fig. 4 and ESM Table 1). There was also a trend towards greater sizes in hyposaline conditions; however, their range did not differ significantly (H_3=3.83, P=0.281). Morphological differences between the tested temperature conditions are shown in Fig. 5. In general, scyphistoma at mid-temperature (Figs. 5b, c) developed the typical slender stalk, with predominantly regular 16 filiform tentacles, with respect to the previously described life cycle in Kienberger et al. (2018), with a mean size of 1.32 ± 0.21mm and 1.56 ± 0.32 mm, at 18 and 23 °C, respectively. At 14 °C, scyphistomae exhibited a regular growth at all salinities, but most of them had a very short stalk, as seen in Fig. 5a. At 28 °C, scyphistomae presented distorted morphologies, they developed an odd number of tentacles (20 and 8, Figs. 5d and e, respectively), which often grew at different rates, resulting in pronounced morphological asymmetries, and scyphistomae with retracted tentacles were also observed.

Experiment B: Asexual reproduction

Formation of podocysts occurred beneath the basal region of the mature scyphistomae (16-tentacles) and at the attachment area of finger-shaped stolons (see Figs. 5a, d and c, respectively), which developed from the lower stalk. Podocysts developed at all temperatures, but not in all salinities, see Fig. 6. The production of podocysts was significantly influenced by thermal and salinity conditions (Kruskal-Wallis test, H_3=23.3, P<0.001; H_3=10.9, P=0.012, respectively, see ESM Table 1). Producing the greatest amount at 23 °C and in all hyposaline conditions, apart from 14 °C, podocysts developed only at the salinity of 36.5. No other forms of asexual reproduction, such as budding and/or fission, were observed during the entire experiment (6 weeks).

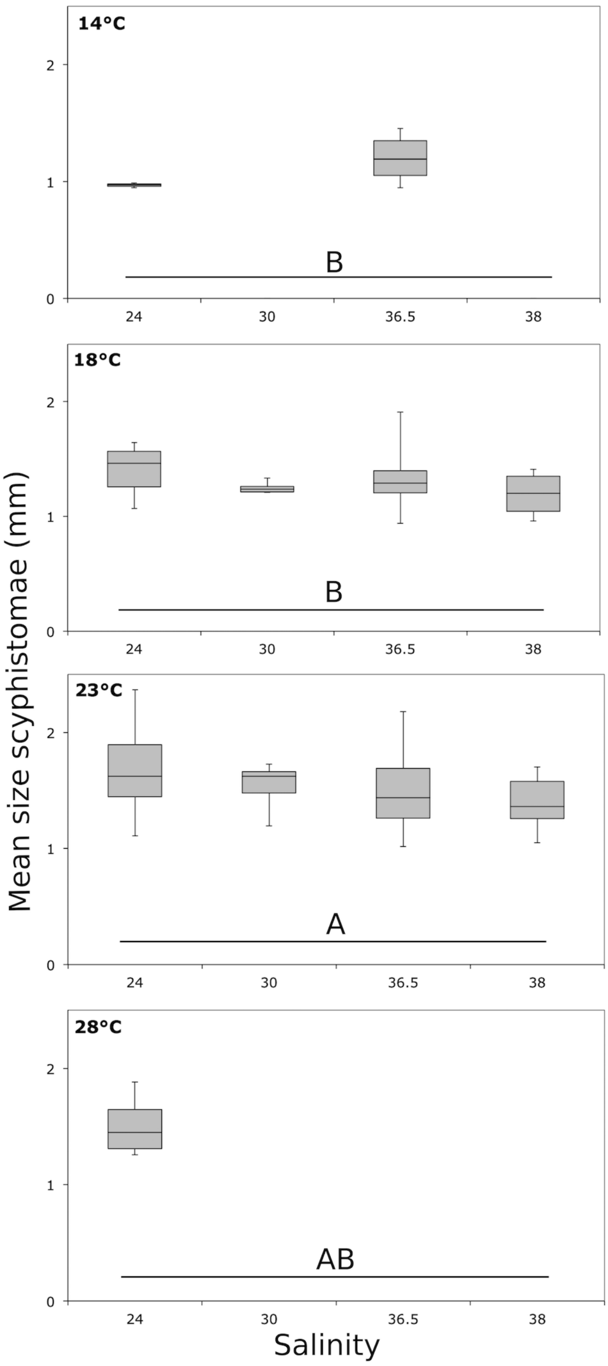

Figure 4 Rhizostoma luteum mean scyphistomae size. Only full-grown scyphistomae (16 tentacles) were measured on the last day of the experiment, which were not present in all treatments at 14 and 28°C. Boxes are median and interquartile range, whiskers are minimum and maximum size. Error bars show the maximum and minimum size. Dunn's pair-wise differences at α=0.05 for temperature are indicated by uppercase letters

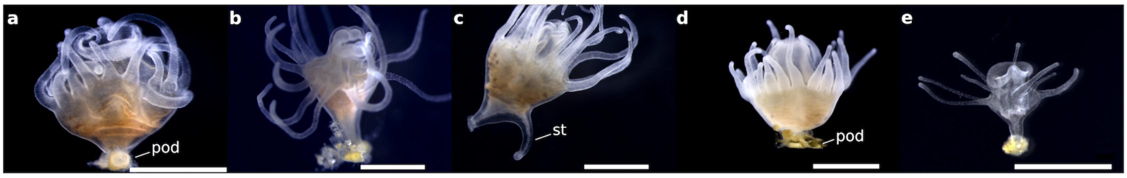

Figure 5 Fully grown *Rhizostoma luteum* scyphistomae reared at various T-S conditions. a scyphistoma grown at 14°C-24 with one podocyst (pod) at the base of the stalk, total body length (TBL) 1.21 cm; b 18°C-24 scyphistoma with typical slender stalk, TBL 1.73 cm; c 23°C-24 production of podocyst by means of stolon (st), TBL 1.98 cm; d 28°C-24 scyphistoma with 20 tentacles and podocyst (pod) at the base of the stalk, TBL 1.43 cm; e 28°-36.5 scyphistoma revealing distorted morphology, TBL 0.87 cm. Scale bare 1mm. Photos by K. Kienberger

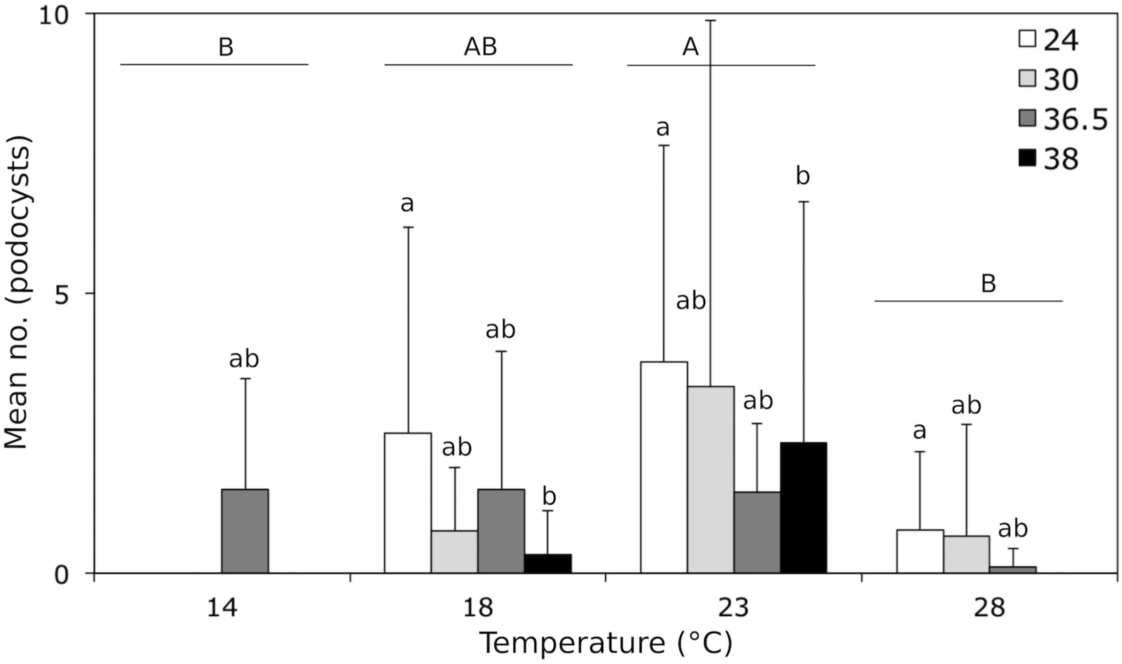

Figure 6 *Rhizostoma luteum* podocysts production. Mean number of podocysts produces counted under different T-S combinations. Colours stand for different salinities tested. Error bars are the upper standard deviation of the 6, 12, 9 and 9 replicates at 14, 18, 23 and 28°C, respectively. Dunn's pair-wise differences at α=0.05 for temperature are indicated by uppercase letters and by lowercase letters for salinity

Transition from benthic to pelagic phase

Experiment B: Strobilation

Free-swimming juvenile medusae (ephyrae) were liberated, but only in the two intermediate temperature groups at 18 (35%) and 23 °C (65%). The 1st ephyra released was in week 3 in the treatment at 23°C with salinity of 24. From week 4 onwards, ephyrae were also liberated into the lower temperature. At 18 °C, ephyra developed in all salinities, however, at 23 °C none developed in the saltiest treatment (Fig. 7). The production of ephyrae was significantly affected by temperature (Kruskal-Wallis test, H_3=19.34, P<0.001), and salinity (H_3=13.60, P=0.003; ESM Table 1). The mean number of ephyrae for treatments at 24 (1.2 ± 1.6) was significantly higher than at the upper salinities (36.5: 0.2 ± 0.4; 38: 0.1 ± 0.2). During our observation, two scyphistomae again produced one ephyra in treatment 23 °C with salinity of 24, being the strobilation time between two liberations of 8 and 14 days.

There was no significant difference between the mean sizes of freshly released ephyra at the temperature, salinity and interaction levels (ANOVA, all P>0.2, see ESM Table 1). Newly liberated ephyrae reached between 3.07 to 4.82 mm from lappet tip to lappet tip when extended (TBD total body diameter; n=31; 3.9 ± 0.5 mm). Typical ephyra had 8 marginal lappets, although 18% were irregular and mostly with 6, 9 or 10 marginal lappets. Apart from one ephyra that was liberated from the side of a flask, being located approximately 1 cm from the bottom, all others were from scyphistomae that were attached to the bottom of the experimental flasks.

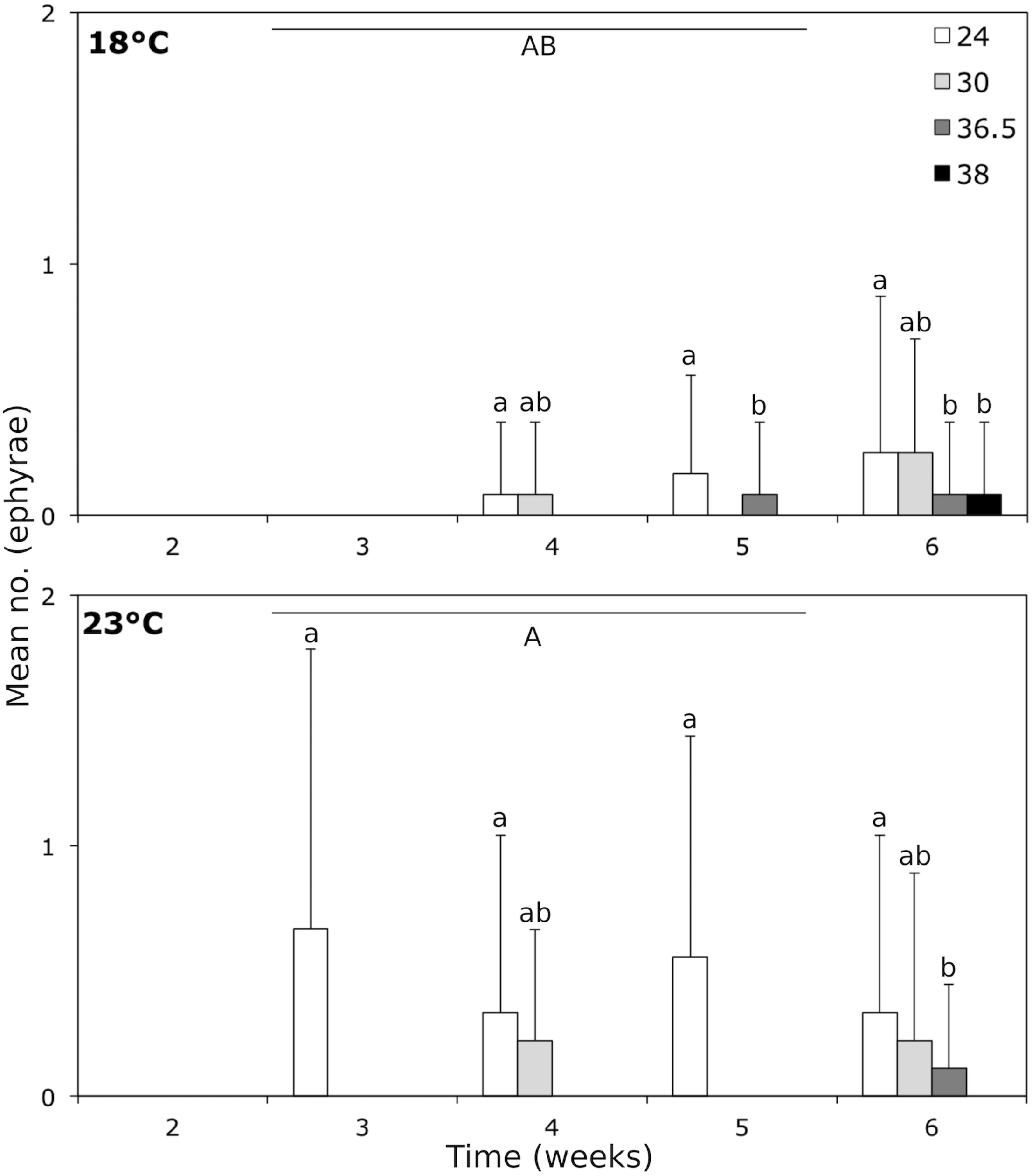

Figure 7 *Rhizostoma luteum* ephyrae production through time. Mean number of ephyrae liberated under different T-S combinations over 6 weeks. Colours stand for different salinities tested. Error bars are the upper standard deviation of the 12 and 9 replicates at 18 and 23°C. Dunn's pair-wise differences at α=0.05 for temperature are indicated by uppercase letters and by lowercase letters for salinity

Pelagic phase

Experiment C: Growth of early medusa stages

A two-way ANOVA showed that the total body diameter (TBD) and the development stage differed significantly among the temperature, salinity and interaction treatments after 7, 14 and 21 days post-liberation (all $p<0.01$, see Electronic Supplement Material ESM Table 3). Seven-days (7-days) post-liberation, the ephyra TBD increased with warmer temperatures and they were better developed, except in the highest temperature group (28 °C), where ephyra size was smaller than in the other treatments (see Fig. 8). In general, ephyra reared at ≤23 °C developed a regular morphology (Figs. 9a-f) and increased diameter since the first day of liberation. In the saltiest treatments (38), all ephyrae decreased in size during the first few days, even though the ephyrae were feeding and, in appearance, they seemed to be in good condition (Figs. 9c and f). At 28 °C, some ephyrae also had a negative growth and the bell (mesogloea) developed in a very transparent manner (somehow, seeming to be thinner than in the other treatments) and irregular (Figs. 9g and h). At 28 °C with salinity 38, various specimens everted their bells and were in poor condition (Fig. 9i).

Fourteen-days (14-days) post-liberation, the mean TBD was significantly greater at 23 °C, and also for the treatments at lower salinities (24 and 33, ESM Table 3). At 28 °C, the morphology of the ephyrae was irregular, various specimens everted their bells and were in poor condition. Six (6) ephyrae died in the condition at 28 °C in the second week of the experiment (28 °C: 2, 1 and 3 ephyrae at salinity 24, 33 and 38, respectively).

Twenty-one-days (21-days) post-liberation, ephyrae in the treatment at 23 °C with salinity 33 had the greater diameter (Fig. 8), however, the difference between the two lower salinities (24 and 33) was not significant (ESM Table 3). After three weeks, all ephyrae in the saltiest incubating condition (38) and in all temperature groups, presented an irregular development, and they everted their bells, therefore, the taking of measurements was not always possible. At 14 and 23 °C, the mortality rate was nil, though, 2 ephyrae died at 18 °C and all died at 28 °C.

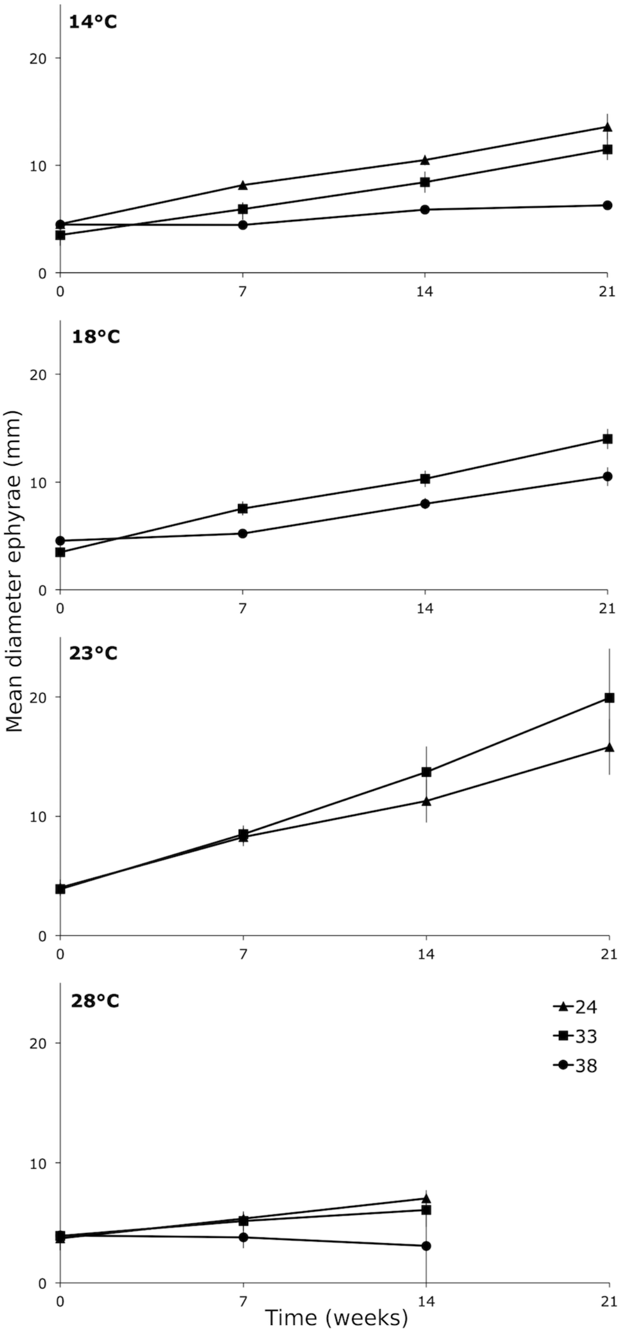

Figure 7 *Rhizostoma luteum* ephyrae production through time. Mean number of ephyrae liberated under different T-S combinations over 6 weeks. Colours stand for different salinities tested. Error bars are the upper standard deviation of the 12 and 9 replicates at 18 and 23°C. Dunn's pair-wise differences at α=0.05 for temperature are indicated by uppercase letters and by lowercase letters for salinity

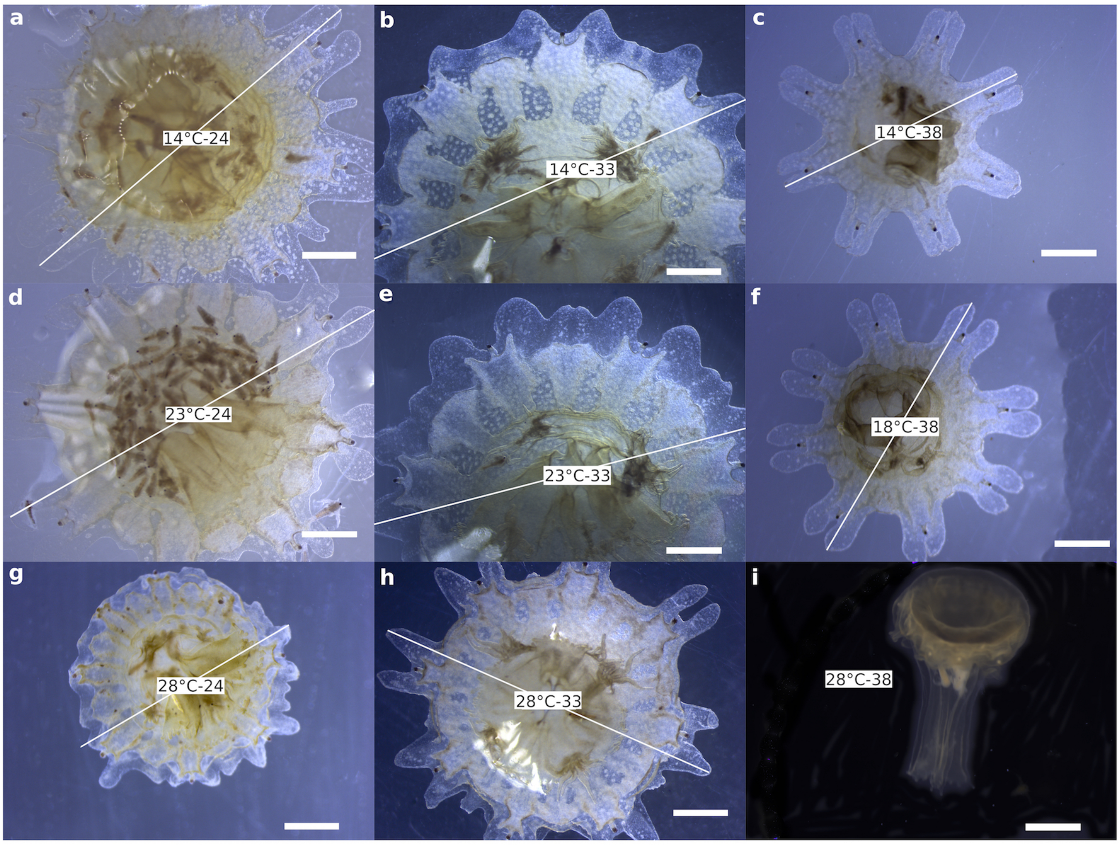

Figure 9 *Rhizostoma luteum* ephyrae 7-days post-liberation reared under different T-S conditions. Scale bare 1mm. Photos by K. Kienberger

The time period that was needed to reach the metaephyra (stage 7) was strongly controlled by temperature ($p<0.001$, ESM Table 3), however, this was not significant between salinities. Stage 7 was rapidly attained at 23 °C (18-20 days), and more time was needed at 14° C (32-38 days). The metaephyra stage was never achieved in the conditions at a temperature of 28 °C and salinity of 38. In general, ephyrae had positive linear growth over time in all incubating conditions. The fastest growing group was at 23 °C with salinity 33 and with an average diameter increase of 0.72 mm per day (Linear regression, R^2= 0.91, $F_{1,108}$=1031, P<0.001).

DISCUSSION

Rhizostoma luteum is a temperate coastal species (see Fig. 1), occurring in regions with strong upwelling episodes and found in shelf ecosystems, which are connected to variations in surface water runoff from land (rainfall and submarine groundwater discharges). Consequently, it is expected that all or some life stages would be exposed to thermal and salinity oscillations and to seasonal temperature fluctuations throughout the year. Our results support the fact that the early life stages of *R. luteum* in laboratory conditions were, on the one hand, highly tolerant to wide ranges of temperature and salinity. On the other hand, some stages showed some significant differences in response to the environmental factors that they were exposed during our laboratory experiments (see Table 2).

Table 2 Summary of the responses of *Rhizostoma luteum* early life stages to environmental factors temperature and salinity. The interaction between both factors was never significant

Response variable	Environmental variable							
	Temperature				Salinity			
	14°C	18°C	23°C	28°C	24	30	36.5	38
Planulae settlement time	*	*	*	*	*	*	*	*
Survival scyphistoma	*	*	*	*	*	*	*	*
Size scyphistoma	*	*	*	*	NS	NS	NS	NS
Total no. of podocysts	*	*	*	*	*	*	*	*
Total no. of ephyrae	*	*	*	*	*	*	*	*
Size ephyrae	NS	NS	NS	NS	NS	NS	NS	NS

* Significant difference ($P < 0.05$), *NS* no significant difference ($P > 0.05$)

Transition from pelagic to benthic phase: planula settlement

Our results suggest that the planula phase of *R. luteum* is highly resilient to a wide thermal window (14 to 28 °C), as none of the tested temperatures limited the settlement capacity and/or its further transformation into scyphistoma. Planulae settled significantly faster in the warmest treatments (23 and 28 °C, Fig. 2). The fact that warmer temperatures decreased the period of swimming, was also reported for other temperate scyphozoan species, such as *Cotylorhiza tuberculata* (Prieto et al. 2010), *Aurelia aurita* (Webster and Lucas 2012), *Cyanea lamarckii* and *Chrysaora hysoscella*

(Gambill et al. 2015). Having a faster settlement time is important, as planulae are lecithotrophic, and they need to settle rapidly in order to metamorphose into feeding scyphistoma, before their energy reserves are exhausted.

Planulae from various species from the North Sea showed a high tolerance to low salinities, although they had diminished swimming and they did not settle on the underside of floating substrate (<20, Holst and Jarms 2010). Further, *Aurelia coerulea* planulae from the China Sea were less active under reduced salinity conditions of 22 (Dong et al. 2018). Conley and Uye (2015) observed that planulae of *Aurelia aurita* from the Inland Sea of Japan extended their planktonic stage when under severe osmotic stress. The authors conclude that this might be an advantage, being more optimal for prolonging the swimming stage rather than metamorphosing into a scyphistoma that is incapable to develop feeding tentacles. Our results suggest that there was no osmotic stress in the lowest salinity tested, as the settlement time was the shortest and planula larvae were active swimming as they settled in all three locations. Contrarily, its hypersaline limits of 38 seemed to induce osmotic stress in prolonging the settlement time, while not actually inhibiting settlement.

The selection of suitable settlement sites is a crucial process for the success of survival and reproduction of planula larvae. Several authors have demonstrated that larvae prefer to settle upside down on solid surfaces (reviewed in Lucas et al. 2012) and even attached to man-made structures (e.g. *Aurelia* sp. from the northern Adriatic, Malej et al. 2012). *Rhizostoma octopus* (Holst and Jarms 2007) planulae settled predominantly on substrate plates (74.6%) (underside 99.8%, topside 0.1%, sides 0.1%), 24.1% on the air-water interface, and nil on the bottom and the wall of experimental bowls. Similarly, we observed that planula larvae of *R. luteum,* which settled on the glass side, did so predominantly on the underside. While the planulae showed a clear spatial preference for the bottom of the flasks (ESM Table 2), we observed the tendency to settle in confined spaces (e.g. near the edge of the flask or right under the glass slide). In the wild they might, therefore, favour gap spaces. Some planulae settled on the air-water interface during the first days of the study, but no further development was observed.

Benthic phase: scyphistomae development

Our results show that scyphistomae of *R. luteum* were highly tolerant to a wide range of environmental conditions (Fig. 3 and ESM Table 1). Nonetheless, salinity appears to have the greatest effect on the survival and development of the benthic stage. A greater number of scyphistomae developed and survived until the end of the experiment in all treatments at hyposaline conditions (24), and in the saltiest treatments (38) scyphistomae showed physiological stress and exhibited distorted morphologies. This was also noted in the warmest temperature groups (28 °C, see Fig. 5d and e). There was a 100% mortality rate within 3 weeks of exposure in the conditions of 28 °C with salinity of 38. Deterioration of tentacles, which reduced feeding capability, was reported by Schiariti et al. (2014) for *Cassiopea* sp., *Cephea cephea*, and *Aurelia* sp. at the lowest temperature (15 °C). Similarly, *Aurelia aurita* scyphistomae in hyposaline treatments (≤20) also exhibited distorted morphologies (Conley and Uye 2015). As no deterioration of tentacles was observed in *R. luteum* in the lowest temperature group (Fig. 5a), we conclude that 14 °C was not the minimum threshold for its development. In other experiments (Kienberger et al. 2018) scyphistomae were exposed to a drastic drop in temperature (48 h at 4 °C), thus showing a high tolerance to colder conditions. Comparable results were reported for *Rhizostoma pulmo* from the NW Mediterranean Sea, whereby 100% of the scyphistomae survived 70 days at the lowest temperature (14 °C), 30% at 21 °C and revealing also the highest mortality at 28 °C (at constant salinities 38-39; Purcell et al. 2012). On the contrary, *Cotylorhiza tuberculata* from the Mar Menor Lagoon (southeastern Spain, Prieto et al. 2010) were very sensitive to temperatures below 16 °C, although salinity had little influence. Some studies have shown the tendency that scyphistoma size is negatively correlated with temperature (Willcox and al. 2007; Han and Uye 2010; Chiaverano and Graham 2017). However, *R. luteum* scyphistoma exhibited a larger size in treatments at 23 °C rather than at 18 °C (see Fig. 4). Larger scyphistomae have been correlated to higher asexual propagation capacity (Willcox et al. 2007), which would be in agreement with the results of our study (see below).

Benthic phase: Asexual reproduction

Various studies reported that temperature was an important factor in producing podocysts, although the optimal temperature was species dependent (Schiariti et al. 2014; Widmer et al. 2016; Lee et al. 2017). Preliminary results showed that encystment of *R. luteum* occurred earlier with a drastic temperature drop. Notwithstanding, after 16 weeks the difference between encystment with and without a drop in temperature was not significant (Kienberger et al. 2018). In the present study, fully-grown scyphistomae of *R. luteum* (feeding *ad libitum*) produced the formation of podocysts by means of stolon in a significantly greater amount at 23 °C and in hyposaline (24) conditions (Fig. 6 and ESM Table 1). We believe that zero podocysts that were developed in some treatments at 14 °C, was mostly due to the fact that most scyphistomae were not yet fully developed (16 tentacles) by the end of the experiment (6 weeks).

During the entire experiment, no excystment was observed, which was likely due to a lack of time or the need for environmental stimulation to produce new scyphistoma (e.g. drastic drop in temperature). Our results are consistent with various studies in the genus *Rhizostoma*; *R. pulmo* and *R. octopus* which both produced podocysts without changes in temperature (*R. pulmo*: 21 °C and salinity of 37.5, Fuentes et al. 2011*; R. octopus*: 20 °C and salinity of 35 and fewer podocysts at a lower temperature, Holst et al. 2007), however, no information concerning excystment was given. Other studies have also reported that they observed the production of podocysts in a wide range of temperatures, however, warmer constant temperature and food availability proved to be beneficial for greater encystment (*R. pulmo, Lychnorhiza lucerna* and *Rhopilema esculentum* (Schiariti et al. 2014); *Nemopilema nomurai* (Feng et al. 2015). Kawahara et al. (2013) suggest that podocysts of *N. nomurai* were responsible for bloom versus non-bloom years and, moreover, that excystment increased significantly under exposure to an abnormally high temperature (≥27 °C) and hyposaline (8-24) conditions. Our results suggest that *R. luteum* would need only one set of environmental stimulation in order to produce new scyphistoma (only for excystment). We speculate that *R. luteum* scyphistomae density might increase their population size via podocyst encystment during summer to autumn (higher temperature), and excystment might occur in spring (higher food availability).

Transition from benthic to pelagic phase: Strobilation

During the entire duration of the experiment, the only type of strobilation observed was monodisc (one ephyra per strobila), which is consistent with results of earlier research. In this preliminary study, the strobilation time was reported to be 5-6 days at 17.5 °C with salinity of 37 (Kienberger et al. 2018). *Rhizostoma luteum* scyphistomae started strobilation spontaneously at 23 and 18 °C in weeks 3 and 4, respectively (Fig. 7). In the lowest temperature group, strobilation was not observed. This might be due to the short experimental time. In the warmest temperature group (28 °C), strobilation was inhibited as many scyphistomae presented distorted morphologies, short stalk and they also developed an odd number of tentacles, as shown in Figs. 5d and e. At 23 °C, ephyrae were released one week earlier and there was a significantly greater amount of ephyrae at a salinity of 24. *Rhizostoma pulmo* also spontaneously strobilated under constant temperature, however, this was only in the spring over a 3-year period (21 °C temperature, 37-38 salinity and 12:12 light cycle (Fuentes et al. 2011). In another laboratory experiment conducted by Purcell et al. (2012), *R. pulmo* strobilated at all tested temperatures (constant: 14, 21 and 28 °C, and salinities 38-39), and it was observed that the colder temperature increased the time until the first ephyra was released (mean no. of days: 85.5 d (14 °C); 15 d (21 °C); 9.5 d (28 °C). For *Cotylorhiza tuberculata* from the Mar Menor Lagoon (southeastern Spain), strobilation rate increased with temperature (optimal 23 °C, max 30 °C), and was inhibited below 19 °C (Prieto et al. 2010). To initiate strobilation in laboratory experiments, *Cotylorhiza tuberculata* required a trigger via a temperature increase, with an optimal change from 17.5 to 23 °C at a seasonal scale. In *R. octopus*, strobilation was also triggered by changes in temperature (15 to 10 °C, 5 to 10 °C, 10 to 15 °C, Holst et al. 2007) and in *Nemopilema nomurai* the increase was triggered by the range of 5 to 10-13 °C (Feng et al. 2015).

Some authors have reported that relatively larger scyphistoma produced larger ephyra (*Aurelia aurita* from Japan; Ishii and Waltanabe 2003) and even released ephyra exhibiting different shapes (*Aurelia* from the Gulf of Mexico, Chiaverano and Graham 2017), suggesting that asexual reproduction is limited by body size. However, newly released ephyrae of *R. luteum* (-24 hours) showed no significant size differences between treatments, and also their morphology was in the same size range as previously reported in Kienberger et al. (2018). Ninety-seven percent (97%) of ephyrae were liberated from the bottom of the glass flask, and this may be because of the higher level of nutrition that is available to the scyphistoma. As it has been observed in laboratory conditions that more ephyrae were produced per scyphistoma when under well-fed conditions (e.g. *Stomolophus meleagris* (Calder 1982), *Rhopilema nomadica* (Lotan et al. 1992), *Lychnorhiza lucerna* (Schiariti et al. 2008) and *Nemopilema nomurai* (Feng et al. 2015)). Due to the low mortality rate of the scyphistomae of *R. luteum* after strobilation (lower than 1% in the present study; nil in the study of Kienberger et al. 2018), it might be possible to have various peaks of ephyrae liberation under field conditions. Being able to strobilated repeatedly represents a crucial supplement for monodisc species, as the low output per strobila cycle is countered by multiple strobilation events over a period of time so that the total output becomes higher (reviewed in Lucas et al. 2012).

Pelagic phase: Growth of early medusa stages

The growth of the early free-swimming medusa stages was strongly controlled by thermal and salinity conditions. Overall, warmer temperature and hyposaline conditions enable faster development, with the potential to accelerate sexual maturity and spawning, notwithstanding the fact of the temperature being somewhat limiting at ~28 °C and salinity ≥38, with a 100% mortality rate at 28 °C with salinity of 38 after 21 days of post-liberation (Fig. 8). Our results show that 14 °C was not the minimum threshold for the young medusa, and that ephyra had a better ability to deal with hypersaline conditions at a lower temperature. This might explain how medusae, that were reported from the western coast of Africa, were in water temperatures during the summer that reach 28 °C, but with salinities < 38 (NOAA, 2019). Ephyrae reared in hypersaline (38) concentrations decreased diameter (TBD) in the first days of post-liberation (Fig. 8). However, it seems to be an adjusting time, since most ephyrae started a positive growth rate after a few days. Consequently, young medusae cope better with hyposaline conditions than the benthic stages, and this might explain the great dispersion pattern that is observed in the free-swimming jellyfish population (Prieto et al. 2013; Kienberger and Prieto 2018).

Results from similar experiments on other species showed that the TBD of *Cotylorhiza tuberculata* ephyrae from the Mar Menor Lagoon did not differ between temperatures, but they reached as well the metaephyra stage faster in warmer conditions, as after 21 days ephyrae reared at 20°C none had developed into medusae versus all at 30°C (Astorga et al. 2012). Growth rates of *Aurelia labiata* ephyrae from the NE Pacific were greater at 21 °C (range tested: 8 to 28 °C (Widmer 2005)), ephyrae maintained at 8 °C gradually decreased in size and those specimens that were reared at >22.5 °C everted their bells and were in poor condition.

The following western Mediterranean species have been reported to have a seasonal occurrence: *Cotylorhiza tuberculata* (Prieto et al. 2010; Purcell et al. 2012), *R. pulmo* (Fuentes et al. 2011; Purcell et al. 2012) and *Aurelia* sp. (Purcell et al. 2012). Consequently, the adult medusa will die over the colder winter months. On the contrary, adult *R. luteum* sightings have been reported year-round (Kienberger and Prieto 2018). However, with the lack of historical data, to our knowledge, nothing is known of whether *R. luteum* has been always present throughout the year, or whether this is due to rising ocean temperatures or other unknown factors. Interestingly, its congener *R. octopus* from colder regions was also reported to occur year-round from the Celtic Sea, the Irish Sea (Doyle et al. 2007), and from the coastal waters of Wales and western Scotland (Pikesley et al. 2014).

In the wild, young medusae seam to show seasonality, as smaller individuals have been reported from December to June (Prieto et al. 2013, Kienberger and Prieto, 2018, Kienberger et al. 2018). It can be assumed that the medusa population consists of multiple cohorts, as juvenile medusae overlap with adult medusa. Therefore, strobilation might occur between autumn and spring, producing various cohorts. Interestingly, medusae smaller than ~10 cm (min. TBD 9.8 cm collected on February 2016 from the NE Alboran Sea, described in Kienberger et al. 2018) were never found in the wild, suggesting that there might be a spatial segregation between the early life stages (estuary) and adult jellyfish. Female medusae carrying planulae have been collected in the wild during the months of March to May and in October from the NE Alboran Sea, which would agree with the preference of planulae to settle in warmer condition.

Is *Rhizostoma luteum* a brackish water jellyfish?

Water salinity is an important environmental factor that limits the distribution of marine species in brackish waters. Hence, early life stages could be limited by the inability of planulae to survive and settle, or the incapability of scyphistomae to develop and strobilate at low salinities, depending on their lowest tolerance for each species and even each population (Arai 1997; reviewed in Holst and Jarms 2010). Our data indicate not only that every stage of the early life cycle of *R. luteum* had a high tolerance to hyposaline (24) conditions, but also showed a significantly greater amount of scyphistoma, podocyst and ephyra production during the hyposaline experiments. These results suggest their possible occurrence in estuaries and brackish waters, and would be in line with occasional observations of adult *R. luteum* (> 35 cm of diameter) inside the Tagus estuary (Kienberger and Prieto, 2018; Kienberger unpubl data). Both of its congeners have been reported to also occur in estuarine environments: *R. pulmo* (northern Adriatic, Kogovšek et al. 2010; Indus Delta, Pakistan, Waryani et al. 2015) and *R. octopus* from the mouths of esturies on the coasts of Ireland and Wales (Houghton et al. 2006; Doyle et al. 2007). Hence, we hypothesize that the benthic stage of *R. luteum* might occur in estuaries of the SW Iberian Peninsula (e.g. Tagus estuary, Guadiana estuary, etc.). The Tagus estuary is a partly mixed mesotidal estuary at 23 and 30 km from the mount distance it had a mean salinity of 30.1 ± 5.3 and 25.6 ± 5.0 with an average temperature of 18.7 ± 3.2 °C over the period 1974-2004 (Costa et al. 2007), therefore, having the optimal T-S condition that was tested in the present study.

Results of various studies concerning the reproduction of estuarine jellyfish, agree that salinity conditions occurring during the spawning seasons may affect the recruitment of scyphistomae and, thereby, the medusa population size in the following years (Xian et al. 2005; Lynam et al. 2011; Amorim et al. 2018). Years with reduced freshwater discharge (dry years) are linked to greater salinity intrusions into the estuary. This suggests that higher salinity conditions during winter/spring were followed by important jellyfish outbreaks in the following summer, while severe river discharge (extreme hyposaline condition) minimized the formation of blooms. As planulae are osmoconformers, hyposaline conditions are a natural stressor of brackish water medusae. The lowest salinities permitting normal recruitment was between 10 and 20 in *Rhopilema esculentum*, *Nemopilema nomurai* and *Chrysaora pacifica* from east Asian Sea (Takao and Uye 2018). Assuming that the early life stages of *R. luteum* occur in brackish water, the reduction in rainfall (Gallego et al. 2011, Guerreiro et al. 2017) on the Iberian Peninsula and the construction of dams in recent decades, might explain the increase in population that has been observed in recent decades (Kienberger and Prieto 2018). The lack of information concerning the benthic stage of wild populations remains an obstacle to explaining the spatio-temporal patterns of the occurrence of the benthic stages.

Climate change and *Rhizostoma luteum*

Rising ocean temperatures, due to climate change, have been well documented at the global and local scale (IPCC 2014). According to a study by Shaltout and Omstedt (2014), that analyse sea surface temperature for the period of 1982-2012, there was a significant annual warming of 0.24 °C per decade for the Gulf of Cadiz (NE Atlantic Ocean) and 0.35 °C per decade for the entire Mediterranean basin. Those authors predict for the 2000-2100 period an ongoing warming of 0.3-1.6 °C for the Gulf of Cadiz and the Alboran Sea (SW Mediterranean Sea).

Studies on other jellyfish have revealed that the variable of temperature has proved to be an important driver for most temperate species. Warming oceans may increase the population size and distribution, and change the timing and length of their seasons (Purcell 2005; Lynam et al. 2011; Astorga et al. 2012; reviewed in Purcell et al. 2012). On the other hand, changes in climate pattern have been observed, which has produced less precipitation over the Iberian Peninsula in recent decades (Gallego et al. 2011). Having a direct effect on the brackish water species, which our results indicate as being the potential areas of scyphistoma population colonies. Consequently, the ongoing warming of the oceans and the decrease in rainfall might have influenced the proliferation of *R. luteum* in recent decades in the southern Iberian Peninsula, resulting in a longer and more prolific reproductive season, due to climate change.

CONCLUSION

In this study, we analyzed the effects of thermic and salinity conditions on the early life stages of the temperate scyphistoma *Rhizostoma luteum*. Our results show that *R. luteum* appears to be able to thrive over wide ranges of temperature and salinity. Occurring in regions with strong upwelling episodes might have given *R. luteum* the ability to adapt to frequent ambient fluctuations. This may predispose *R. luteum* to cope better with ongoing environmental changes, whereas, this proves to be a disadvantage to other taxa. Changes in ocean climate are likely to affect some species more than others, particularly those at the boundaries of their geographic ranges, where they may be most susceptible to changes in seasonal temperature, food supply, competitors, or predators.

The results of our study suggest that warming ocean conditions might increase the asexual reproduction rate of *R. luteum*, through an increased podocyst and ephyra production. Moreover, its distributions may broaden or shift its geographic range as a result of temperature and salinity changes. Notwithstanding, that elevated T-S condition in the NW Mediterranean Sea might be a natural barrier for *R. luteum* during its early life stages. The present study confirmed its predisposition to form blooms, as no external stimulation was needed to produce podocysts and ephyrae. Being a large medusa (approximately 70 cm in diameter), a mass development might have negative consequences for the tourist, fishery and aquaculture industries. But, concomitantly, it may become a new target species for the nutraceutical and biomedical industries. Therefore, its biomass should be continually monitored if blooms arise due to ongoing environmental changes, and further research needs to be undertaken to find the early life stages of *R. luteum* in the wild, in order to be able to monitor these crucial stages.

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REFERENCES

Adler L, Jarms G (2009) New insights into reproductive traits of scyphozoans: special methods of propagation in *Sanderia malayensis* GOETTE, 1886 (Pelagiidae, Semaeostomeae) enable establishing a new classification of asexual reproduction in the class Scyphozoa. Mar Biol 156(7):1411-1420. doi: 10.1007/s00227-009-1181-6

Amorim K, Mattmüller RM, Algueró-Muñiz M, Meunier CL, Alvarez-Fernandez S, Boersma M, Morais P, Teodósio MA (2018) Winter river discharge may affect summer estuarine jellyfish blooms. Mar Ecol Prog Ser 591:1-41. doi: 10.3354/meps12356

Arai MN (1997) A Functional biology of Scyphozoa. Chapman and Hall, London

Arai MN (2005) Predation on pelagic coelenterates : a reviewed iew. J Mar Biol Assoc UK 85:523-536

Astorga D, Ruiz J, Prieto L (2012) Ecological aspects of early life stages of *Cotylorhiza tuberculata* (Scyphozoa: Rhizostomae) affecting its pelagic population success. Hydrobiologia 690(1):141-155. doi: 10.1007/s10750-012-1036-x

Calder DR (1982) Life history of the cannonball jellyfish , *Stomolophus meleagris* L. Agassiz, 1860 (Scyphozoa, Rhizostomida). Biol Bull 162(2):149-162

Chiaverano LM, Graham WM (2017) Morphological plasticity in *Aurelia* polyps, with subsequent effects on asexual fecundity and morphology of young medusae. Mar Ecol Prog Ser 582:79-92. doi: 10.3354/meps12314

Conley K, Uye S (2015) Effects of hyposalinity on survival and settlement of moon jellyfish (*Aurelia aurita*) planulae. J Exp Mar Bio Ecol 462:14-19. doi: 10.1016/j.jembe.2014.10.018

Costa MJ, Vasconcelos R, Costa JL, Cabral HN (2007) River flow influence on the fish community of the Tagus estuary (Portugal). Hydrobiologia 587(1):113-123. doi: 10.1007/s10750-007-0690-x

Dong Z, Wang L, Liu Q, Sun T (2018) Effects of salinity and temperature on the recruitment of *Aurelia coerulea* planulae. Mar Biol Res 14(5):454-461. doi: 10.1080/17451000.2018.1459725

Doyle TK, Houghton JDR, Buckley SM, Hays GC, Davenport J (2007) The broad-scale distribution of five jellyfish species across a temperate coastal environment. Hydrobiologia 579(1):29-39. doi: 10.1007/s10750-006-0362-2

Fautin DG (2002) Reproduction of Cnidaria. Can J Zool 1754:1735-1754. doi: 10.1139/Z02-133

Feng S, Zhang F, Sun S, Wang S, Li C (2015) Effects of duration at low temperature on asexual reproduction in polyps of the scyphozoan *Nemopilema nomurai* (Scyphozoa: Rhizostomeae). Hydrobiologia 754(1):97-111. doi: 10.1007/s10750-015-2173-9

Feng S, Wang S, Sun S, Zhang F, Zhang G, Liu M, Uye S (2018) Strobilation of three scyphozoans (*Aurelia coelurea, Nemopilema nomurai,* and *Rhopilema esculentum*) in the field at Jiaozhou Bay, China. Mar Ecol Prog Ser 591:141-153. doi: 10.3354/meps12276

Fuentes V, Straehler-Pohl I, Atienza D, Franco I, Tilves U, Gentile M, Acevedo M, Olariaga A, Gili J (2011) Life cycle of the jellyfish *Rhizostoma pulmo* (Scyphozoa: Rhizostomeae) and its distribution, seasonality and inter-annual variability along the Catalan coast and the Mar Menor (Spain, NW Mediterranean). Mar Biol 158(10):2247–2266. doi: 10.1007/s00227-011-1730-7

Gallego MC, Trigo RM, Vaquero JM, Brunet M, García JA, Sigró J, Valente MA (2011) Trends in frequency indices of daily precipitation over the Iberian Peninsula during the last century. J Geophys Res 116:D02109. doi: 10.1029/2010JD014255

Gambill M, McNaughton SL, Kreus M, Peck MA (2015) Temperature-dependent settlement of planula larvae of two scyphozoan jellyfish from the North Sea. Estuar Coast Shelf Sci 201:64-71. doi: 10.1016/j.ecss.2016.08.042

Guerreiro SB, Birkinshaw S, Kilsby C, Fowler HJ, Lewis E (2017) Dry getting drier - The future of transnational river basins in Iberia. J Hydrol Reg Stud 12:238-252. doi: 10.1016/j.ejrh.2017.05.009

Han C, Uye S (2010) Combined effects of food supply and temperature on asexual reproduction and somatic growth of polyps of the common jellyfish *Aurelia aurita* s.1. Plankt Benthos Res 5(3):98-105. doi: 10.3800/pbr.5.98

Holst S, Jarms G (2007) Substrate choice and settlement preferences of planula larvae of five Scyphozoa (Cnidaria) from German Bight, North Sea. Mar Biol 151:863-871. doi: 10.1007/s00227-006-0530-y

Holst S, Sötje I, Tiemann H, Jarms G (2007) Life cycle of the rhizostome jellyfish *Rhizostoma octopus* (L.) (Scyphozoa, Rhizostomeae), with studies on cnidocysts and statoliths. Mar Biol 151(5):1695-1710. doi: 10.1007/s00227-006-0594-8

Holst S, Jarms G (2010) Effects of low salinity on settlement and strobilation of scyphozoa (Cnidaria): Is the lion's mane *Cyanea capillata* (L.) able to reproduce in the brackish Baltic Sea? Hydrobiologia 645(1):53-68. doi: 10.1007/s10750-010-0214-y

Houghton JDR, Doyle T, Davenport J, Hays GC (2006) Developing a simple, rapid method for identifying and monitoring jellyfish aggregations form air. Mar Ecol Prog Ser 314:159-170

IPCC (2014) Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, RK Pachauri and LA Meyer (eds.)]. IPCC, Geneva, Switzerland. doi: 10.1017/CBO9781107415324

Ishii H, Watanabe T (2003) Experimental study of growth and asexual reproduction in *Aurelia aurita* polyps. Sess Org 20:69-73

Kawahara M, Ohtsu K, Uye S (2013) Bloom or non-bloom in the giant jellyfish *Nemopilema nomurai* (Scyphozoa: Rhizostomeae): roles of dormant podocysts. J Plankton Res 35(1):213-217. doi: 10.1093/plankt/fbs074

Kienberger K, Prieto L (2018) The jellyfish *Rhizostoma luteum* (Quoy and Gaimard, 1827): not such a rare species after all. Mar Biodiv 48:1455. doi: 10.1007/s12526-017-0637-z

Kienberger K, Riera-Buch M, Schönemann AM, Barsch V, Halbauer R, Prieto L (2018) First description of the life cycle of the jellyfish *Rhizostoma luteum* (Scyphozoa: Rhizostomeae). PLoS ONE 13(8). doi: 10.1371/journal.pone.0202093

Kogovšek T, Bogunović B, Malej A (2010) Recurrence of bloom-forming scyphomedusae: wavelet analysis of a 200-year time series. Hydrobiologia 645(1):81-96. doi: 10.1007/s10750-010-0217-8

Lee HE, Han CH, Kim B, Yoon WD (2017) Effects of temperature and salinity on the asexual reproduction of *Nemopilema nomurai* (Scyphozoa: Rhizostomeae). Ocean

Sci J 52(4):573–579. doi: 10.1007/s12601-017-0040-5

Lotan A, Ben-Hillel R, Loya Y (1992) Life cycle of *Rhipilema nomadica*: a new immigrant scyphomedusan in the Mediterranean. Mar Biol 112:237-242

Lucas CH (2001) Reproduction and life history strategies of the common jellyfish, *Aurelia aurita*, in relation to its ambient environment. Hydrobiologia 451:229-246

Lucas CH, Graham WM, Widmer C (2012) Jellyfish life histories: role of polyps in forming and maintaining scyphomedusa populations. Adv Mar Biol 63:133-196. doi: 10.1016/B978-0-12-394282-1.00003-X

Lynam CP, Lilley MKS, Bastian T, Doyle TK, Beggs SE, Hays GC (2011) Have jellyfish in the Irish Sea benefited from climate change and overfishing? Glob Change Biol 17(2):767-782. doi: 10.1111/j.1365-2486.2010.02352.x

Malej A, Kogovšek T, Ramšak A, Catenacci L (2012) Blooms and population dynamics of moon jellyfish in the Northern Adriatic. Cah Biol Mar 53(3):337-342

NOAA (2019) National Ocean and Atmorpheric Administration, CoastWatch Satelite Data Products. https://coastwatch.noaa.gov/cw/satellite-data-products.1.html. Accessed 16 February 2019

Pikesley SK, Godley BJ, Ranger S, Richardson PB, Witt MJ (2014) Cnidaria in UK coastal waters: description of spatio-temporal patterns and inter-annual variability. J Mar Biol Assoc UK:1-8. doi: 10.1017/S0025315414000137

Prieto L, Astorga D, Navarro G, Ruiz J (2010) Environmental control of phase transition and polyp survival of a massive-outbreaker jellyfish. PloS ONE 5(11): e13793. doi: 10.1371/journal.pone.0013793

Prieto L, Armani A, Macías D (2013) Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. Mar Biol 160(12):3241-3247. doi: 10.1007/s00227-013-2293-6

Prieto L, Enrique-Navarro A, Li Volsi R, Ortega MJ (2018) The large jellyfish *Rhizostoma luteum* as sustainable a resource for antioxidant properties, nutraceutical value and biomedical applications. Mar Drugs 16(10): 396. doi:10.3390/md16100396

Purcell JE, White JR, Nemazie DA, Wright DA (1999) Temperature, salinity and food effects on asexual reproduction and abundance of the scyphozoan *Chrysaora quinquecirrha*. Mar Ecol Prog Ser 180:187-196. doi: 10.3354/meps180187

Purcell JE (2005) Climate effects on formation of jellyfish and ctenophore blooms: a review. J Mar Biol Assoc UK 85(3):461-476. doi: 10.1017/S0025315405011409

Purcell JE, Atienza D, Fuentes V, Olariaga A, Tilves U, Colahan C, Gili J (2012) Temperature effects on asexual reproduction rates of scyphozoan species from the northwest Mediterranean Sea. Hydrobiologia 690: 169-180. doi: 10.1007/s10750-012-1047-7

Purcell JE, Juhl AR, Mańko MK, Aumack CF (2018) Overwintering of gelatinous zooplankton in the coastal Artic Ocean. Mar Ecol Prog Ser 591:281-286. doi: 10.3354/meps12289

Quoy JRC, Gaimard JP (1827) Observations zoologiques faites à bord de l'Astrolabe, en mai 1826, dans le détroit de Gibraltar. Ann Sci Nat 10:175-237

Schiariti A, Kawahara M, Uye S, Mianzan HW (2008) Life cycle of the jellyfish *Lychnorhiza lucerna* (Scyphozoa: Rhizostomeae). Mar Biol 156(1):1-12. doi: 10.1007/s00227-008-1050-8

Schiariti A, Morandini AC, Jarms G, von Glehn Paes R, Franke S, Mianzan H (2014) Asexual reproduction strategies and blooming potential in Scyphozoa. Mar Ecol

Prog Ser 510:241-253. doi: 10.3354/meps10798

Shaltout M, Omstedt A (2014) Recent sea surface temperature trends and future scenarios for the Mediterranean Sea. Oceanologia 56(3):411-443. doi: 10.5697/oc.56-3.411

Straehler-Pohl I (2017) Cubozoa and Scyphozoa: The results of 20 years of scyphozoan life cycle research with new results on cubozoan life cycles to suggest a new nomenclature referring to both classes. In: Frontiers in ecological studies of jellyfish (eds. Toyokawa M, Miyake H, Nishikawa J) Seibutsu Kenkyu Sha Co. Ltd. (Organisms Research Co.Ltd.), Tokyo:17-29

Takao M, Uye S (2018) Effects of low salinity on the physiological ecology of planulae and polyps of scyphozoans in the East Asian Marginal Seas: potential impacts of monsoon rainfall on medusa population size. Hydrobiologia 815:165. doi: 10.1007/s10750-018-3558-3

Waryani B, Siddiqui G, Ayub Z, Khan SH (2015) Occurrence and temporal variation in the size-frequency distribution of 2 bloom-forming jellyfishes, *Catostylus perezi* (L. Agassiz, 1862) and *Rhizostoma pulmo* (Cuvier, 1800), in the Indus Delta along the coast of Sindh, Pakistan. Turk J Zool 39(1):95-102. doi: 10.3906/zoo-1401-13

Webster CN, Lucas CH (2012) The effects of food and temperature on settlement of *Aurelia aurita* planula larvae and subsequent somatic growth. J Exp Mar Bio Ecol 436-437:50-55. doi: 10.1016/j.jembe.2012.08.014

Widmer CL (2005) Effects of temperature on growth of north-east Pacific moon jellyfish ephyrae, *Aurelia labiata* (Cnidaria: Scyphozoa). J Mar Biol Ass UK 85:569-573. doi: 10.1017/S002531540511495

Widmer CL, Fox CJ, Brierley AS (2016) Effects of temperature and salinity on four species of north-eastern Atlantic scyphistomae (Cnidaria:Scyphozoa). Mar Ecol Prog Ser 559:73-88. doi: 10.3354/meps11879

Willcox S, Moltschaniwskyj NA, Crawford C (2007) Asexual reproduction in scyphistomae of *Aurelia* sp.: Effects of temperature and salinity in an experimental study. J Exp Mar Bio Ecol 353:107-114. doi: 10.1007/s00227-008-0959-2

Xian W, Kang B, Liu R (2005) Jellyfish blooms in the Yangtze Estuary. Science 307(5706):41. doi: 10.1126/science.307.5706.41c

Chapter V

The first record of *Drymonema dalmatinum* from the northern Alboran Sea (western Mediterranean)

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The large scyphozoan jellyfish *Drymonema dalmatinum* was first described by Haeckel (1880) from material collected off the Dalmatian coast of the Adriatic Sea (eastern Mediterranean). In 1882, Haeckel renamed *D. dalmatinum* as *D. victoria*, describing the species in more detail and adding a single partial sample from material collected by the HMS Challenger expedition in 1873, near Gibraltar, considering this medusa as a deep-water species. Since then, there has been no report of *D. dalmatinum* in the western Mediterranean. Based on genetic and morphological data, Bayha and Dawson (2010) established a new semaeostome family, Drymonematidae, to accommodate three valid *Drymonema* species (Bayha and Dawson 2010): the rare *D. dalmatinum* Haeckel, 1880 from the Mediterranean, *D. larsoni* Bayha and Dawson 2010 from the Caribbean, and *D. gorgo* Müller 1883 from the Brazilian region. Recently, Malej et al. (2014) reported an increased frequency of sightings of *D. dalmatinum* in the last 15 years from the Adriatic and eastern Mediterranean Sea.

We report the sighting of single specimen observed on April 30, 2013, at the dive location known as Marina del Este, La Herradura, southern Spain (36.720278°N, 3.728333°W) by scuba divers (see Fig. 1). The jellyfish was found near the sea floor at a depth of 12 m, with a bell diameter of approximate 70 cm, the orientation of the umbrella was upwards, with its tentacles trailing below and accompanied by many small fishes. The sea temperature and salinity were 14.97°C and 35.082, respectively.

From the photographic material taken in situ, it can be determined that the specimen belongs to *D. dalmatinum* showing the following morphological characters: the bell is milkish-white, shield-shaped and flatly rounded with a thicker central part (Fig. 1a). The numerous tentacles are longer than the diameter of the bell and of unequal lengths and thickness and are not grouped in separated clusters (Fig. 1b). It is to be noted that the exumbrellar markings seen in some Mediterranean animals are not visible in this individual, but may be imperceptible because of the quality of the photographic material (Fig. 1c). Figure 1d shows the complexly folded veil-like oral arms, which are almost as long as the bell diameter. The genus *Drymonema* is distinguished from *Cyanea* and *Desmonema* by the fact that its tentacles arise from the middle zone of the subumbrella and not in separated clusters.

Haeckel's finding from the Strait of Gibraltar has been questioned by Mayer (1910) and being such a rare medusa, it seemed important to document this occurrence.

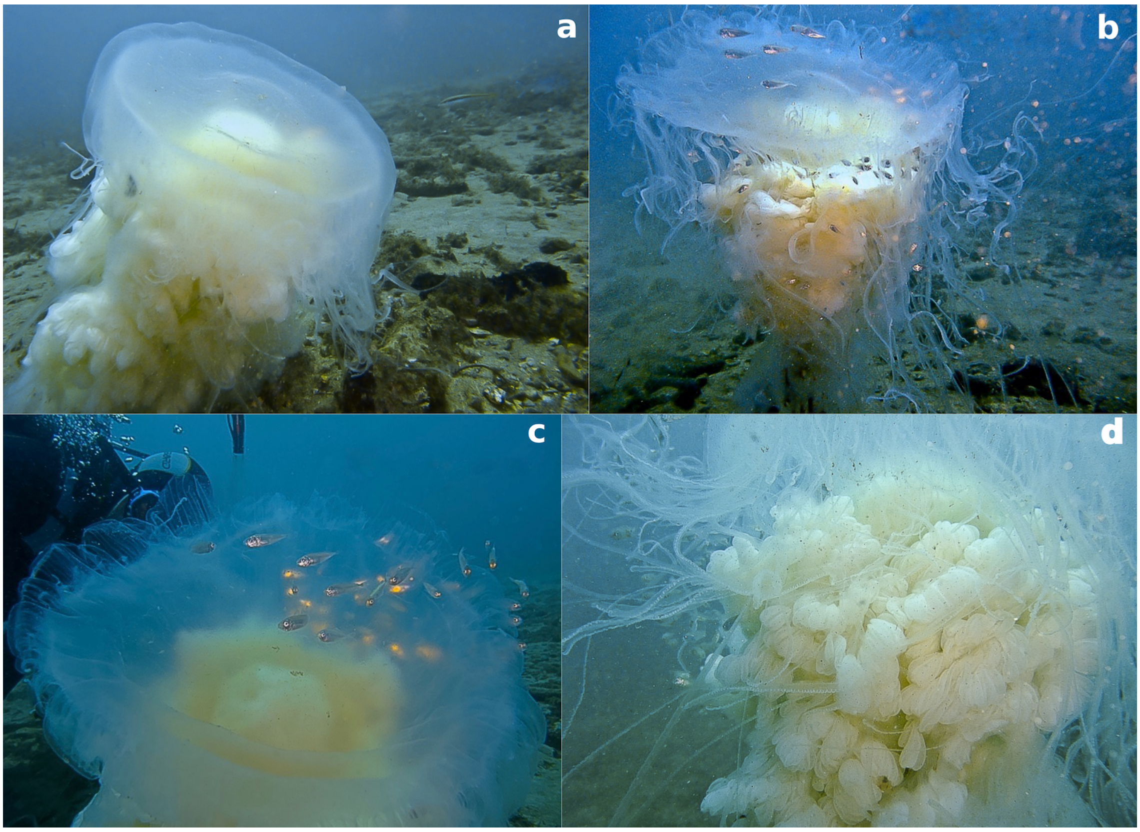

Figure 1 *Drymonema dalmatinum* from La Herradura, NE Alboran Sea, photographed on April 30, 2013. a, b Side view. c Aboral view. d Close-up of the oral arms (Photographic credits: S. Bellamy)

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REFERENCES

Bayha KM, Dawson MN (2010) New family of allomorphic jellyfishes, Drymonematidae (scyphozoa, Discomedusae), emphasizes evolution in the functional morphology and trophic ecology of gelatinous zooplankton. Biol Bull 219:249–267

Haeckel E (1880) System der Acraspeden. Zweite Hälfte des System der Medusen. Verlag von Gustav Fischer, Jena, pp 361–672

Malej A, Vodopivec M, Pestorić B, Lucic D, Onofri I (2014) The lesser-known medusa *Drymonema dalmatinum* Haeckel 1880 (Scyphozoa, Discomedusae) in the Adriatic sea. ANNALES Ser hist nat 9:79–86

Mayer AG (1910) Medusae of the world, vol III: the Scyphomedusae. Carnegie Institution of Washington, Washington, DC.

Chapter VI

Interannual variability and population dynamic of *Pelagia noctiluca* (Forsskål, 1775) (Scyphozoa: Semaeostomeae, Pelagiidae) in the northern Alboran Sea, southwest Mediterranean Sea

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In preparation

ABSTRACT

The mauve stinger *Pelagia noctiluca* is one of the most common jellyfish in the Mediterranean Sea and its proliferation causes significant negative socio-economic effects on the sector of fisheries and tourism. From the northern Alboran Sea (SW Mediterranean) little is known of this holoplanktonic scyphomedusae. Hence, the aim of this present research is to describe *P. noctiluca* population dynamic and to investigate its interannual and seasonal fluctuations. During 4½ year we recorded the presence/absence of gelatinous organisms from one location in southern Spain (La Herradura, NE Alboran Sea). 1748 medusae were collected between July 2012 and December 2016, the gender was determined and the bell diameter was measured. To estimate the growth parameters from length-frequency data the von Bertalanffy growth function (VBGF) was fitted using a non-parametric, robust approach known as ELEFAN (Electronic Lengths Frequency Analysis). Jellyfish were present in almost all the sampling months, and its abundance fluctuated significantly between years and seasons. Showing only for the winter months a clear lower abundance at an interannual level. Nonetheless, our results show that to have a summer with a great abundance of mauve stinger two requisites are necessary: 1) during spring (April-June) a high abundance of jellyfish need to be present; 2) during summer the wind stress needs to change between strong westerlies (activating the coastal upwelling mechanism) and episodes of calm and/or weak easterlies (pushing the jellies near coast). Consequently, that *Pelagia* is present during spring might not necessarily mean, greater abundances during the following summer. Young jellies were present (<3.5 cm) from May-October, showing an increase in growth (bell diameter) from April-September, as during this period individuals moved through length-frequency classes rapidly. Large medusae (> 15 cm) were collected from February to March and then disappeared during the summer months. The predicted extreme length of the stock was estimated to be 17.67 cm, which is one of the biggest sizes reported from the western Mediterranean Sea.

Keywords: Interannual variability, growth, length-frequency analysis, von Bertalanffy growth function, ELEFAN, population dynamics, jellyfish blooms

INTRODUCTION

In the Mediterranean Sea the presence of jellyfish blooms (sudden outbreaks of one species which comes to dominate the plankton for a period) is known for a long time, but it is not until the proliferation of the mauve stinger *Pelagia noctiluca* (Forsskål 1775) in the early '80s (CIESM 2001; Mariottini et al. 2008) that caused significant socio-economic effects and produced major concerns for the sectors of fisheries (reviewed in Purcell et al 2007) and tourism (Nastav et al. 2013; Donno et al. 2014), that scientist have begun to study the possible cause of these blooms. *Pelagia noctiluca* is widely spread in the Mediterranean Sea and occurs in both the western and the eastern basin. This scyphistoma is considered one of the most common species in the Mediterranean Sea (CIESM 2001; Boero 2013; Canepa et al. 2014) and large blooms have been reported in the last decades (Gili and Pagès 2005; Daly Yahia et al. 2010; Kogovšek et al. 2010, Licandro et al. 2010; Bernard et al. 2011). During summer 2006 tens of thousands of bather were stung by jellyfish on Spain's Mediterranean beaches and some beached were even forced to close temporarily (Pingree and Abend 2006).

A study by Goy and coauthors (1989) reported that over the past 200 years (1785-1985) blooms of *P. noctiluca* have occurred about every 12 years in the western Mediterranean basin and were related to an excess of anticyclonic metrological situations (high pressure) in particular during late spring- early summer. However, in the last decades there seem to have occurred a shift indicating that blooms appear more frequent in the western basin. (Bernard et al. 2011) results indicate a long lasting abundance of about 15 years between 1994 and 2008 in the Ligurian Sea. Since the 1998 a higher frequency of blooms was as well reported from the Balearic Sea and the Gulf of Tunis (Daly Yahia et al. 2010).

The mauve stinger *P. noctiluca* is an oceanic species and widely distributed in all warm and temperate waters (Russel 1970). This Semaeostomeae is a holoplanktonic species and has direct development, lacks the scyphistoma (benthic) phase, and therefore, it is not restricted to coastal waters. The appearance of a swarm of jellyfish at some moment and in a specific location near the coast depend on the levels of abundance of the metapopulation at basin-scale, but also on the local physical conditions (wind direction, shelf topography and food conditions) forcing individuals to achieve a particular beach right at that moment (Daly Yahia et al. 2003; Sabatés et al. 2018; Canepa et al. 2014; Aouititen et al. 2019).

The Alboran Sea (see Fig. 1) is the westernmost basin of the Mediterranean Sea and is an area of transition between the Atlantic Ocean and the Mediterranean Sea. It has been called the Alboran Channel, which is an apt name for this elongated, semienclosed body of water, lying between the Iberian Peninsula and the north of Africa. It is bordered on the north by Spain, on the south by Morocco and western Algeria, on the west by the Strait of Gibraltar (15 km), and on the east by the line joining Cabo de Gata (Spain) and Cabo Figalo (Algeria, as defined by the International Hydrographic Organization, 1953). The semipermanent Azores anticyclone is strongly influencing the seasonal weather pattern in the Alboran Sea. During winter (November-February), migrating cyclones and anticyclones cause the weather to be unsettled, wet, and windy. During summer (June-September) the Azores anticyclone extends northeastwards, causing warm and dry weather with light winds. Spring extends from March through May and is characterized by an alternation of stormy winter and calm summer weather. Autumn is usually very short and last during the month of October (Parrilla and Kinder 1987). Dominant winds, mainly westerlies or easterlies (called "*Poniente*" or "*Levante*", respectively, in the local terminology). Westerlies are common during winter and spring (November-March), bringing humid and colder weather. Easterlies can occur in every season. These winds blow from the east and the southeast and are associated with high-pressure north of the peninsula. The northern Alboran Sea is a highly dynamic system, which presents great variations in hydrological and biogeochemical variables at different spatial and temporal scales, presenting of quasi-permanent structures such as fronts, anticyclonic and cyclonic eddies etc. It is considered an upwelling region (Sarhan et al. 2000 and reference therein). Upwelling can be forced by several mechanisms: 1) wind induced (westerlies), as the water transported offshore sinks, 2) unsteadiness of the Atlantic Jet-front position. The later is the main forcing agent that modulates the hydrological processes in this area and across the Alboran Sea basin, which enters through the Strait of Gibraltar (Macías et al. 2008; Navarro et al. 2011; Ruiz et al. 2013). Though, this inflow of Atlantic Water is nutrient-poor. Therefore, the importance of coastal upwelling in the Spanish shore as the main source of chlorophyll to the region throughout the year (Macías et al. 2007). Higher nutrient concentrations and phytoplankton biomass are observed during spring, which are associated with wind-driven upwelling events. During summer the water column is characterized by a marked thermal stratification which extended from the surface down to 20-30 m (Ramírez et al. 2005). The strong stratification of the water column prevents the injection of nutrient into the layer above the pycnocline, leading to the drop of nutrient concentrations, as the chlorophyll profiles during the summer present the typical feature of oligotrophic systems.

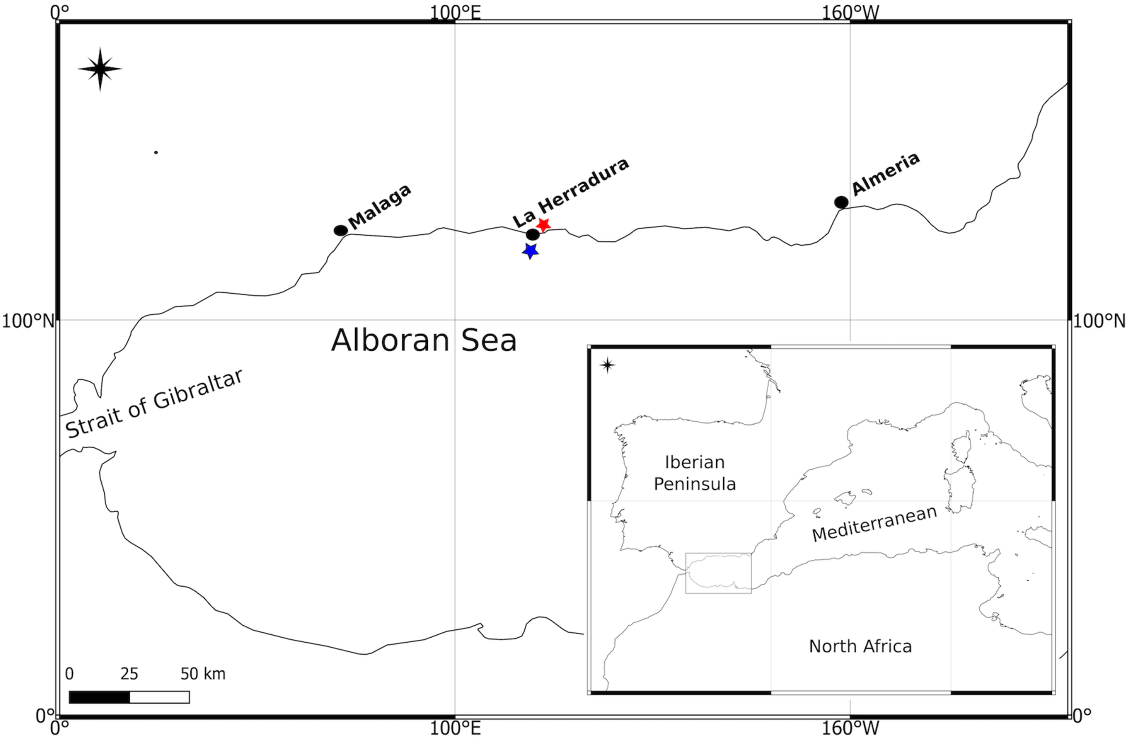

Figure 1 Location of La Herradura in the northeast Alboran Sea. Blue star represent SIMAR network point 2039080 (Spanish National Ports Authority). Red star represent meteorological stations Almuñécar (*Junta de Andalucía*)

Various studies have concluded that warmer water temperature, lack of rain during spring-early summer, and with a subsequent water column stratification increase the abundance of *P. noctiluca* (Goy et al. 1989; Molinero et al. 2005, 2008; Daly Yahia et al. 2010; Canepa et al. 2014). Thought, lower abundance of jellyfish during the warmer month has been reported from several locations (e.g. Catalan Coast (Canepa et al. 2014); Strait of Messina, central Mediterranean (Rosa et al. 2013); northern Tunisia (Aissi et al. 2014)). It has been suggested that the warmer temperature causes metabolic stress (Rosa et al. 2013) and the reduction of zooplankton prey make surface waters unfavourable for *P. noctiluca*, which during the warmer months might migrate to colder mid-water depths (Canepa et al. 2014). Nevertheless, as *P. noctiluca* is an offshore species, it is clear that its abundance near the coast depends on the level of abundance of the metapopulation at basin-scale and that there is a fluctuation at a local and regional level depending on the external stress factors.

It is important to understand the population dynamic of jellyfish to better understand its bloom pattern, though, only a few authors who have tackled this question. They have often used a confusing mix of methods, which cannot be used for comparisons within species and even less for comparisons between species. In this present study we have applied the method proposed by Palomares and Pauly (2009) using the von Bertalanffy Growth Function (VBGF). The analysis of length-frequency data is a reliable way of obtaining growth parameters of fish and has been used for a long time in fisheries management. The authors have found that the growth rates of jellyfish are largely comparable to those of small fishes such as guppies and anchovies.

Our knowledge of the mechanisms triggering the proliferation of *P. noctiluca* is still incomplete (reviewed in Canepa et al. 2014) and limits our ability to predict their population oscillations. Understanding how jellyfish populations behave (decrease, increase or stay the same) is imperative, as many of these gelatinous species influence ecosystem processes, human activities and diverse economic sectors. Therefore, actual data of coastal jellyfish blooms requires long-term studies; consequently investigating the population dynamics of these organisms throughout all seasons of the year. The aim of the present study is to describe for the first time the interannual and seasonal fluctuations of *P. noctiluca* from the SW Mediterranean Sea and to describe its population dynamics through the estimation of the parameters of the von Bertalanffy growth function.

MATERIALS AND METHODS

In situ observation and sampling collection

Study area

The beach known as Marina del Este, La Herradura, NE Alboran Sea (36.720278˚ N, 3.728333˚ W, Fig. 1 Map) was selected amongst other locations of the southern coast of Spain due to its geographical position in front of an upwelling area, its exposure to easterly and westerly winds and its rather steep sea bottom slope.

Visual observation

To record the presence or absence of *Pelagia noctiluca* during 4½ years (between 1 July 2012 to 31 December 2016) the same transect was walked daily (weather permitting). We adapted the survey strategy described by (Doyle et al. 2007; Houghton et al. 2007), where it was used for the study of the distribution of five jellyfish species across the Irish and Celtic Sea shores. In this present study, the survey method consisted of walking along a designated beach transect to record the presence or absence of *P. noctiluca*, recording individuals stranded and the ones swimming near the shore. The starting point of the transect was at the western point of the beach; walking along the water edge (270 m length) this area was constituted of small gravel (approx. 165m) and sand. The transect's end was on top of a small cliff (3 m over sea level). The field of view covered 180° with a horizontal distance of approx. 8 m. As the tidal coefficient is not important in this zone, surveys were not timed to coincide with the low tide as done in the following studies (Doyle et al. 2007; Fleming et al. 2013).

Data Set

To compare our collected data at local level with abundance reported at regional level (Alboran Sea) and interbasin NW Mediterranean we analysed the dataset collected by the *Instituto de Ciencias Marinas de Andalucía* (ICMAN-CSIC) between 2012 and 2016 (Ministerio del Medio Ambiente, Consejería de Medio Ambiente de Andalucía, town hall, diving schools, national and regional media and part of a citizen science project.

Environmental parameters

Oceanographic parameters

Sea surface temperature (SST) and salinity (SSS) were measured daily during the summer months using a conductivity-temperature-depth instrument (CTD, NKE Instrumentation) taken from the top of the cliff at the end of the sampling transect at 1m depth.

Hourly wind and wave data were obtained from the nearest SIMAR network point 2039080 (36.67˚ N, 3.75˚ W) data provided by *Puertos del Estado* (entity of Spanish National Ports Authority), which was the nearest point to our study zone. The data consisted of hourly wind speed (m/s) and direction, and wave high (m) and direction. Wind and current roses were constructed using hourly values obtained from the SIMAR network.

Meteorological parameters

Daily rain accumulation and average air temperature were obtained from the meteorological stations (Junta de Andalucía), Almuñécar (36.75˚ N, 3.68˚ W) this being the closest one to our study zone (https://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController?action=Static&url=coordenadas.jsp&c_provincia=18&c_estacion=11).

Statistical analysis

All data were tested for normality (Shapiro-Wilks test) and homoscedasticity of variances was evaluated using the Barlett test and the Levene test; data with normal distribution were tested by two-way analysis of variance (ANOVA). Significant ANOVA results were tested using Turkey's pair-wise comparisons. Data that were not normally distributed, and even after applying various transformations, were tested by a non-parametric analogue (Kruskal-Wallis ANOVA on rank) and using Dunn's pair-wise comparisons in post hoc tests. A significant level of $\alpha = 0.05$ was chosen for all tests that were performed in this study. All statistical analyses of the data were performed using R (v3.4.1) software.

Laboratory analysis

Species collection

Pelagia noctiluca were collected during visual observations when there was a great number of jellyfish presents. We sampled specimens that were swimming near shore or were freshly stranded at the beach. Some medusae were captured directly inside the marina (Puerto Deportivo Marina del Este, La Herradura) using a dip net. Bell diameters were measured across the lappets of the collected jellyfish to the nearest millimetre (0.1 mm), oral side facing up, using a ruler. Body volume was identified by water displacement from the calibrated cylinder, measured to the nearest 5 ml.

Comparison of sexes

The gender was determined by visual analysis of the gonads. The male gonad has a dark pink to purple colour and is composed of a series of small cylindrical follies. The female gonad is browner in colour with eggs that can be easily distinguished individually. For medusae whose gender determination was uncertain visually, a small piece (~0.5 cm) of gonad was removed and confirmed under microscopic analysis.

Growth estimation and cohort identification

From July 2012 to December 2016 a total of 1748 jellyfish were sampled. A minimum of 30 medusae was measured each month. However, owing to the scarcity of medusae, sometimes fewer or even none where collected. Bell diameters were measured across the lappets of the collected jellyfish to the nearest millimetre (0.1 mm), oral side facing up, using a ruler to establish mean size and diameter-frequency histograms. The data were grouped into bell size classes by 0.5 cm intervals.

To describe the growth of *P. noctiluca* we applied the method proposed by (Palomares and Pauly 2009). Bell diameters were arranged sequentially in time and fitted with the von Bertalanffy growth function (VBGF) using a non-parametric, robust approach known as ELEFAN (Electronic Lengths Frequency Analysis) employing FAO-ICLARM Stock Assessment Tools (FiSAT II, Version1.2.2, Gayanilo et al. 2005, available at www.fao.org/fishery/topic/16072/en).

The VBGF is one of the standard models of fishery managers and has the form:

$$L_t = L_\infty(1 - e^{-K(t-t_0)})$$

where, L_t is the length at age t, L_∞ is the asymptotic length i.e. the mean length the animal would reach if they were to grow indefinitely (corresponding to the maximum length in the population in question), K is the growth coefficient, t_0 is the age when length equals zero (parameter setting the origin of the curve on the X-axis).

To account for seasonal oscillation of growth we used a variant of the VBGF described in Palomares and Pauly (2009). This equation includes two parameters more than the standard VBGF, *C* and *WP*. *C* is the amplitude of growth oscillations, C = 0.25, 0.5 and 0.75 for populations sampled from sub-tropical, temperate and boreal localities, respectively. Therefore, as *P. noctiluca* is a temperate species $C = 0.5$. WP is the "winter point" being the period of the year when growth is slowest, this is usually during the coldest month of the year, February in the northern hemisphere ($WP = 0.1$).

In FiSAT II the analysis was carried out in the following stages:

1. Analysis of the data using ELEFAN I to estimate K and L_∞.
2. The means and cohorts were estimated following Bhattacharya's method (Bhattacharya 1967).
3. To refine parameter estimation NORMSEP (Hasselblad, 1966) was applied employing the maximum likelihood concept.

Values of *K* and L_∞were then used to estimate growth performance index (Φ') (Pauly & Munro 1984) using the following equation:

$$\Phi' = 2\ log_{10}L_\infty + log_{10}\ K$$

RESULTS

Temporal distribution

During the 54 months (4½ years) of monitoring, the presence of *Pelagia noctiluca* was recorded in almost all the sampling months (Fig. 2). Only during three of them, we registered zero jellyfish (April 2013, September and November 2016). The jellyfish abundance peak was highly variable, with the tendency of lower abundance from November to February, though, a clear pattern was not visible. The highest abundance was recorded on 29 July 2012 (848 ind). This quantity recorded in one day is higher than the entire abundance recorded for the entire year 2013 or 2014, see Table 1. During 2015 *Pelagia* was present during more than 1/3 (137 d) of the year. The interannual variation among the jellyfish abundance was significantly different (Kruskal-Wallis test, H_3=105.1, P<0.001; Fig. 3), with more than a 10-fold difference between 2015 and 2013.

Table 1 Abundances of Pelagia noctiluca recorded between 2013 and 2016 from La Herradura, NW Alboran Sea.

Year	Total abundance (ind)	Mean±SD abundance (ind)	Max abundance (day)	Max abundance (month)	No. days present
2013	564	1.68±10.05	141 (Dec)	201 (Dec)	44
2014	709	2.33±7.67	65 (Apr)	113 (Apr)	81
2015	5594	18.34±59.95	638 (Oct)	1676 (Oct)	137
2016	2534	7.82±40.29	412 (May)	879 (Apr)	62

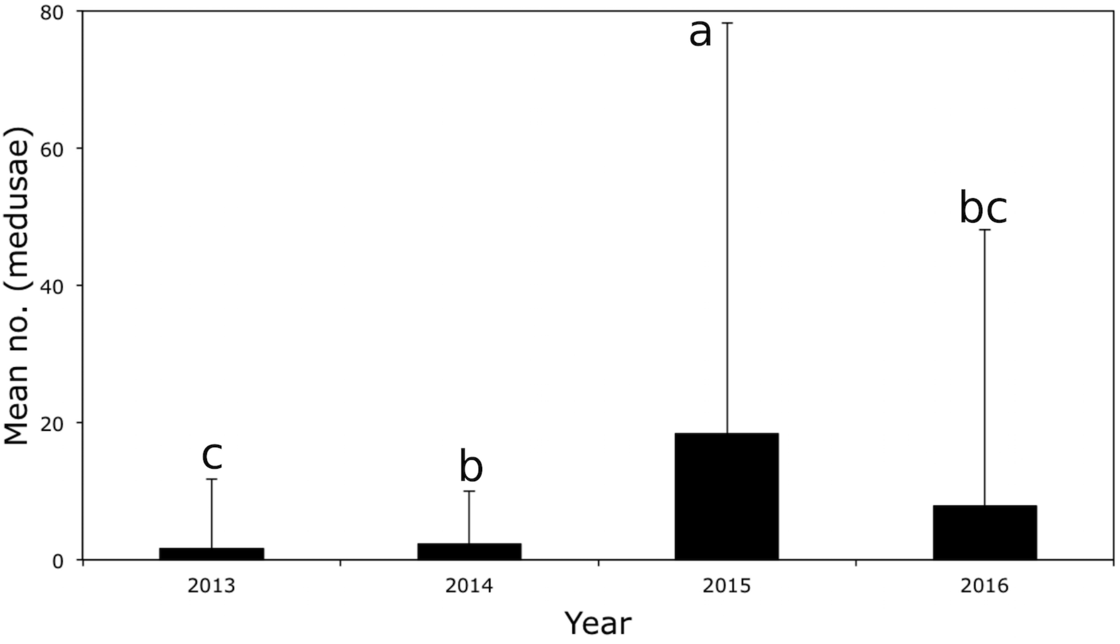

Figure 3 Interannual abundance of *Pelagia noctiluca* from La Herradura, NW Alboran Sea. Error bars are the upper standard deviation. Years marked by different letters differ significantly (*p*<0.05 by post hoc Dunn's test).

At the interseasonal level the abundance was as well highly variable, being significantly different in spring, summer and autumn (Kruskal-Wallis test, Spring: H_3=62.6; Summer: H_4=86.4; Autumn: H_4=38.1, all P<0.001), and not significant in winter (Kruskal-Wallis test, Winter: H_3=2.8, P=0.420; Fig. 4). The peak monthly abundances were in spring (2014 and 2016) and autumn (2013 and 2015).

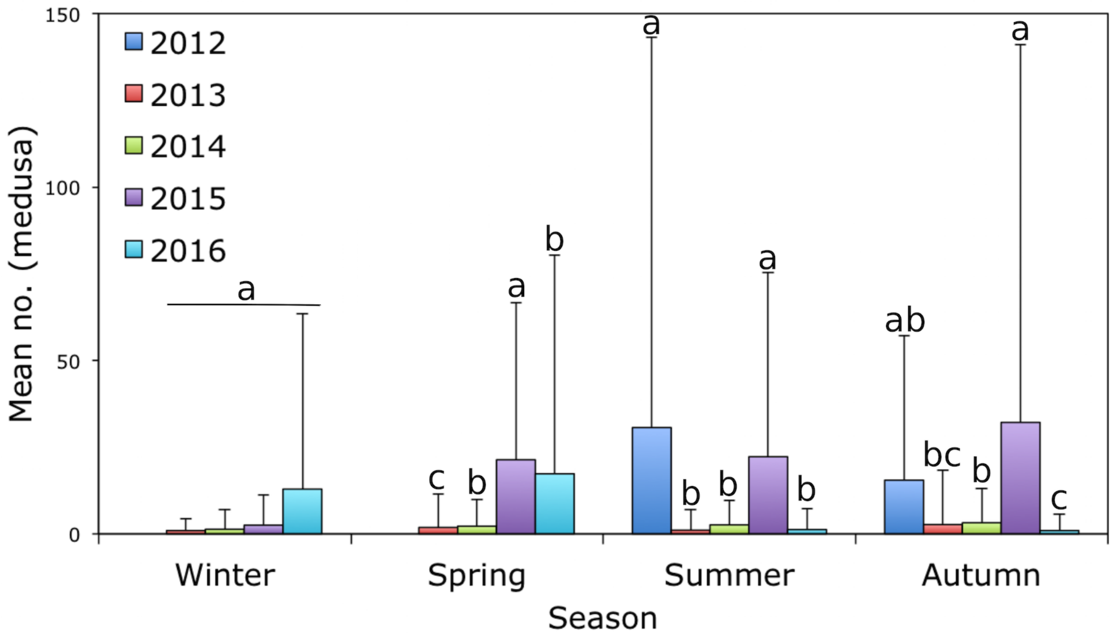

Figure 4 Interseasonal abundance of *Pelagia noctiluca* from La Herradura, NW Alboran Sea. No data available for winter and spring 2012. Error bars are the upper standard deviation. Seasons marked by different letters differ significantly (*p*<0.05 by post hoc Dunn's test)

Spring showed two clear tendencies: during 2013 and 2014 relative low abundances of medusae were recorded (1.86±9.75 ind and 2.21±9.75 ind) with a similar maximum abundance of 61 and 65 ind, respectively (Fig. 4). During 2015 and 2016 the abundance was 10-fold higher 21.38 ±47.63 and 17.34±63.03 ind, respectively. The peak abundances for spring were observed on the 6 May 2016 (412 ind) and on 29 June 2015 (302 ind). During spring 2015 we recorded 48 days with *Pelagia* (total abundance 1646 ind) and 30 d (1439 ind) during 2016. Even thought we do not have daily abundances for spring 2012, the data set at regional level shows reports of great abundances.

Summer 2012 and 2015 had significantly higher abundance than the other three summers (2013, 2014, and 2016, Fig. 4). Summer 2012 had a mean abundance of 30.65±112.58 ind and jellies was recorded during 42 days (Table 2). During summer 2015 the total abundance was higher then during spring with a mean abundance of 22.23±53.11 ind, and *Pelagia* was recorded during 54 d. For 2016 already by the end of spring the abundance degreased considerably, and the mean abundance dropped to 1.25±6.08 ind during summer and was seen only during 9 days.

Inter-basin

Comparing abundance during summer between the Spanish coast of the NW Mediterranean basin and the Alboran Sea during the summer 2012-2015, maintaining about 47% of the abundance for the Alboran Sea. Thought, during summer 2016, 22% were reported from the Alboran Sea (Fig. 5).

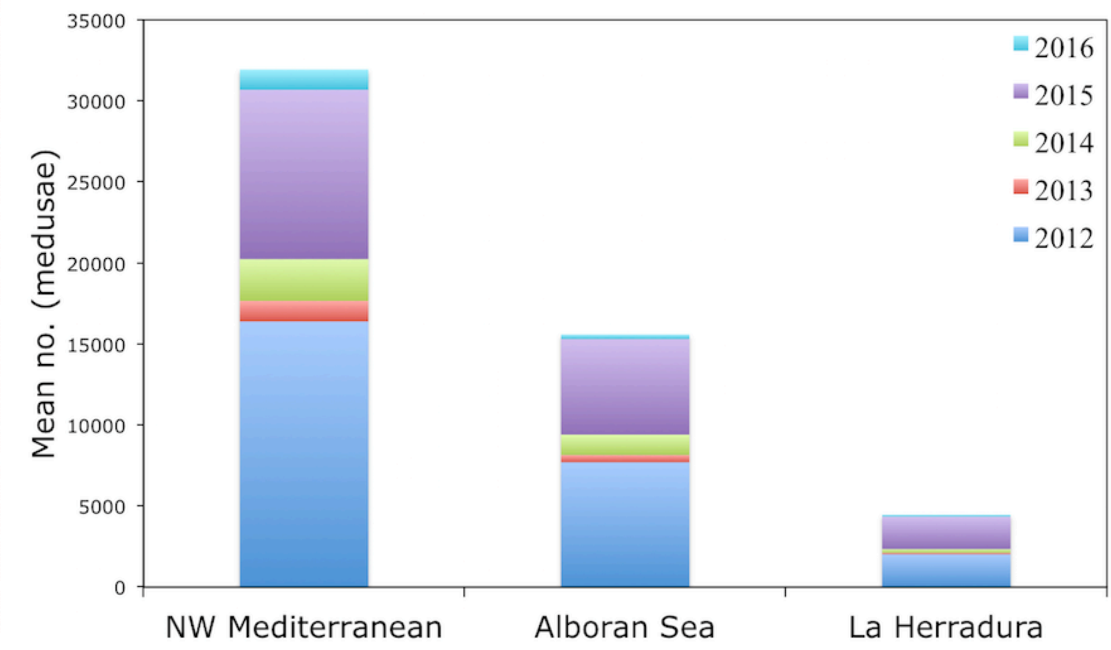

Figure 5 Summer abundances of *Pelagia noctiluca* from the NW Mediterranean (Spanish Coast), Alboran Sea (SW Mediterranean) and La Herradura (NE Alboran Sea)

Environmental parameters

Air temperature

There was no significant difference between the air temperature variability between the three-tested summers (one-way ANOVA, $F_{(2)}$=2.28, P=0.110). Thought, the average temperature during summer 2015 was warmer than the other two (2015: 24.17±2.58 °C; 2016: 23.88±1.74 °C; 2012: 23.51±2.02°C) see Figure 6 and Table 2.

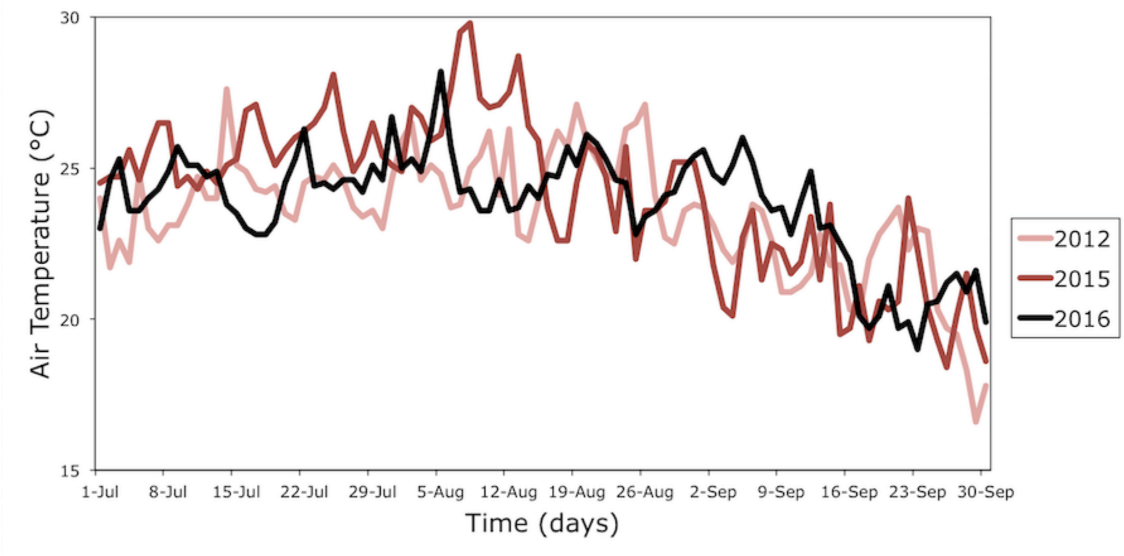

Figure 6 Average air temperatures over the summer 2012, 2015 and 2016

Table 2 Seasonal average of the main environmental variables during summer 2012, 2015 and 2016 from La Herradura, NE Alboran Sea

Summer	Mean±SD abundance (ind)	No. days present	Mean Air Temperature (°C)	Mean SST (°C)	Mean SSS	Mean wind velocity (m/s)	Mean wave height (m)/ direction
2012	30.65±112.58	42	23.51±2.02	21.67±1.30	37.101±0.121	3.28±2.36 WSW	0.67±0.45 WSW
2015	22.23±53.11	54	24.17±2.58	21.96±3.73	37.347±0.314	3.36±3.21 WSW	0.63±0.41 WSW
2016	1.25±6.08	9	23.88±1.74	22.34±2.44	37.336±0.121	2.78±2.57 WSW	0.73±0.50 ESE

SST Sea Surface Temperature, *SSS* Sea Surface Salinity

Sea surface temperature

The variation between the sea surface temperature (SST) during the summer months was not significant (one-way ANOVA, $F_{(2)}$=1.059, P=0.349). Thought, the average temperature was highest during 2016 (22.34±2.44°C), and was for 2012 and 2015, 21.66±1.30°C and 21.96±3.73°C, respectively (Fig. 7). Drops of SST consider with strong westerlies wind episodes.

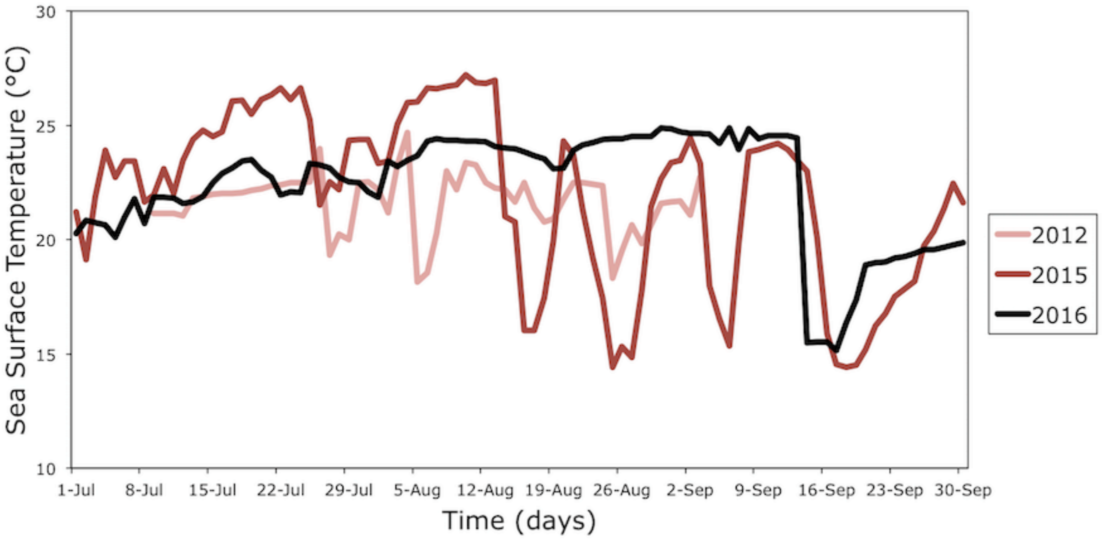

Figure 7 Sea surface temperatures over the summer 2012, 2015 and 2016

Sea surface salinity

The variation between the sea surface salinity (SSS) during the summer months showed significantly difference between the tested summers (one-way ANOVA, $F_{(2)}$=27.11, P>0.001). Summer 2012 and 2015 had lower SSS, 36.84 and 36.86, respectively, and 37.06 for 2016. The highest salinity (38.04) was recorded during summer 2015 (Fig. 8). Increasing of SSS consider with strong westerlies wind episodes.

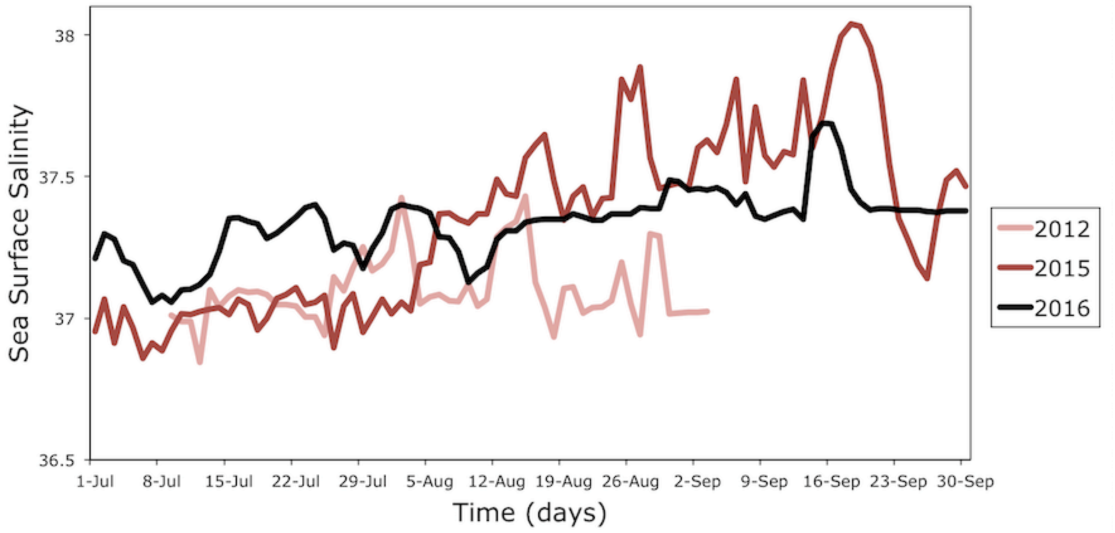

Figure 8 Sea surface salinity over the summer 2012, 2015 and 2016

Wind intensity

The wind speed was significantly different between the three-tested summers (Kruskal-Wallis ANOVA, $H_{(2)}$=38.42, P>0.001), as well the wind direction (Kruskal-Wallis, $H_{(2)}$=28.76, P>0.001). During summer 2012 westerlies winds predominated about 61% of the times against 34% easterlies. The average wind speed was 3.28±2.36 m/s (max 13.90 m/s), wind stronger than 8.8 m/s were 3% of the time westerlies and less than 1% was easterlies (Fig. 9 and Fig. 10). In summer 2015 westerlies winds predominated about 58% of the times and approx. 36% was easterlies. The average wind speed was 3.19±2.61 m/s (max 13.50 m/s), with winds stronger than 8.8 m/s (6.2%) being mostly westerlies, almost no strong easterly was blowing 0.1%. During 2016 the predominate wind forcing was as well from the west (50%), but the amount of easterly was higher (45%). The average wind was 2.87±2.24 m/s (max 12.94 m/s), and winds blowing stronger than 8.8 m/s were 1.9% of the times easterlies and 1.3% westerlies.

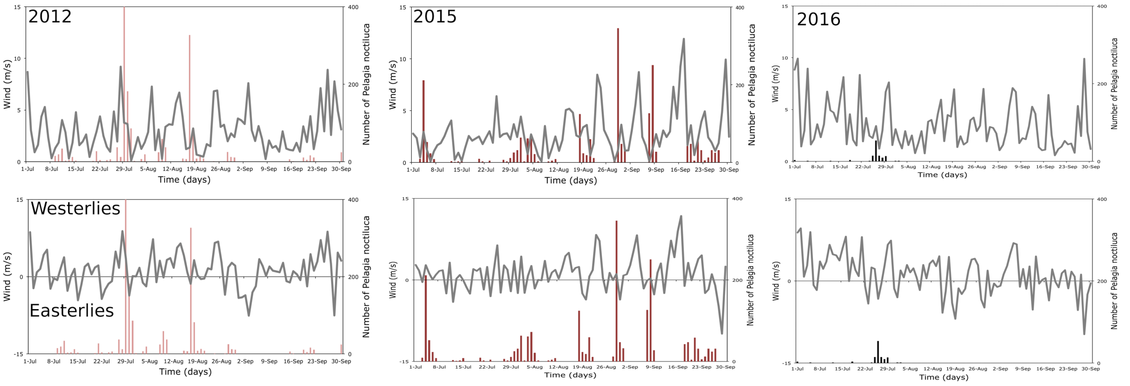

Figure 9 Time series of local wind speed (m/s) and daily abundance of *Pelagia noctiluca* from the NE Alboran Sea during summer 2012, 2015 and 2016 (Top). Zonal wind components (low) show positive and negative values (westerlies and easterlies, respectively)

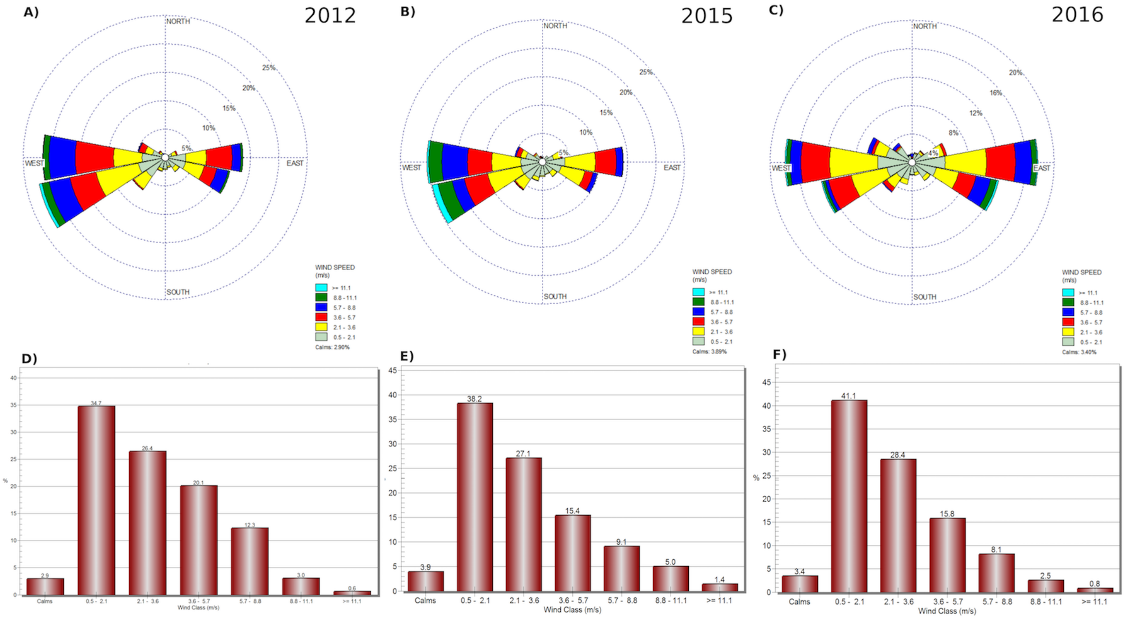

Figure 10 Wind rose diagram for summer 2012 (A), 2015 (B) and 2016 (C). Persent frequency distribution of wind speed for summer 2012 (D), 2015 (E) and 2016 (F)

Wave intensity

The wave height was significantly different between the three-tested summers (Kruskal-Wallis ANOVA, $H_{(2)}$=52.04, *P*>0.001), as well the wave direction (Kruskal-Wallis, $H_{(2)}$=253.93, *P*>0.001). During summer 2012 the predominant current was from SW (31%) and ESE (27%). Wave higher than 2.1 m were 0.5% from SW and 0.3% ESE. The average height was 0.68±0.45 m. The predominant current during summer 2015 was from SW (30%) and ESE (25%), with an average of 0.63±0.41 m. There was no waves higher than 2.1m from the E, 0.4% was from SW. During 2016 the principal current was from ESE (50%) and only 15% of the times from SW. The mean wave height was 0.73±0.47 m. Waves higher than 2.1 m were 2% from ESE, without any recorded from the W (Fig. 11).

Comparison of sexes

The gender was determined from a total of 1294 *P. noctiluca*, 571 females and 723 males. Over the total amount of medusae sampled the male were dominant, being the difference statistically significant (X^2 = 17.85, *P*<0.01; Table 3. Though, the overall sex ratio per month was approximately 1:1 for most moths, and was significantly different for only three months. There was not a significant difference in the bell diameter size between female (7.26±2.13 cm) and male (7.21±2.06 cm) jellyfish (t_{828} = 0.3, p = 0.7), and neither in the mass between female (40.84±37.29 ml) and male (40.90±38.77 ml) medusae (t_{848} = -0.1, p = 0.9). The relationship between bell diameter and mass could be fitted to a power function ($M = 0.124B^{2.816}$, R^2 = 0.853, n = 868, Fig. 12).

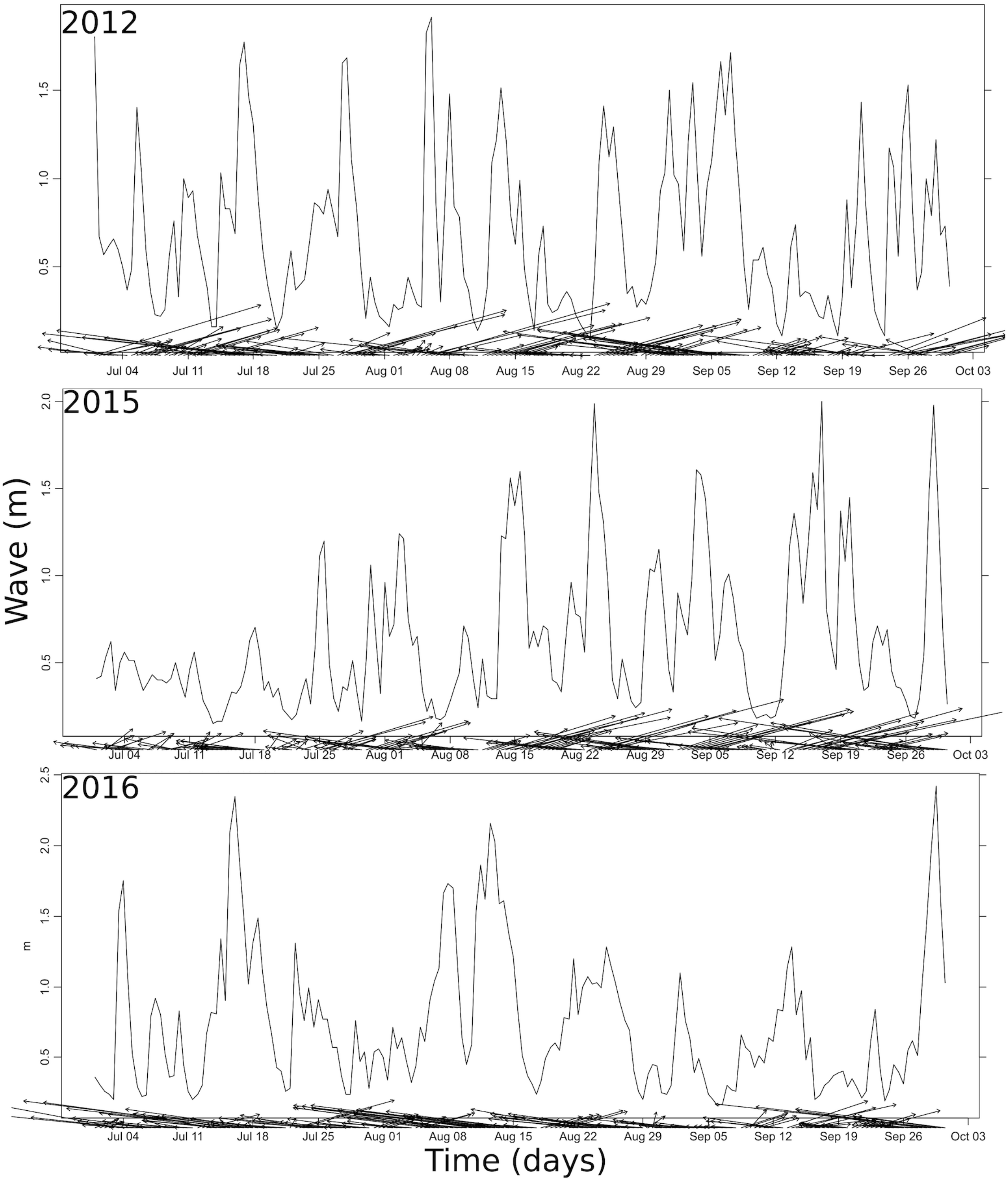

Figure 11 Stick plot of the daily mean wave height for summer 2012, 2015 and 2016

Table 3 Chi-square analyses to test for significant differences in gender distribution of *Pelagia noctiluca* from the Alboran Sea (SW Mediterranean Sea)

	Female	Male	Sum	Expected Values	X^2	*P*
October 2012	6	14	20	10	3.2	0.074
December 2012	10	26	36	18	7.11	*0.008
January 2013	9	15	24	12	1.50	0.221
June 2013	31	33	64	32	0.06	0.802
August 2013	24	24	48	24	0.00	1.000
September 2013	12	18	30	15	1.20	0.273
November 2013	8	20	28	14	5.14	*0.023
December 2013	27	21	48	24	0.75	0.386
January 2014	20	16	36	18	0.44	0.505
March 2014	16	16	32	16	0.00	1.000
June 2014	6	14	20	10	3.2	0.074
July 2014	14	24	38	19	2.63	0.105
August 2014	12	16	28	14	0.57	0.450
September 2014	18	30	48	24	3.00	0.083
November 2014	6	11	17	8.5	1.47	0.225
December 2014	17	12	29	14.5	0.86	0.353
January 2015	16	12	28	14	0.57	0.450
March 2015	18	26	44	22	1.45	0.228
April 2015	54	67	121	60.5	1.40	0.237
June 2015	16	40	56	28	10.29	*0.001
July 2015	35	49	84	42	2.33	0.127
August 2015	36	36	72	36	0.00	1.000
September 2015	26	26	52	26	0.00	1.000
October 2015	14	24	38	19	2.63	0.105
February 2016	14	20	34	17	1.06	0.304
March 2016	15	23	38	19	1.68	0.194
April 2016	17	21	38	19	0.42	0.516
May 2016	37	33	70	35	0.23	0.633
October 2016	25	24	49	24.5	0.02	0.886
December 2016	12	12	24	12	0.00	1.000
TOTAL	**571**	**723**	**1294**	**647**	**17.85**	***<0.001**

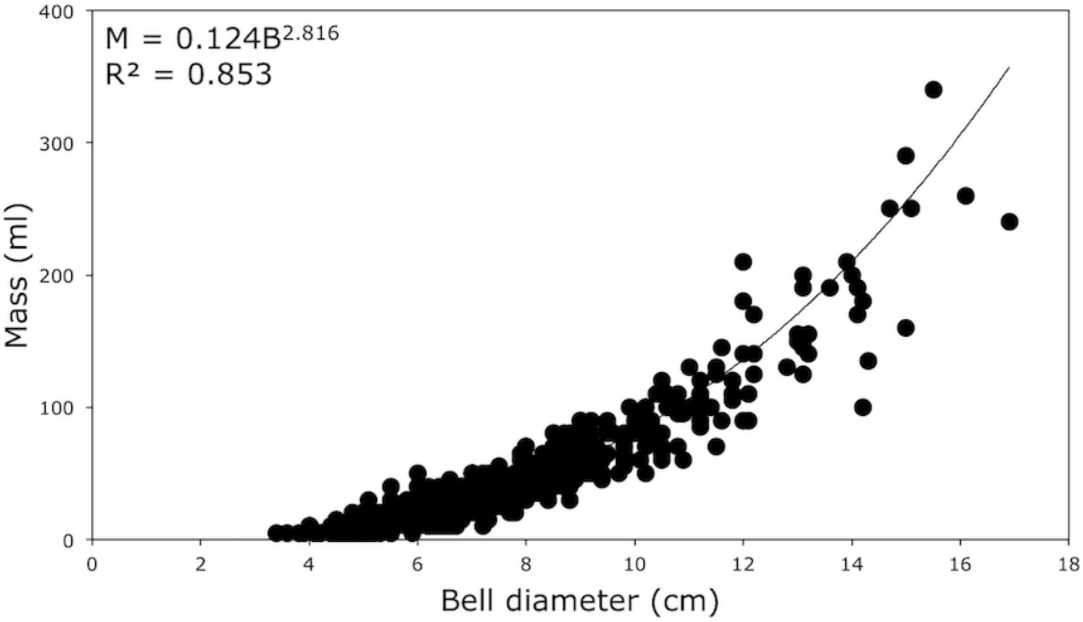

Figure 12 The relation between bell diameter (D) and mass (M) of *Pelagia noctiluca* from the Alboran Sea (SW Mediterranean Sea) *n* = 976

Growth estimation and cohort identification

Over the 4 ½ years period *P. noctiluca* an intermediate cohort of 6-7 cm was present in almost all the sampling months, except in winter where mostly larger medusae were present. A total of 1748 specimens were collected during the period of July 2012 to December 2016 ranging in size from 1.1 to 16.9 cm, collected in October 2014 and March 2016, respectively. The average bell diameter was 7.3±2.3 cm and the frequency distribution histogram showed the highest frequency in the 6.0 – 6.5 cm size range (Fig. 13).

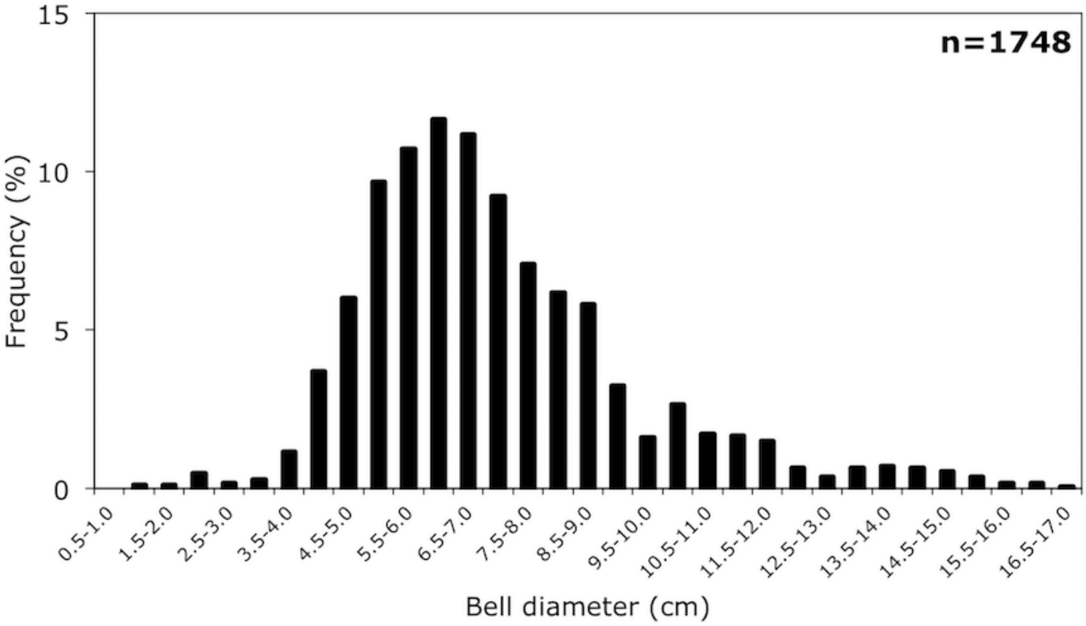

Figure 13 Size-frequency distribution of the bell diameter of *Pelagia noctiluca* from the Alboran Sea (SW Mediterranean Sea) from July 2012 until December 2016

Based on the analysis through FiSAT II program, sub program ELEFAN I, using the K-scan technique (Fig. 14) we obtained that for *P. noctiluca* the asymptotic length (L_∞) was 17.85 cm, with a growth coefficient (K) of 0.97 $year^{-1}$ and a growth performance index (ϕ') of 2.49. Parameters of the von Bertalanffy growth function are presented in Table 4, and the predicted extreme length is represented in Figure 15. These values are the base for the von Bertalanffy growth curve of *P. noctiluca* (Fig. 16). Monthly length frequency data showed distinct modes during the 4½ year of sampling with a clear progression of these modes over this time period. Examination of the temporal size distribution illustrated in Figure 17 reveals that an influx of small medusae (< 3.5 cm) was observed between later spring to early autumn (May-October), though, being highly variable between years. The presence of juveniles overlaps with adults from the previous generations. There was an increase in growth of individuals is apparent from April till September (increased slope), during this period individuals moved through length-frequency classes rapidly. Larger medusae were collected from February to March (>15 cm). The predicted extreme length of the stock was estimated to be 17.67 cm. Thought, some size categories contained to few observations to be recognised as cohort.

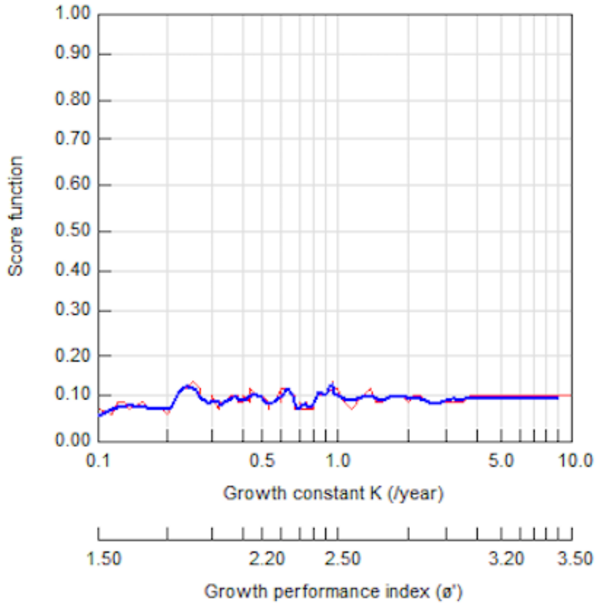

Figure 14 *K*-scan values curve by Shepherd's method for *Pelagia noctiluca* from the Alboran Sea (SW Mediterranean Sea)

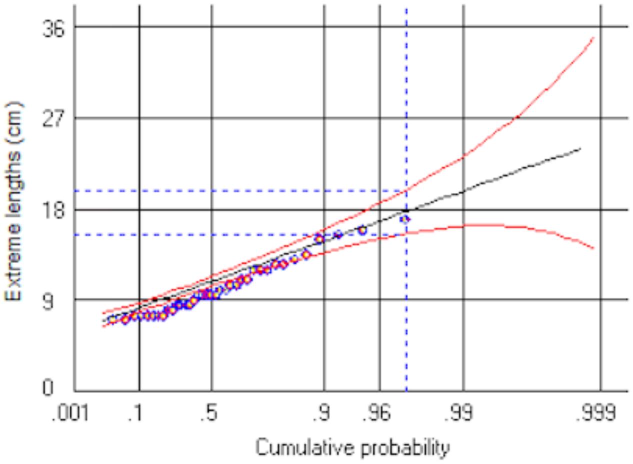

Figure 15 Predicted extreme length of *Pelagia noctiluca* from the Alboran Sea (SW Alboran Sea)

Table 4 Population parameters of *Pelagia noctiluca* in the Alboran Sea (SW Mediterranean Sea)

Characteristic	**Value**
Asymptotic length $\boldsymbol{L_\infty}$ (cm)	17.85
Growth coefficient *K* ($\boldsymbol{year^{-1}}$)	0.97
to	-0.991
Observed maximum length (cm)	16.9
Predicted extreme length	17.67
Range at 95% confidence interval (cm)	15.55-19.79
Growth performance Φ'	2.490
Sample number *n*	1748

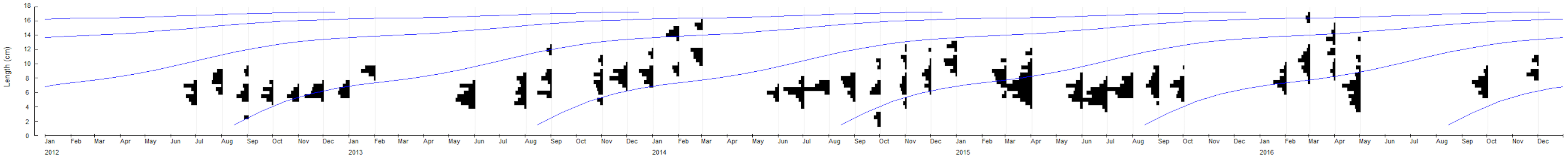

Figure 16 Length-frequency distribution output generated by ELEFAN I with superimposed growth curve for *Pelagia noctiluca* from the Alboran Sea (SW Mediterranean Sea). The relevant parameters are: $\boldsymbol{L_\infty}$=17. 85 cm , K=0.97 $\boldsymbol{year^{-1}}$, C=0.5, WP=0.1

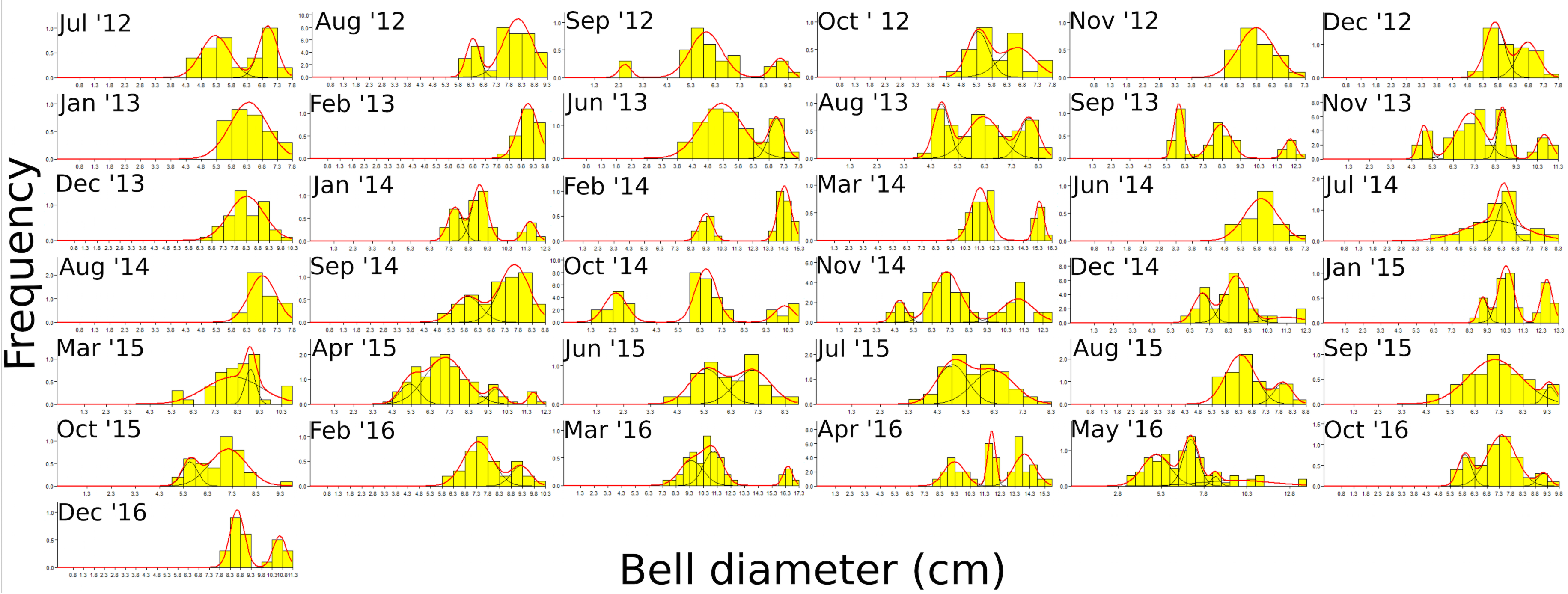

Figure 17 Monthly size classes distribution of *Pelagia noctiluca* medusae from the Alboran Sea (SW Mediterranean Sea) from July 2012 until December 2016. Growth parameters were estimated by means of ELEFAN I. During some months because of the scarcity the medusae to few ore none where collected

DISCUSSION

The mauve stinger *Pelagia noctiluca* is considered to be one of the most common and notorious species in the Mediterranean Sea. In spite of only a handful of studies have investigated its population dynamic and from the northern Alboran Sea (SW Mediterranean) nothing is known about its interannual and seasonal oscillations. (Madin 1991) stated that it was the most abundant scyphomedusae in the Alboran Sea, and its presence was reported during summer 2008 (Licandro et al. 2010), thought without any further information.

In this study, we monitored during 4½ years the presence/absence of *P. noctiluca* using visual observation from the beach known as Marina del Este, La Herradura (NE Alboran Sea, see map Fig. 1). The jellyfish presence was recorded in almost all months, thought the abundance was highly variable between years and seasons (Figure 2). The number of jellyfish during summer 2012 and 2015 was significantly higher as during the other three summers (2013, 2014 and 2016), which was as well reflected in the number of days recording the presence of the target species (see Fig. 4 and Table 2). A similar study recording stranded jellyfish from the Moroccan SW Alboran coastline reported as well lower abundance during 2013, 2014 and 2016 and a 10-fold higher abundance for 2012 and 2015 (Aouititen et al. 2019). Thought, they did not record any jellyfish during summer 2013. Nevertheless, these results suggest a similar interannual oscillation pattern occurring from the northern and southern shores of the Alboran basin. Furthermore, since 2007 the presence of the mauve stinger has been monitored from the coast of Andalucía (southern Spain), and between 2007 and 2012 by far the highest abundance was reported during summer 2012 (Prieto and Navarro 2013). Resulting, that since 2007 until 2016 the mauve stinger has been present every year in the Alboran Sea. Whereby blooms during the summer months occurred during 2007, 2012 and 2015 (present study). Our results confirm also the suggestion that any presence of *P. noctiluca* in the western Mediterranean Sea may indicate its presence in the entire western basin (Goy et al. 1989) (Bernard et al. 2011).

The year-round presence of *Pelagia* has been reported from various locations from the western Mediterranean basin: Catalan Coast (Canepa et al. 2014), northern Tunisia (Aissi et al. 2014), Strait of Messina (Rosa et al. 2013; Milisenda et al. 2018), and higher abundances were reported during winter-spring. Lower abundance or even the complete disappearance of jellyfish has been reported during the summer period

(Rosa et al. 2013; Canepa et al. 2014). The "disappearing" of *Pelagia* has as well occurred during summer 2016 since high numbers of medusae were present from February to May, and then its abundance drastically dropped during the summer months (Fig. 2). We hypothesised, therefore, that one prerequisite to have an important summer presence of *Pelagia* is to have a high abundance during spring. Henceforth, depending on the prevalent wind stress during summer the jellyfish are maintained near the coast (summer 2012 and 2015) or disappear (summer 2016).

Summers with *Pelagia* have persistent westerlies, activating the wind-driven upwelling and resulting in injecting nutrients in the usually oligotrophic system during this time of the year (Ramírez et al. 2005). Maintaining the jellyfish population in surface layer with abundant food resource during summer. Additionally, episodes of easterlies winds push the jellyfish population near the coast (stranding events, see Fig. 9). These results agree with various studies that at the local level, wind direction influences the stranding events of *P. noctiluca* (Berline et al. 2013; Canepa et al. 2014; Aouititen et al. 2019). Furthermore, our results indicate that the mauve stinger does not get "washed ashore" by wave generated by strong easterly winds, but occurred near coast mostly during low energy period (Fig. 9). A similar observation was made by Ferraris et al. (2012), which noted that *Pelagia* tended to stay at a deeper depth when the wave height exceeded 0.20 m. Summer 2016 had the lowest abundance of jellyfish during our sampling period (Fig. 2), however, the abundance during spring was important. During summer 2016 the predominant winds were as well westerlies, being less intense than during summer 2012 and 2015, and strong episodes of easterlies were recorded (Figures 9 and 10). This is as well reflected in the wind-induced currents, as during summer 2016 the principal wave direction was from ESE.

We sampled a total of 1748 jellyfish from July 2012 to December 2016 the population intermediate cohort from the NE Alboran Sea was 6-7 cm, which is consistent to the one reported from southern Italy (5-7 cm, Rosa et al. 2013; 6-8 cm, Milisenda 2014). Though, *Pelagia* from the Alboran Sea grows to larger sizes as large specimens (> 12 cm) were present from September to May with bell size bigger than 14 cm collected in February-March 2014 and March-April 2016, the largest medusa we have measured was 16.9 cm (March 2016). The biggest medusa collected from the Strait of Messina were reported during May (>12 cm, Milisenda et al. 2018; 14.5 cm May 2009, Rosa et al. 2013). Bigger sizes were as well reported from the Ligurian Sea were the mean bell size was 15.6 cm, with a range from 12 to 21 cm, Lilley et al. 2014). The population from northern Tunisia (Aissi et al. 2014) had as well bigger diameter over the winter 8-10 cm, however, the biggest sizes were reported from June (max 13±0.4 cm).

In the Mediterranean Sea *P. noctiluca* might reproduce year-round when conditions are optimal (Malej et al. 1992; Sandrini and Avian 1991; Canepa et al. 2014). From the Strait of Messina sexual reproduction was reported throughout the year, with two seasonal peaks in spring and autumn (Milisenda et al. 2018). In the Adriatic Sea (eastern Mediterranean) ephyrae were observed from April to December, with a peak in late summer and autumn (Malej et al. 1992). We observed young medusae (< 3.5 cm) between late spring to early autumn (May-October) and during May 2016 young medusa (< 3 cm) were collected (Fig. 17). According to a recent study (Ramondenc 2019), juveniles of 2.7 cm have been reported to be about 2½ months under laboratory conditions Therefore, our results would agree with observations done in two aquaculture facilities in the northern Alboran Sea (Bosch-Belmar et al. 2017). The presences of ephyrae were reported year-round at low-medium densities (from 1.50 to 15 ind m^{-3}), with higher densities in February, June and October. During most of the months, multiple cohorts were present (up to 4 were estimated), however, a clear pattern was not visible, as our results showed a great fluctuation at interannual and seasonal level. Only one cohort was present during winter 2013, though, multiple (up to 3 cohorts) were estimated during the successive winters.

From the Strait of Messina during a one year observation bimodal distribution was reported in February, June and July and three distinct cohorts in April and May (Milisenda et al. 2018). The *Pelagia* population from Tunisia showed a seasonal occurrence with multiple cohorts, consisting of over wintering jellyfish until February (8-10 cm) and young medusae started to appear by mid-February (2- 3 cm). *Pelagia noctiluca* life cycle was suggested to be between 9 months (Kogovšek et al. 2010) and a year (Morand et al. 1992), agreeing with our results, as bigger jellies disappeared by late spring (> 12 cm). *Pelagia noctiluca* had a sex ratio of approximately 1:1 for most moths, thought the male percentage dominated over the entire sampling period (44% female, 56% male, n= 1294). The same gender distribution was also observed in the population from the Strait of Messina (45% female, 55% male, n=1054, Milisenda et al.

2014), which the authors concluded that the sex ratio might be influenced by the slight gender-specific foraging behaviour.

CONCLUSIONS

Our results suggest that one of the prerequisites of a greater abundance of *Pelagia noctiluca* during the summer months near the northern coast of the Alboran Sea requires a high abundance of jellies during the spring. Still, the presence of mauve stinger during spring does not necessarily lead to jelly blooms during summer. As the second requisite is the activation of the wind-induced upwelling mechanism through episodes of Westerlies "*Poniente*" during the summer months and episodes of weaker easterlies "*Levante*" pushing the jellies near the coast. However, to predict *P. noctiluca* fluctuation over the years a longer dataset is necessary, as our 4 ½ years show a great interannual and seasonal fluctuation recording the presence/absence of this jellyfish.

REFERENCES

Aissi M, Touzri C, Gueroun SKM, Kefi-daly Yahia O, Daly-Yahia MN (2014) Persistent occurrence and life cycle of Pelagia noctiluca in the Channel of Bizerte (Northern Tunisia). Ecology, Environment and Conservation 20(4):1453–60

Aouititen M, Bekkali R, Nachit D, Luan X, Mrhraoui M (2019) Predicting jellyfish strandings in the Moroccan North-West Mediterranean Coastline. European Scientific Journal 15(2):72–84 doi.org/10.19044/esj.2019.v15n2p72

Berline L, Zakardjian B, Molcard A, Ourmières Y, Guihou K (2013) Modeling jellyfish *Pelagia noctiluca* transport and stranding in the Ligurian Sea. Marine Pollution Bulletin 70 (1–2): 90–99. https://doi.org/10.1016/j.marpolbul.2013.02.016

Bernard P, Berline L, Gorsky G (2011) Long term (1981-2008) monitoring of the jellyfish *Pelagia noctiluca* (Cnidaria, Scyphozoa) on Mediterranean Coasts (Principality of Monaco and French Riviera). Journal of Oceanography, Research and Data 4: 1–10

Bhattacharya CG (1967) A simple method of resolution of a distribution into Gaussian Components. Biometrics 23(1):115–35. doi.org/10.2307/2528285

Boero F (2013) Review of Jellyfish Blooms in the Mediterranean and Black Sea. Studies and Reviews. General Fisheries Commission for the Mediterranean 92, FAO, Rome, pp 54

Bosch-Belmar M, Milisenda G, Girons A, Taurisano V, Accoroni S, Totti C, Piraino S, Fuentes V (2017) Consequences of stinging plankton blooms on finfish mariculture in the Mediterranean Sea. Frontiers in Marine Science 4: 1–11.

doi.org/10.3389/fmars.2017.00240

Canepa A, Fuentes V, Sabatés A, Piraino S, Boero F, Gili, J.M. (2014) *Pelagia noctiluca* in the Mediterranean Sea. In: Pitt, K.A. & Lucas, C.H. (Eds.), Jellyfish Bloom. Springer, Netherlands, pp. 237–266. doi.org/10.1007/978-94-007-7015-7_11

CIESM (2001) Gelatinous zooplankton outbreaks: theory and practice. CIESM Worshop series 14, Mediterreanan Science Commission, Monaco, 112 pp

Daly Yahia MN, Goy J, Daly Yahia-Kéfi O (2003) Distribution et écologie des méduses (Cnidaria) du golfe de Tunis (Méditerranée sud occidentale). Oceanol Acta. 26(5-6):645–655. doi:10.1016/ j.oceact.2003.05.002

Daly Yahia MN, et al. (2010) Are the outbreaks of *Pelagia noctiluca* (Forskal, 1775) more frequent in the Mediterranean basin? ICES Co-operative Research Report 300: 8-14

De Donno A, Idolo A, Bagordo F, Grassi T, et al. (2014) Impact of stinging jellyfish proliferations along South Italian Coasts : Human health hazards, treatment and social costs. International Journal of Environmental Research and Public Health 11:2488–2503. doi.org/10.3390/ijerph110302488

Doyle TK, Houghton JDR, Buckley SM, Hays GC, Davenport J (2007) The broad-scale distribution of five jellyfish species across a temperate coastal environment. Hydrobiologia 579(1):29–39. doi.org/10.1007/s10750-006-0362-2

Ferraris M, Berline L, Lombard f, Guidi L, Elineau A, Mendoza-Vera JM, Lilley MKS. (2012) Distribution of *Pelagia noctiluca* (Cnidaria , Scyphozoa) in the Ligurian Sea (NW Mediterranean Sea). Journal of Plankton Research 34 (10): 874–85. doi.org/10.1093/plankt/fbs049

Fleming NEC, Harrod C, Houghton JDR (2013) Identifying potentially harmful jellyfish blooms using shoreline surveys. Aquaculture Environment Interactions 4(3): 263–72. doi.org/10.3354/aei00086

Gayanilo FC, Sparre P, Pauly D (2005) FAO-ICLARM Stock Assessment Tools (FiSAT II). Revised version. User's guide. FAO Computerized Information Series (Fisheries) 8. Rome, FAO. pp 168

Gili JM, Pagés F 2005. Les proliferacions de meduses. Bol Soc Hist Nat Baleares 48:9-22

Goy J, Morand P, Etienne M (1989) Long-term fluctuations of *Pelagia noctiluca* (Cnidaria, Scyphomedusa) in the Western Mediterranean Sea. Prediction by climatic variables. Deep Sea Research Part A. Oceanographic Research Papers 36(2): 269–79. doi.org/10.1016/0198-0149(89)90138-6

Hasselblad V (1966) Estimation of parameters for a mixture of normal distributions. Technometrics 8:431-444

Houghton JDR, Doyle TK, Davenport J, Lilley MKS, Wilson RP, Hays GC (2007). Stranding events provide indirect insights into the seasonality and persistence of jellyfish medusae (Cnidaria: Scyphozoa). Hydrobiologia 589 (1):1–13. doi.org/10.1007/s10750-007-0572-2

Kogovšek T, Bogunovic B, Malej A (2010) Recurrence of bloom-forming

Scyphomedusae : Wavelet Analysis of a 200-Year Time Series. Hydrobiologia 645(1): 81–96. doi.org/10.1007/s10750-010-0217-8

Licandro P, Conway DVP, Daly Yahia MN, Fernandez de Puelles ML, Gasparini S, Hecq JH, Tranter P, Kirby RR (2010) A blooming jellyfish in the northeast Atlantic and Mediterranean. Marine Biology 6: 688–91

Lilley MKS, Ferraris M, Elineau A, Berline L, Cuvilliers P, Gilletta L, Thiéry A, Gorsky G, Lombard F (2014) Culture and growth of the jellyfish *Pelagia noctiluca* in the laboratory. Marine Ecology Progress Series 510: 265–73. doi.org/10.3354/meps10854

Macías D, Navarro G, Echevarría F, García CM, Cueto JL (2007) Phytoplankton pigment distribution in the northwestern Alboran Sea and meteorological forcing: A remote sensing study. Journal of Marine Research 65(4): 523–43. doi.org/10.1357/002224007782689085

Macías D, Bruno M, Echevarría F, Vazquez A, Garcia CM (2008) Meteorologically-induced mesoscale variability of the North-western Alboran Sea (Southern Spain) and related biological patterns. Estuarine, Coastal and Shelf Science 78: 250–66. doi.org/10.1016/j.ecss.2007.12.008.

Madin LP (1991) Distribution and taxonomy of zooplankton in the Alboran Sea and adjacent Western Mediterranean. A literature survey and field guide. Woods Hole Oceanographic Institution

Malej A, Malej M (1992) Population dynamics of the Jellyfish *Pelagia noctiluca* (Forsskal, 1775). Marine Eutrophication and Population Dynamics, 215–19.

Mariottini GL, Giacco E, Pane L (2008) The mauve stinger *Pelagia noctiluca* (Forsskal, 1775). Distribution, ecology, toxicity and epidemiology of stings. A Review. Marine Drugs 6:496–513. doi.org/10.3390/md20080025

Milisenda G, Martínez-Quintana A, Fuentes V, Aglieri G, Bosch-Belmar M, Boero F, Piraino (2018) Reproductive and bloom patterns of *Pelagia noctiluca* in the Strait of Messina, Italy. Estuarine, Coastal and Shelf Science 201: 29–39. doi.org/10.1016/j.ecss.2016.01.002

Milisenda G, Rosa S, Fuentes V, Boero F, Guglielmo L, Purcell J, Piraino S (2014) Jellyfish as Prey: Frequency of predation and selective foraging of *Boops Boops* (Vertebrata, Actinopterygii) on the mauve stinger *Pelagia Noctiluca* (Cnidaria, Scyphozoa). PloS One 9 (4): e94600. doi.org/10.1371/journal.pone.0094600

Molinero JC, Ibanez F, Nival P, Buecher E, Souissi S (2005) North Atlantic climate and Northwestern Mediterranean plankton variability. Limnology and Oceanography 50(4):1213–20. doi.org/10.4319/lo.2005.50.4.1213

Molinero JC, Ibanez F, Souissi S, Buecher E, Dallot S, Nival P (2008) Climate control on the longterm anomalous changes of zooplankton communities in the Northwestern Mediterranean. Global Change Biol 14:11–26

Morand P, Goy J, Dallot S (1992). Recrutement et Fluctuations à long-terme de *Pelagia noctiluca* (Cnidaria, Scyphozoa). Annales de l'Institut Océanographique 68(1–2):151–58

Nastav B, Malej M, Malej Jr, Malej A (2013) Is it possible to determine the economic impact of jellyfish outbreaks on fisheries ? A Case Study – Slovenia. Mediterranean

Marine Science 14: 214–23.

Navarro G, Vázquez Á, Macías D, Bruno M, Ruiz J (2011) Understanding the patterns of biological response to physical forcing in the Alborán Sea (Western Mediterranean). Geophysical Research Letters 38: 1–5. doi.org/10.1029/2011GL049708

Palomares MLD, Pauly D 2009. The Growth of Jellyfishes. Hydrobiologia 616: 11–21. doi.org/10.1007/s10750-008-9582-y

Pauly D, Munro JL, (1984) Once more on the comparison of growth in fish and invertebrate. ICLARM Fishbyte 2(1):21

Parrilla G, Kinder TH (1987) The physical oceanography of the Alboran Sea.

Pingree G, Abend L (2006) Spain's beaches and flora feel the heat. Available at http://www.csmon- itor.com/2006/0914/p07s02-woeu.html

Prieto L, Navarro G (2013) Avistamientos de medusas en el litoral andaluz. ISBN: 978-84-695-8463-7, Cadiz (Spain), pp 36. http://www.marinera-medex.eu/index.php?option=311&file=Prieto_Navarro_2013.pdf

Purcell J, Uye S, Lo W (2007) Anthropogenic causes of jellyfish blooms and their direct consequences for humans : A Review. Marine Ecology Progress Series 350: 153–74. doi.org/10.3354/meps07093

Ramírez T, Cortés D, Mercado JM, Vargas-Yañez M, Sebastián M, Liger E (2005) Seasonal dynamics of inorganic nutrients and phytoplankton biomass in the NW Alboran Sea. Estuarine, Coastal and Shelf Science 65(4): 654–70. doi.org/10.1016/j.ecss.2005.07.012

Ramondenc S, Ferrieux M, Collet S, Benedetti F, Guidi L, Lombard F (2019) From egg to maturity: A closed sysem for complete life cycle studies of the holopelagic jellyfish *Pelagia noctiluca*. Journal Of Plankton Research 00:1–11. doi.org/10.1093/plankt/fbz011

Rosa S, Pansera M, Granata A, Guglielmo L (2013) Interannual Variability, growth, reproduction and feeding of *Pelagia noctiluca* (Cnidaria: Scyphozoa) in the Straits of Messina (Central Mediterranean Sea): Linkages with temperature and diet. Journal of Marine Systems 111–112: 97–107. doi.org/10.1016/j.jmarsys.2012.10.001

Ruiz J, Macías D, Rincón MM, Pascual A, Catalán IA, Navarro G (2013) Recruiting at the Edge: Kinetic energy inhibits anchovy populations in the Western Mediterranean." PLoS ONE 8 (2). doi.org/10.1371/journal.pone.0055523

Russell FS (1970) The medusae of the British Isles II, Pelagic Scyphozoa with a supplement to the first volume on hydromedusae. Cambridge University Press, Cambridge

Sabatés A, Salat J, Tilves U, Raya V, Purcell J, Pascual M, Gili JM, Fuentes V (2018) Pathways for *Pelagia noctiluca* jellyfish intrusions onto the Catalan Shelf and their interactions with early life fish stages." Journal of Marine Systems 187:52–61. doi.org/10.1016/j.jmarsys.2018.06.013

Rottini SL, Avian M (1991) Reproduction of *Pelagia noctiluca* in the Central and Northern Adriatic Sea. Hydrogiologia 1987: 197–202

Sarhan T, Lafuente JG, Vargas M, Vargas JM, Plaza F (2000) Upwelling mechanisms in the Northwestern Alboran Sea. Journal of Marine Systems 23: 317–31.

Chapter VII

The First record of beaching events for a calycophoran siphonophore: *Abylopsis tetragona* at the Strait of Gibraltar

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ABSTRACT

Two beaching events of the calycophoran siphonophore *Abylopsis tetragona* (Otto, 1823) were observed in two different areas of the Strait of Gibraltar during the cold season. The first was discovered on November 2014, on Getares Beach (Algeciras Bay, Mediterranean part of the Strait of Gibraltar), where more than 700 colonies were found deposited along the tideline. The second event was discovered on January 2015, on Paloma Baja Beach (Tarifa, Atlantic part of the Strait of Gibraltar) where an average density of 170 colonies m^{-2} was spread the seashore. Both events seemed to be promoted by strong easterly winds, preceded by upwelling episodes that may have concentrated high densities of the siphonophore in superficial layers. This study represents the first report of a calycophoran siphonophore mass stranding. The records were made thanks to citizen science and jellyfish outreach at secondary schools (PERSEUS@school initiative), illustrating the importance of citizen science projects in observing natural phenomena. We consider the monitoring and recording of cnidarian stranding events especially important in highly productive and biologically active areas such as the Strait of Gibraltar.

Keywords: Mass stranding; gelatinous zooplankton; citizen science; outreach; secondary school

INTRODUCTION

Siphonophores are long pelagic colonial hydrozoans (Cnidaria) found throughout the world's oceans. They constitute a diverse and abundant group of gelatinous animals that are still poorly understood, due to their fragility and their, mostly, oceanic distribution (Pugh 1989; Haddock 2004). Nevertheless, some species are mainly coastal and neritic and they can reach very high abundances (Greve 1994; Pugh 1999; Mapstone 2014). To the suborder Calycophorae belong some of the smallest and most coastal living siphonophores, like the species *Abylopsis tetragona* (Otto, 1823). It is an abundant epipelagic siphonophore inhabitant of temperate and warm waters of the Atlantic, Pacific and Indican oceans and the Mediterranean Sea (Alvariño 1971; Mapstone 2014).

In the Strait of Gibraltar area there are a few studies on the distribution and abundance of siphonophores, made more than 20 years ago. From them we know that *A. tetragona* is a common and predominant species among the gelatinous zooplankters in the area (Bigelow and Sears 1937; Dallot et al. 1988; Mills et al. 1996). In the Mediterranean Sea and the Atlantic Ocean, this species is distributed mainly in the upper 50 m although it can be spread beyond 200 m depth (Pugh 1974; Gili et al. 1987a; Lučić et al. 2011) and occurs both in coastal and open waters (Bigelow and Sears 1937; Alvariño 1971; Gili et al. 1987a). According to Andersen et al. (1992) it can perform diel vertical migration of about 400 m, occurring in the upper 100 m at night. However, Buecher (1999) pointed out that in coastal waters the behaviour of *A. tetragona* must be different from offshore observations, with vertical migration being limited or negligible.

In the Mediterranean, *A. tetragona* is known to live all year around (Buecher 1999; Bouillon et al. 2004) with a main breeding period during spring (Bigelow and Sears 1937; Gili et al. 1987b). In the northwestern Mediterranean the lowest abundances are found during the winter and the maxima during spring (Buecher 1999) and summer (Gili et al. 1987a). There are no studies on the feeding of this species. However, it is known that calycophoran siphonophores are voracious predators of zooplankton and they may constitute a considerable trophic impact in certain areas (Purcell 1982; Purcell and Kremer 1983; Pagès et al. 2001). In addition, they compete with the co-existing plankton, including higher trophic levels such as fish (e.g.: Purcell and Arai, 2001).

The Strait of Gibraltar (Fig. 1) is located at the southern tip of the Iberian Peninsula and connects the Gulf of Cadiz (Atlantic Ocean) with the Alboran Sea (Mediterranean Sea). Powerful local westerly and easterly winds affect the oceanography of the Strait, activating upwelling and downwelling episodes, respectively. During a westerly wind episode, Ekman transport typically drags the surface waters outwards and a deep current moves towards the surface (upwelling), bringing up low temperature waters with high nutrient concentrations. In contrast, when easterly winds dominate the surface currents flow inshore and a downwelling event can occur (Macías et al. 2008; Echevarria et al. 2009; Navarro et al. 2011). This scenario of high complex oceanography, where different water masses coexist and many frontal zones develop accumulating important phytoplankton biomasses, results in a hotspot region for marine biodiversity (e.g.: Macías et al., 2006; 2009; Navarro et al., 2006).

Today, many reports of exceptional marine events and species sighting are published thanks to citizen participation in science (e.g.: Boero et al., 2013; Kienberger and Prieto, 2016, 2017). More and more projects have been specifically designed to give citizens a role, some of them for the outreach and educational benefit of the volunteers, like the case of the PERSEUS project which involves scientists and secondary schools, (http://www.perseus-net.eu/site/content.php?locale=1&sel=519; Silvertown, 2009). In addition, citizen science has become a request in many international project calls, like those under the European Commission's Horizon 2020 programme. Undoubtedly, the best way for the public to understand and appreciate nature and science is to participate in it (Silvertown 2009; Boero 2016). In the case of monitoring gelatinous macroplankton, citizen science has already been proved an effective tool (Boero et al. 2009; Kienberger and Prieto 2017).

This work reports on the first mass stranding events of a calycophoran siphonophore, *Abylopsis tetragona*. It occurred at the tip of the southern Iberian coast, the Strait of Gibraltar, on both the Atlantic and Mediterranean sides, during the cold season, November 2014 and January 2015. The records were made thanks to citizen science and outreach at a secondary school.

MATERIAL AND METHODS

Study area

Algeciras Bay, on the Mediterranean side of the Strait of Gibraltar, is a semi-enclosed bay that opens into the waters of the northeastern boundary of the Strait (Fig. 1). The bay faces south-southeast and the bathymetry is characterized by a central canyon that reaches its maximum depth (about 450 m) and width at the mouth of the bay (Sammartino et al. 2014). The beach of Getares is located in the west side of the bay, just after the entrance, and faces east. On the Atlantic side, Paloma Baja Beach (Fig. 1) is situated at the most western point of the Strait of Gibraltar. It faces southwest and the continental shelf seaward is characterized by a very smooth bathymetry with depths shallower than 100 m (Luján et al. 2011).

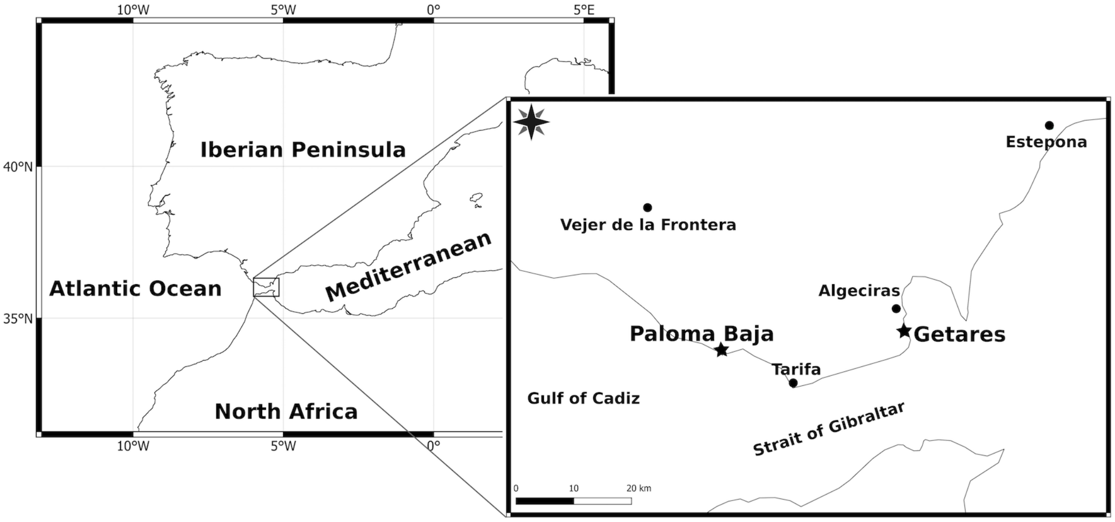

Figure 1 Geographical location of the study area in the Strait of Gibraltar, southern tip of the Iberian Peninsula. Black stars represent the location of both beaching events, Getares Beach, Algeciras Bay, on November 21st and 22nd, 2014 and Paloma Baja Beach, Tarifa, on January 11th, 2015

Sampling

From October 2014 through February 2015, students of 1st bachelor of the high-school *María Auxiliadora* (Algeciras, Cádiz, Spain; Fig. 1) carried out daily beach surveys in order to monitor jellyfish strandings. These surveys were part of their school research project focused on gelatinous plankton, led by their biology teacher (Ana Villaescusa) and the *Instituto de Ciencias Marinas de Andalucía* (ICMAN-CSIC). This activity was inserted within the European PERSEUS@school project, an initiative working with school teachers to help and enhance marine environmental education and outreach on jellyfish in secondary schools. During these surveys, the first *A. tetragona* beaching event was recorded. The second event was discovered fortuitously by students of the same high-school during a geology field trip. Sample collecting was only possible during the first event, during which a total of 200 specimens were taken. Of these, some were preserved in 5% formalin for taxonomic identification at the Marine Science Institute (ICM-CSIC).

Meteorological and oceanographical conditions

Daily wind vector values were obtained from two meteorological stations (Junta de Andalucía), Estepona (36°26'40''N, 05°12'35''W), and Vejer de la Frontera (36°17'06''N, 05°50'24''W), these being the closest ones to Getares and Paloma Baja beaches, respectively. Both stations are relatively low-lying, at short distances off the coast and well exposed to the predominant winds of the Strait and the Gulf of Cadiz (http://www.juntadeandalucia.es/agriculturaypesca/ifapa/ria/servlet/FrontController?action=Init). Daily sea surface temperature satellite images were obtained to observe possible upwelling events in the studied area. The images were acquired from a 1km Advanced Very High-Resolution Radiometer (AVHRR) Ocean Pathfinder Version 5 sea surface temperature (SST) dataset, belonging to the Physical Oceanography Distributed Active Archive Center at the NASA Jet Propulsion Laboratory (http://podaac.jpl.nasa.gov/accessed 2015 Jun 11). STT images were obtained from both previous days to the beaching and the closest possible day to each event. Additionally, daily water temperature average for the studied period was calculated from the *in situ* hourly sea surface temperature dataset obtained from the meteorological buoy placed in the NW part of the Strait of Gibraltar (Tarifa buoy; entity of Spanish Harbours).

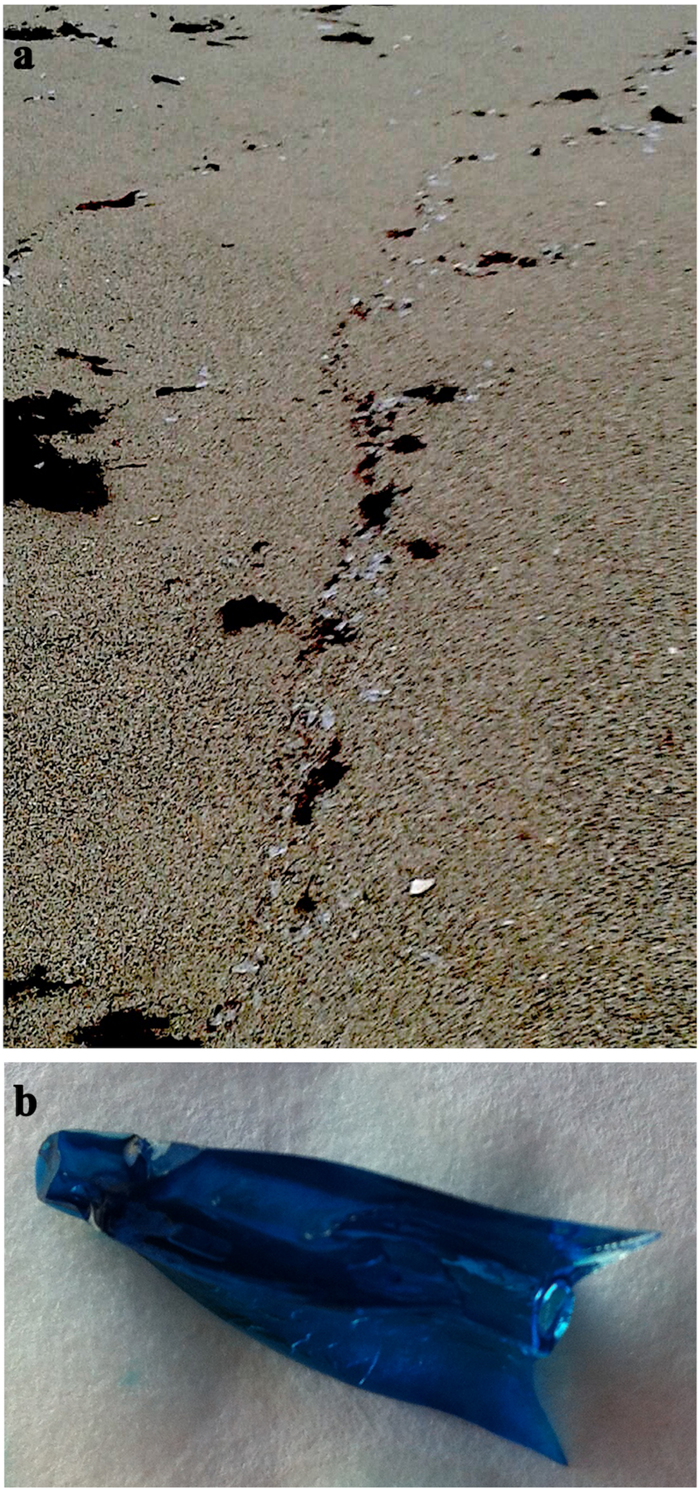

Figure 2 Beaching event at Getares Beach, Algeciras Bay, on November 21st and 22th, 2014. a *Abylopsis tetragona* colonies deposited along the tideline at the sandy Getares Beach. b One of the colonies sampled by the students, dyed with methylene blue. Low-resolution pictures taken with a cell phone

RESULTS

The siphonophore samples and pictures from both beaching events were taxonomically identified as *Abylopsis tetragona* (Fig. 2 and 3). The morphological identification of this species is unmistakeable due to the characteristic shape of both the anterior and the posterior nectophores (Bigelow and Sears 1937; Mills et al. 1996). The first beaching event was found both on November 21st and 22nd, 2014 at Getares beach (36°05'30''N, 5°26'37''W; Fig. 1) in the Bay of Algeciras (Fig. 2) during an episode of stormy easterly winds with velocities up to 4.5 m s^{-1}, which lasted 3 days (Fig. 4). Here more than 700 colonies were found deposited along the tideline for about 400 m of the beach (Fig. 2). Siphonophores were found together with the red algae *Asparagopsis armata* Harvey 1855 (Fig. 2). This is a sandy beach, with a typical winter beach profile at the time of the stranding. During fifteen days previous to this episode, continuous westerly winds with velocities up to 4.4 m s^{-1} were registered in the area (Fig. 4b). Simultaneously, the SST satellite images showed cold water in the coastal area of Algeciras Bay, supporting the concept of an upwelling event, while during the strong easterlies warmer water was present in the area (Fig. 6).

The second beaching event was found on January 11th, 2015 at Paloma Baja Beach (36°03'43''N, 5°43'28''W; Fig. 1) in Tarifa during a windless day and just after 3 days of strong easterly winds with velocities up to 5.0 m s^{-1} (Fig. 5). Here an estimated average density of 170 colonies m^{-2} extended for about 550 m along the coastline (Fig. 3) and no other organisms were found. This is a similar sandy beach, with a low profile at the moment of the event. In this case, no westerly winds were registered before the episode (Fig. 5b), however cold water in the coastal area of the beach was observed in the SST satellite images, unlike the warmer water present during the easterlies (Fig. 6). Confirming this pattern, the SST data from Tarifa's buoy showed drops in water temperature during westerlies and increments during easterlies, framed in the general descent pattern due to the seasonal evolution of water temperature (Fig. 5).

In the two cases only polygastric stages (anterior and posterior nectophores) were found but no eudoxids (sexual stage). No other gelatinous or non-gelatinous zooplankton organisms were found beached during the episodes (Figs. 2 and 3). In both cases, stranded colonies were relatively big in size (ca. 3 cm), probably all adult individuals (see Fig. 3).

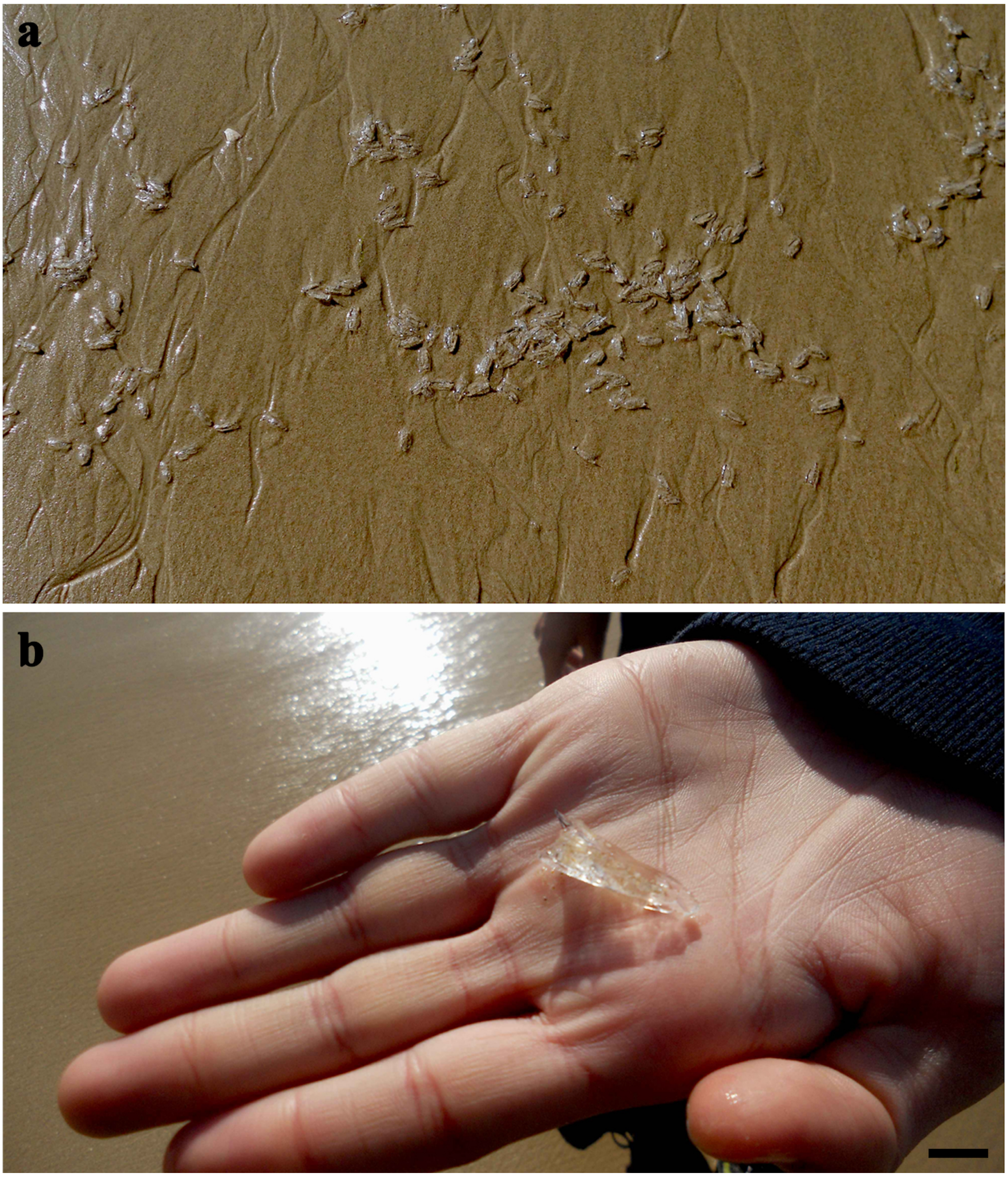

Figure 3 Beaching event at Paloma Baja Beach, Tarifa, on January 11th, 2015. a *Abylopsis tetragona* colonies extended along the coastline of the sandy beach of Paloma Baja. b Detail of one of the beached colonies, with an estimated size of ca. 3 cm, in one of the students' hand (17.5 cm long). Scale bar: b = 1.0 cm

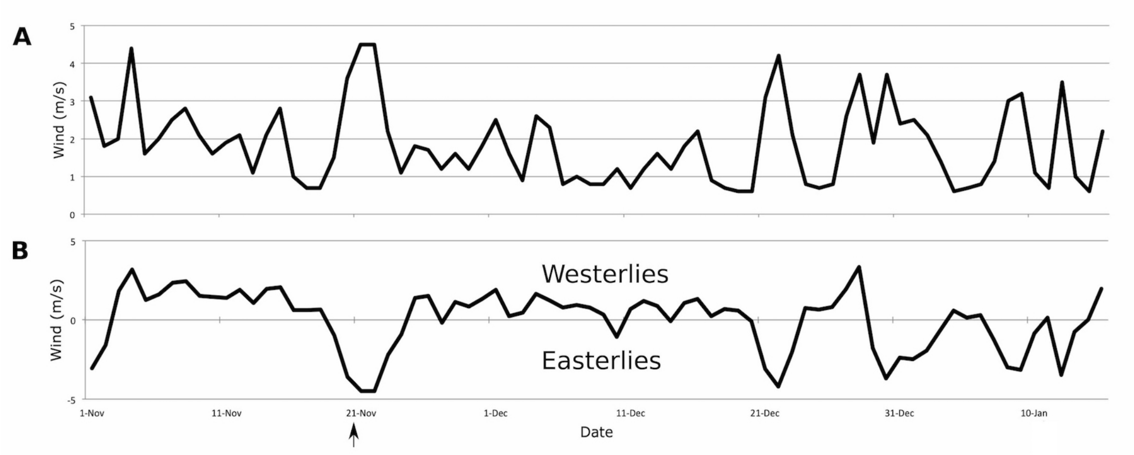

Figure 4 Estepona time series of local winds. a Wind speed expressed as m/s. b zonal component of the wind showing westerlies (positive values) and easterlies (negative values)

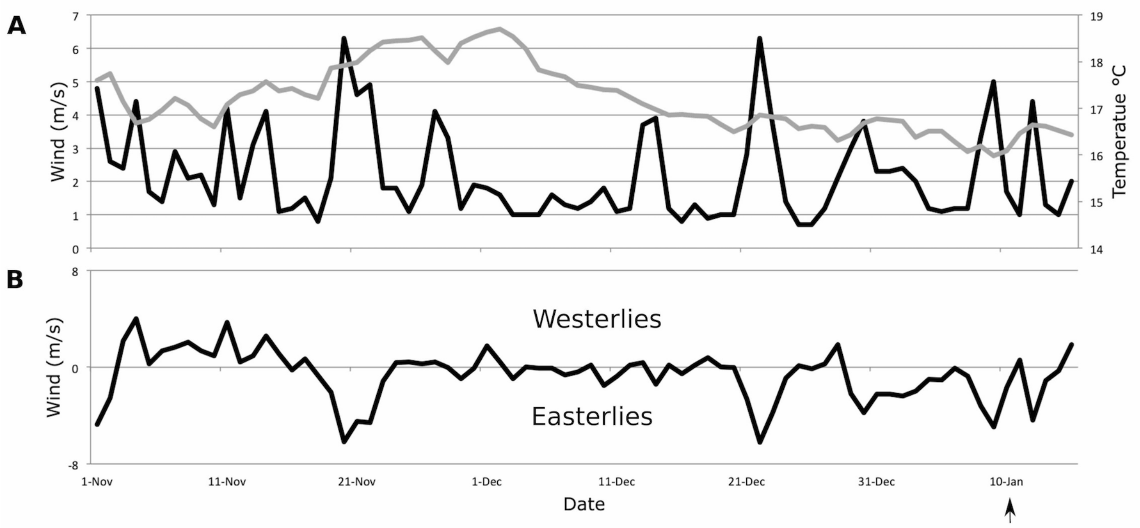

Figure 5 Vejer de la Frontera time series of local winds. a Wind speed expressed as m/s (black line) and sea surface temperature (SST) recorded from the buoy offshore of Tarifa (grey line). b zonal component of the wind showing westerlies (positive values) and easterlies (negative values)

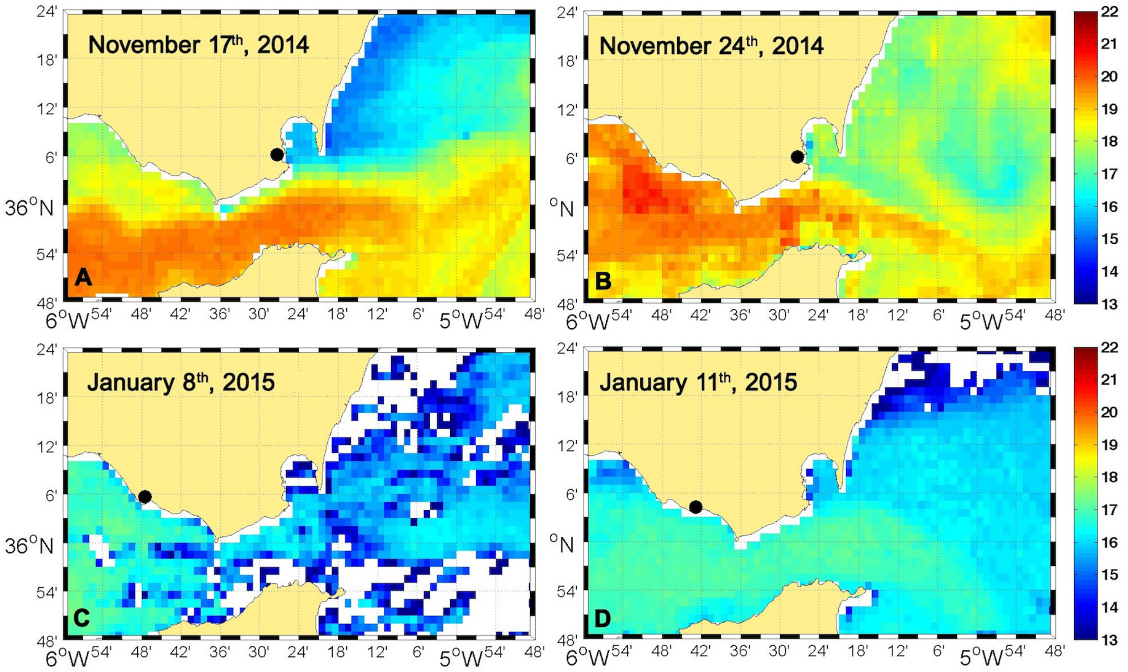

Figure 6 Sea surface temperature (ºC) satellite images (AVHRR sensor, 1 km^2 spatial resolution). a 4 days before the 1st beaching, in Getares Beach (black dot), while continuous westerly winds, showing cold water in the area. b 3 days after the 1st beaching, showing warmer SST after the intense easterly wind. c 3 days before the 2nd beaching event, in Paloma Baja Beach (black dot), showing cold water in the area. d The same day of the 2nd beaching, showing warmer SST after the intense easterly wind

DISCUSSION

To the best of our knowledge, these events are the first records for a calycophoran siphonophore mass stranding. Among siphonophores only the pleustonic Portuguese Man-of-War, *Physalia physalis* (Linnaeus 1758), has been reported stranded on shore (e.g.: Araya et al. 2015; Prieto et al. 2015). Mass stranding events of gelatinous zooplankton organisms along shorelines are a natural and relatively common phenomenon strongly influenced by the direction and energy of prevailing winds and surface currents (Graham et al. 2001). In both cases reported here in the South coast of Spain, *A. tetragona* was found beached during or just after the course of 3 days of strong easterly winds, which might have been one of the main drivers for the stranding of the individuals. In addition, we think that the upwelling episodes detected in the previous days in both areas could have promoted the accumulation of the existing populations of *A. tetragona* throughout the water column to the surface layers and thus facilitated a high density of individuals being washed ashore during the easterly winds. In this context, substantially increased abundance of zooplankton organisms like medusae and copepods has been reported close to the coast during upwellings in other areas (Dunstall et al. 1990; Pagès and Gili 1992).

Recent studies in the area have reported stranding of the scyphomedusan *Rhizostoma luteum* (Quoy and Gaimard 1827) and the pleustonic siphonophore *P. physalis* on both sides of the Strait of Gibraltar (Prieto et al. 2013; Prieto et al. 2015). These two studies concluded that strong westerly winds were responsible for the stranding events in contrast to the easterly winds found to be responsible in the present study. We think the difference between these results could be for two reasons. Firstly, the natural habitat of the stranded animals and secondly, the associated upwelling events previous to the beachings. For the first reason, *A. tetragona* is an epipelagic species presenting coastal populations (Bigelow and Sears 1937; Gili et al. 1987a) thus most probably inhabiting the nearby waters of the beaches where stranded. However, the stranded *P. physalis* and *R. luteum* found in the Strait of Gibraltar area come from the more open-ocean north Atlantic waters pushed by both the dominant currents, towards the Mediterranean, and the westerly winds, towards the southern Iberian coast (Prieto et al. 2013; Prieto et al. 2015). Nevertheless, other jellyfish species like the scyphomedusan *Pelagia notiluca*, when occurring in waters of Algeciras Bay, had also presented stranding events during strong easterly winds episodes on a regular basis (Kienberger, per. obs.). Regarding the second reason, the existence of a high abundance of animals in the surrounding waters at the moment of the stranding is the primordial factor for observing these natural events (Graham et al. 2001; Houghton et al. 2007). In this context, the occurrence of upwelling events in both areas prior to strong easterly winds may be of importance in observing plankton strandlines (Davenport 1995).

Whether these calycophoran beaching events are usual or not in the area of the Strait of Gibraltar and whether they are seasonal episodes we cannot know from the information recorded here. Nevertheless, the observations made by Buecher (1999) in the Bay of Villefranche who found *A. tetragona* essentially in a mixed and cold water column and becoming less numerous in the surface layer when the seasonal thermocline develops, support the fact that the reported events occurred during the winter season, when the water column is homogeneous. Also, the higher frequency of strong and stormy winds during the cold season makes these events potentially seasonal as observed for other gelatinous zooplankton (Graham et al. 2001).

The fact that only polygastric stages (asexual) specimens of *A. tetragona* were found stranded on both beaches is an interesting result that could indicate the composition of overwintering populations of this group of siphonophores in the area (Houghton et al. 2007). Coincident with this result, Dallot et al (1988) found basically polygastric stages on the Atlantic side of and in the Strait of Gibraltar itself during the months of October and November. Furthermore, the main breeding period for this species has been described as occurring during spring in the Mediterranean Sea (Bigelow and Sears 1937; Gili et al. 1987b).

We consider monitoring and recording of cnidarian stranding events of importance, since they may give information on life cycles, seasonality, spatial distributions, and historical occurrence (Houghton et al. 2007; Kienberger and Prieto 2017), especially in highly productive and biologically important areas such as the Strait of Gibraltar (Gómez et al. 2004; Navarro et al. 2011) where zooplanktivorous siphonophores may have a considerable trophic role (Purcell 1982; Pagès et al. 2001). Furthermore, some authors have pointed out the usefulness of documenting mass strandings as an index of future global changes (Flux 2009).

This work highlights the importance of marine and jellyfish scientific knowledge outreach in schools, the existence of projects promoting outreach and also the interaction between researchers, school teachers and students. This promotion of citizen science to increase the ocean literacy of people living near the sea is essential, as well as our sense of responsibility for the ecosystems that sustain us (Boero 2016).

In summary, this study represents the first report of a calycophoran siphonophore mass stranding event. The individual events seemed to be promoted by strong easterly winds, preceded by upwelling episodes that may have concentrated high densities of the siphonophore in superficial layers. This is a good example of the importance of citizen science projects, with cooperation between scientists and society and schools to improve knowledge of regular or exceptional natural phenomena.

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REFERENCES

Alvariño A (1971) Siphonophores of the Pacific with a review of the world distribution. Bull Scripps Inst Ocean 16:1–432.

Andersen V, Sardou J, Nival P (1992) The diel migrations and vertical distributions of zooplankton and micronekton in the Northwestern Mediterranean Sea. 2. Siphonophores, hydromedusae and pyrosomids. J Plankton Res 14:1155–1169. doi: 10.1093/plankt/14.8.1155

Araya JF, Aliaga JA, Araya ME (2015) On the distribution of *Physalia physalis* (Hydrozoa: Physaliidae) in Chile. Mar Biodivers. doi: 10.1007/s12526-015-0417-6

Bigelow HB, Sears M (1937) Siphonophorae. Copenhagen

Boero F (2016) Teaching zoology in the age of machines. Ital J Zool 83:283–284. doi: 10.1080/11250003.2016.1223610

Boero F, Belmonte G, Bracale R, et al (2013) A salp bloom (Tunicata, Thaliacea) along the Apulian coast and in the Otranto Channel between March-May 2013. F1000Research 2:1–7. doi: 10.12688/f1000research.2-181.v1

Boero F, Putti M, Trainito E, et al (2009) First records of *Mnemiopsis leidyi* (Ctenophora) from the Ligurian, Tyrrhenian and Ionian Seas (Western Mediterranean) and first record of *Phyllorhiza punctata* (Cnidaria) from the

Western Mediterranean. Aquat Invasions 4:675–680. doi: 10.3391/ai.2009.4.4.13

Bouillon J, Medel MD, Pagès F, et al (2004) Fauna of the Mediterranean Hydrozoa. Sci Mar 68:438. doi: 10.3989/scimar.2004.68s25

Buecher E (1999) Appearance of *Chelophyes appendiculata* and *Abylopsis tetragona* (Cnidaria, Siphonophora) in the Bay of Villefranche, northwestern Mediterranean. J Sea Res 41:295–307. doi: 10.1016/S1385-1101(99)00005-2

Dallot S, Goy J, Carré C (1988) Peuplements de carnivores planctoniques gélatineux et structures productives en Méditerranée occidentale. Oceanol Acta 193–209.

Davenport J (1995) Upwelling-generated plankton strandlines: important predictable food sources for seabirds at Husvik, South Georgia. Mar Biol 123:207–217. doi: 10.1007/BF00353612

Dunstall TC, Carter CH, Monroe BP, et al (1990) Influence of upwellings, storms, and generating station operation on water chemistry and plankton in the Nanticoke Region of Long Point Bay, Lake Erie. Can J Fish Aquat Sci 47:1434–1445.

Echevarria F, Zabala L, Corzo a., et al (2009) Spatial distribution of autotrophic picoplankton in relation to physical forcings: the Gulf of Cadiz, Strait of Gibraltar and Alboran Sea case study. J Plankton Res 31:1339–1351. doi: 10.1093/plankt/fbp070

Flux JEC (2009) First mass stranding of *Velella velella* in New Zealand. Mar Biodivers Rec 1:e84. doi: 10.1017/S175526720700872X

Gili J-M, Pagès F, Riera T (1987a) Distribución de las especies más frecuentes de sifonóforos calicóforos en la zona norte del Mediterráneo occidental. Investig Pesq 51:323–338.

Gili J-M, Pagès F, Vives F (1987b) Distribution and ecology of a population of planktonic cnidarians in the western Mediterranean. In: Bouillon J, Boero F, Cicogna F, Cornelius PFS (eds) Modern trends in the systematics, ecology, and evolution of hydroids and hydromedusae. Oxford University Press, Oxford, UK, pp 157–170

Gómez F, Gorsky G, García-Górriz E, Picheral M (2004) Control of the phytoplankton distribution in the Strait of Gibraltar by wind and fortnightly tides. Estuar Coast Shelf Sci 59:485–497. doi: 10.1016/j.ecss.2003.10.008

Graham WM, Pagès F, Hamner WM (2001) A physical context for gelatinous zooplankton aggregations: a review. Hydrobiologia 451:199–212.

Greve W (1994) The 1989 German Bight invasion of *Muggiaea atlantica*. ICES J Mar Sci 51:355–358. doi: 10.1006/jmsc.1994.1037

Haddock SHD (2004) A golden age of gelata: past and future research on planktonic ctenophores and cnidarians. Hydrobiologia 530–531:549–556. doi: 10.1007/s10750-004-2653-9

Houghton JDR, Doyle TK, Davenport J, et al (2007) Stranding events provide indirect insights into the seasonality and persistence of jellyfish medusae (Cnidaria: Scyphozoa). Hydrobiologia 589:1–13. doi: 10.1007/s10750-007-0572-2

Kienberger K, Prieto L (2016) The first record of *Drymonema dalmatinum* from the northern Alboran Sea (western Mediterranean). Mar Biodivers 0–1. doi:

10.1007/s12526-016-0620-0

Kienberger K, Prieto L (2017) The jellyfish *Rhizostoma luteum* (Quoy & Gaimard, 1827): not such a rare species after all. Mar Biodivers. doi: 10.1007/s12526-017-0637-z

Lučić D, Benović A, Batistić M, et al (2011) A short-term investigation of diel vertical migrations of the calycophoran Siphonophora in the open south Adriatic Sea (July 2003). Acta Adriat 52:159–172.

Luján M, Crespo-Blanc A, Comas M (2011) Morphology and structure of the Camarinal Sill from high-resolution bathymetry: Evidence of fault zones in the Gibraltar Strait. Geo-Marine Lett 31:163–174. doi: 10.1007/s00367-010-0222-y

Macías D, Bruno M, Echevarria F, et al (2008) Meteorologically-induced mesoscale variability of the North-western Alboran Sea (southern Spain) and related biological patterns. Estuar Coast Shelf Sci 78:250–266. doi: 10.1016/j.ecss.2007.12.008

Macías D, García CM, Echevarría Navas F, et al (2006) Tidal induced variability of mixing processes on Camarinal Sill (Strait of Gibraltar): A pulsating event. J Mar Syst 60:177–192. doi: 10.1016/j.jmarsys.2005.12.003

Macías D, Navarro G, Bartual A, et al (2009) Primary production in the Strait of Gibraltar: Carbon fixation rates in relation to hydrodynamic and phytoplankton dynamics. Estuar Coast Shelf Sci 83:197–210. doi: 10.1016/j.ecss.2009.03.032

Mapstone GM (2014) Global Diversity and Review of Siphonophorae (Cnidaria: Hydrozoa). PLoS One 9:e87737. doi: 10.1371/journal.pone.0087737

Mills CE, Pugh PR, Harbison GR, Haddock SHD (1996) Medusae, siphonophores and ctenophores of the Alboran Sea, south western Mediterranean. Sci Mar 60:145–163.

Navarro G, Ruiz J, Huertas IE, et al (2006) Basin-scale structures governing the position of the deep fluorescence maximum in the Gulf of Cádiz. Deep Sea Res Part II Top Stud Oceanogr 53:1261–1281. doi: 10.1016/j.dsr2.2006.04.013

Navarro G, Vázquez Á, Macías D, et al (2011) Understanding the patterns of biological response to physical forcing in the Alborán Sea (western Mediterranean). Geophys Res Lett 38:1–5. doi: 10.1029/2011GL049708

Pagès F, Gili J-M (1992) Influence of Agulhas waters on the population structure of planktonic cnidarians in the southern Benguela Region. Sci Mar 56:109–123.

Pagès F, González HE, Ramón M, et al (2001) Gelatinous zooplankton assemblages associated with water masses in the Humboldt Current System, and potential predatory impact by *Bassia bassensis* (Siphonophora: Calycophorae). Mar Ecol Prog Ser 210:13–24.

Prieto L, Armani A, Macías D (2013) Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. Mar Biol 160:3241–3247. doi: 10.1007/s00227-013-2293-6

Prieto L, Macías D, Peliz A, Ruiz J (2015) Portuguese Man-of-War (*Physalia physalis*) in the Mediterranean: A permanent invasion or a casual appearance? Sci Rep

5:11545. doi: 10.1038/srep11545

Pugh PR (1989) Gelatinous zooplankton - the forgotten fauna. Prog Underw Sci 14:67–78.

Pugh PR (1999) Siphonophorae. In: Boltovskoy D (ed) South Atlanctic Zooplankton. Backhuys Publishers, Leiden, The Netherlands., pp 467–511

Pugh PR (1974) The vertical distribution of the siphonophores collected during the Sond Cruise, 1965. J mar biol Ass UK 54:25–90.

Purcell JE (1982) Feeding and growth of the siphonophore *Muggiaea atlantica* (Cunningham 1893). J Exp Mar Bio Ecol 62:39–54.

Purcell JE, Arai MN (2001) Interactions of pelagic cnidarians and ctenophores with fish: a review. Hydrobiologia 451:27–44.

Purcell JE, Kremer P (1983) Feeding and metabolism of the siphonophore *Sphaeronectes gracilis*. J Plankton Res 5:95–106. doi: 10.1093/plankt/5.1.95

Sammartino S, Lafuente JG, Garrido JCS, et al (2014) A numerical model analysis of the tidal flows in the Bay of Algeciras, Strait of Gibraltar. Cont Shelf Res 72:34–46. doi: 10.1016/j.csr.2013.11.002

Silvertown J (2009) A new dawn for citizen science. Trends Ecol Evol 24:467–71. doi: 10.1016/j.tree.2009.03.017

3. CONCLUSIONS

GENERAL CONCLUSIONS

Gelatinous organisms are key members of the marine environment and outbreaks are natural phenomena that occur seasonally in many marine ecosystems. The aim of this Doctoral Thesis was to investigate the spatio-temporal of some of the gelatinous organisms occurring in the southern Iberian Peninsula. To achieve this goal, a combination of literature review, field study and experimental works has been performed.

- To disentangle the historical status of *Rhizostoma luteum*

A literature review of *R. luteum* has been undertaken, which included an extended revision of the scientific literature since its first description in 1827 and we reviewed the grey literature (e.g. Underwater Field Guides). Citizen science data (photographic and videographic material) were analysed as far back as 1987. Our results show that *R. luteum* has been systematically misidentified in the past with it sister species from the Mediterranean Sea *Rhizostoma pulmo*, and from the Atlantic Ocean with *Rhizostoma octopus* and *Catostylus tagi*. Hence, until this present Doctoral Thesis *R. luteum* was mostly referred being a rare species, however, our study shows clearly that in the last decades this medusa has been observed regularly (annually since 2007 from the southern Iberian coasts). We suggest that the reasons why this *Rhizostoma* went scientifically unnoticed for more than 60 years are: 1) the confusion over its identity and 2) the lack of scientific studies, rather than an absence of this large jellyfish.

- To define the geographic distribution of *R. luteum*

We were able to update the geographic range to the Bay of Biscay, reaching as far north as Brest (Brittany), to the Coast of Murcia and the Balearic Basin (NW Mediterranean Sea). The most eastern observation was from the Strait of Sicily (southern Italy) this medusa was evidently transported by strong Westerlies. Nonetheless, our results show that the environmental conditions are not optimal for its reproduction in the eastern Mediterranean basin.

Because of the lack of scientific records of this species, the use of citizen science data proved to be a powerful tool to apply to study its geographic range. In an ever-changing environment, and especially in the framework of climate changes, it is important to know which species were/are present in a specific region.

- To reproduce the life cycle of *R. luteum* in the laboratory and describe all development stages of its early life stages

For the first time, the complete life cycle of *R. luteum* has been reproduced in the laboratory. All the early life stages from the planula, to the young medusa have been described and compared to the other species in the same genus (*Rhizostoma pulmo* and *Rhizostoma octopus*). The life cycle resembles that of its congeners, with the distinction that it has the unique features of being a brooding species (internal fertilization with subsequent release of planulae) and the strobilation type observed was monodisc (one ephyra per strobila). Being a brooding species is important as it makes this jellyfish more accessible to culture in captivity as planulae can easily be collected directly from the female medusae in a controlled culture.

- To parameterize environmental control (thermal and salinity) on the early life stages and asexual reproduction of *R. luteum*

Laboratory experiments have been conducted to determine the response of the early life stages to different thermal and salinity concentrations. The results of our experiments suggest that the early life stages can thrive over a wide range of temperature and salinities; this may predispose them to cope better with ongoing environmental changes. Moreover, we concluded that warming oceans conditions might: 1) increase the asexual reproduction rate; 2) displace its distribution range.

In the wild, the benthic and ephyra stages have never been detected. Whereby, this Doctoral Thesis might give a clue regarding where to start searching for these crucial stages. As our results arise the possible occurrence of some or all reproductive stages in estuaries or other brackish environments. Therefore we hypothesize, if it turns out to be really a brackish water jellyfish, the lower precipitation and in the building of dam structures in the last decades, might have favoured the asexual reproduction stages, subsequently, a real increase of this species has occurred.

- To categorize other large jellyfish presented in the Alboran Sea

We identified three other large jellyfish which have been sporadically observed in the northern Alboran Sea which where *Rhizostoma pulmo*, *Catostylus tagi* and *Drymonema dalmatinum*. Our results indicate that *R. pulmo* seems to have been more abundant in the 1990s in the Alboran Sea. *C. tagi* is very abundant during summer on the shores of Portugal, and depending on the dominant currents it might enter occasionally the Alboran Sea via the Strait of Gibraltar. The observation we have reported in this Doctoral Thesis of *D. dalmatinum* is the only scientific record of this species since 1873 made by Haeckel.

- To determine the variability of the coastal proliferation of *Pelagia noctiluca* at high temporal resolution and to characterize its interannual and seasonal cycle at a local and regional scale

During the 54 months (4½ years) of monitoring, the presence of *Pelagia noctiluca* was recorded in almost all the sampling months and its fluctuation was highly variable at an annual and seasonal level. Therefore a clear pattern was not visible. Only the winter months showed a clear lower abundance. In general, summer 2012 and 2015 had a greater abundance of jellyfish then the other three summers (2013, 2014 and 2016). During summer 2012 and 2015 the mauve stinger was present from La Herradura (NE Alboran Sea) during 42 and 54 days, respectively. Versus years with a low abundance only 14, 27 and 9 days (2013, 2014 and 2016, respectively) were recorded. At a regional scale (Alboran Basin) our results suggest the same fluctuations; evidently, the abundance in a particular location depends on the external stress factors. Resulting, that the mauve stinger has been annually present between 2007 and 2016, and in great affluence during summer 2007, 2012 and 2015.

- To identify the environmental variables associated with *P. noctiluca* presence near the coast

During the summer months (2012 and 2015) we correlated the presence of *P. noctiluca* to the predomination of strong westerly winds "*Poniente*", which in this region activate the near coast upwelling mechanism, therefore injecting nutrient-rich water to an oligotrophic system. Additionally, low episodes of easterlies "*Levante*" are needed to push the jellyfish near the coast. Our results indicate that the prerequisite is to have during spring high abundance of *P. noctiluca.* Hence, this might not necessarily mean as well great abundances during summer. Out of 3 springs with a high abundance of *Pelagia*, subsequent only two summers maintained jellyfish near the coast. Summer 2016 with low abundance of jellies had a greater amount of stronger easterly winds, and less westerly.

- To estimate the growth parameters of *P. noctiluca*

We estimated the growth parameter of *P. noctiluca* from the length-frequency (bell diameter) data fitting the von Bertalanffy growth function (VBGF). Monthly length frequency data showed distinct modes during the 4½ year of monitoring with a clear progression of these modes over this time period. Young medusae (< 3.5 cm) were observed between later spring to early autumn (May-October), though, was highly variable between years. Large medusae (> 15 cm) were collected from February to March and then disappeared during the summer months. The predicted extreme length of the stock was estimated to be 17.67 cm this is one of the biggest sizes reported from the western Mediterranean Sea.

- To identify the physical forcing on the mass beaching of *Abylopsis tetragona*

Mass stranding of gelatinous organisms can be correlated to external stress factors like strong easterlies "*Levante*", preceded by upwelling mechanism activated by strong westerlies "*Poniente*" in the Strait of Gibraltar. As it is not realistic to think that scientist can be on spot, during mass beaching events of gelatinous organisms, using citizen science data is an important tool to improve knowledge of regular or exceptional natural phenomena of gelatinous organisms beaching events.

FUTURE RESEARCH

The above-mentioned conclusions led to the following lines for further research:

1. The genus *Rhizostoma* should be revised as there is the necessity to clarified for each species its geographic range, morphological characters and molecular analyses as their might be a cryptic species hiding between the three established species.
2. The scyphistoma (benthic) and ephyra (pelagic state) of the *Rhizostoma luteum* should be found and studied in the wild.
3. The Genus *Drymonema* should be investigated and find some further record of the species *D. dalmatinum* from the southern Iberian if possible should be found.
4. Describing the life cycle for missing species is important as our results showed even in the same genus important differences might appear. Additionally, in the framework of climate change to parameterize the environmental control of all stages of the life cycle is crucial for the most common gelatinous organisms.
5. In general gelatinous organisms should be monitored on a regular basis using opportunistic or specific citizen science project, and systematic, robust monitoring programs using standardized methodologies. Reports of gelatinous organisms might include not just enthusiastic naturalist, but fisherman, offshore aquaculture operators, dive clubs, marine protected area supervisors, lifeguards etc.
6. Citizen science initiatives need to coordinate and inter-share data (data-sharing policy or the use of open source database).
7. In the Alboran Sea, systematic non-coastal observations and monitoring of gelatinous organisms are vital.
8. It is important to keep on up to date database about gelatinous organisms with experts in taxonomy verifying the accurate identification.
9. Collaboration between Institute/University and Zoo facilities should be encouraged as jellyfish aquarists have acquired precious know-how over the years working closely with these species and the use of the zoo facilities and infrastructure is a great benefit.

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Finally to Darius Enayati, without whom, I would not have had the courage to embark on this journey in the first place. Last but not least, I would like to thank my family for their unconditional support.

We can not live on this planet with dead oceans.
If the Oceans Die, We Die!

Paul Watson

APPENDICES

Appendix A

Appendix A ST1 Records of *Rhizostoma luteum*, *Rhizostoma pulmo* and *Catostylus tagi* from 1993 to 2015. Abbreviation: s – swimming; b – beached.

Records of *R. luteum* between 1998 to 2015

Record No.	Record date	Location	Obser-vations	Latitude	Longitude	References
1	October 4, 1998	Peñon de las Caballas, La Herradura, Granada, Alboran Sea	s	36,7261	-3,7250	Present study (photo credit J. C. Moreno Robledo, Red de Vigilantes Marinos)
2	September, 2000	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit M. Campillo)
3	October, 2001	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	Present study (photo credit M. Campillo)
4	October, 2006	La Garita, Tarifa, Strait of Gibraltar	s	35,9972	-5,6122	Present study (photo credit V. Pérez)
5	April 20, 2007	Malaga, Alboran Sea	b	36,6614	-4,3547	http://www.todosurf.com/foro/viewtopic.php?f=1&t=6397
6	July 18, 2007	Caleta de Velez, Malaga, Alboran Sea	b	36,7453	-4,0603	Campaña Medusas - Ministerio de Medio Ambiente
7	September 8, 2007	La Barrosa, Cadiz, Gulf of Cadiz	b	36,3709	-6,1188	Campaña Medusas - Ministerio de Medio Ambiente
8	December 3, 2007	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	Present study (photo credit L. Sanchez Tocino)
9	January 31, 2008	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	Present study (photo credit L. Sanchez Tocino)
10	July 27, 2008	Fuengirola, Malaga, Alboran Sea	s	36,5639	-4,5983	http://www.panoramio.com/photo/19335251
11	July, 2009	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	Present study (photo credit M. Campillo)
12	September, 2009	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	Present study (photo credit M. Campillo)
13	October 4, 2009	Calahonda, Granada, Alboran Sea	s	36,7017	-3,4044	Present study (photo credit A. Exposito)
14	November, 2009	Punta Entinas-Sabinar, Almeria, Alboran Sea	s	36,6631	-2,7000	Present study (photo credit A. Terrón, Asociación Hombre y Territorio)
15	November 15, 2009	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	https://m.flickr.com/#/photos/8783763@N04/4856852420/
16	June 21, 2010	Chorillo, Ceuta, Strait of Gibraltar	s	35,8852	-5,3206	http://elfarodigital.es/ceuta/medio-ambiente/11241-los-barcos-de-limpieza-encuentran-un-aguamala-de-casi-diez-kilos.html
17	June 26, 2010	Fuengirola, Malaga, Alboran Sea	b	36,5639	-4,5983	https://www.youtube.com/watch?v=5lqKUyr2ZCE
18	July 10, 2010	Malaga, Alboran Sea	b	36,6614	-4,3547	http://objetivomalaga.diariosur.es/fotos-JuanjoFernandez/playas-malaga/medusa-gigante-691657.html
19	September 29, 2010	Torrox Costa, Malaga, Alboran Sea	b	36,7283	-3,9442	Campaña Medusas - Ministerio de Medio Ambiente
20	July, 2011	Almuñécar, Granada, Alboran Sea	s	36,7250	-3,6856	Present study (photo credit R. Bermejo Lacida)
21	July 25, 2011	Roquetas del Mar, Almeria, Alboran Sea	s	36,8178	-2,5484	Present study (photo credit A. Perez, Aquatours Almeria)
22	August 7, 2011	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	http://delmontealamar.wordpress.com/2011/09/13/snorkel-en-playa-de-los-berengueles-punta-de-la-mona/
23	August 12, 2011	Aguadulce, Almeria, Alboran Sea	s	36,8176	-2,5458	Present study (photo credit A. Perez, Aquatours Almeria)
24	August 13, 2011	Playa Cabria, Almuñécar, Granada, Alboran Sea	s	36,7250	-3,6856	http://www.surfcastinggranada.com/t1875-foto-de-medusa-en-cabria-almunecar
25	August 30, 2011	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7211	-3,7361	Present study (photo credit J. J. Merlos)
26	September 3, 2011	Roquetas de Mar, Almeria, Alboran Sea	s	36,8178	-2,5484	Present study (photo credit A. Perez, Aquatours Almeria)
27	September 6, 2011	Puerto Santa Maria, Gulf of Cadiz	b	36,5689	-6,2253	http://www.jellywatch.org/node/1054
28	September 11, 2011	Castell de Ferro, Granada, Alboran Sea	s	36,7017	-3,4044	Present study (photo credit J. M. Osorio Rodriguez)
29	October 16, 2011	Cabo Espichel, Setubal, Portuguese Coast	s	38,4425	-9,2203	Present study (photo credit M. Pereira)
30	November 21, 2011	Coast of Granada, Alboran Sea	s	36,7203	-3,7281	https://www.youtube.com/watch?v=FpmBxP3oOnw
31	November 27, 2011	Cabo Espichel, Setubal, Portuguese Coast	s	38,5056	-8,8950	Present study (photo credit S. Martins)
32	June 3, 2012	Almeria, Alboran Sea	b	36,8153	-2,4606	http://almeria360.com/actualidad/03062012_una-medusa-gigante-aterroriza-a-los-banistas-de-una-playa-de-almeria_23446.html
33	June 5, 2012	Agadir, Morocco, NW African Coast	s	30,4309	-9,6519	Prieto et al., 2013
34	June 12, 2012	Doñana National Park, Gulf of Cadiz	b	36,8124	-6,3990	Prieto et al., 2013

Records of *R. luteum* between 1998 to 2015

Record No.	Record date	Location	Obser-vations	Latitude	Longitude	References
35	June 17, 2012	Marbella, Malaga, Alboran Sea	b	36,5044	-4,8660	http://www.laopiniondemalaga.es/malaga/2012/06/18/medusas-gigantes-costa-costa-malaga/513600.html
36	June 17, 2012	Malaga, Alboran Sea	b	36,7214	-4,4019	http://www.laopiniondemalaga.es/malaga/2012/06/19/lluvias-calor-alimento-medusas/513769.html
37	June 17, 2012	Algarrobo, Malaga, Alboran Sea	b	36,7460	-4,0604	http://www.laopiniondemalaga.es/malaga/2012/06/18/medusas-gigantes-costa-costa-malaga/513600.html
38	June 18, 2012	El Puerto de Santa Maria, Gulf of Cadiz	b	36,5769	-6,2277	Prieto et al., 2013
39	June 18, 2012	Caleta de Velez, Malaga, Alboran Sea	s	36,7228	-3,9775	https://www.youtube.com/watch?v=9E-rVu_jmZM
40	June 18, 2012	Torrenueva, Granada, Alboran Sea	s	36,6956	-3,4900	https://www.youtube.com/watch?v=dnv5IJ7Cjio
41	June 18, 2012	Salobraña, Granada, Alboran Sea	b	36,7322	-3,5981	http://waste.ideal.es/medusas-plaga-ideal.htm
42	June 19, 2012	Motril, Granada, Alboran Sea	b	36,6943	-3,4423	http://www.abc.es/20120619/sociedad/abci-medusa-playa-granada-201206191220.html
43	June 20, 2012	Almuñécar, Granada, Alboran Sea	b	36,7250	-3,6856	https://www.youtube.com/watch?v=2zOjX-Te13M
44	June 20, 2012	Fuengirola, Malaga, Alboran Sea	b	36,5639	-4,5983	https://www.youtube.com/watch?v=78d2fU7A_BY
45	June 21, 2012	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	https://www.youtube.com/watch?v=9LfmY5ft2D0
46	June 24, 2012	Nerja, Malaga, Alboran Sea	s	36,7458	-3,8644	Present study (photo credit M. Martinez Peralta)
47	June 24, 2012	Malaga, Alboran Sea	b	36,7214	-4,4019	https://www.youtube.com/watch?v=Jj0shh2iATM
48	June 26, 2012	Marbella, Malaga, Alboran Sea	b	36,4897	-4,9456	https://www.facebook.com/10150127838175354/photos/a.10150454459120354.638245.10150127838175354/10151882101600354
49	June 28, 2012	Marbella, Malaga, Alboran Sea	s	36,5019	-4,9015	Present study (photo credit Y. Zamora Paneca, Observadores del Mar)
50	June 29, 2012	Malaga, Alboran Sea	b	36,7214	-4,4019	https://www.youtube.com/watch?v=dzsi6ma1p_8
51	June 30, 2012	Torremolinos, Malaga, Alboran Sea	b	36,6225	-4,4922	http://www.diariosur.es/20120630/local/malaga/medusa-gigante-aparece-playa-201206301259.html
52	July 1, 2012	Huelin, Malaga, Alboran Sea	b	36,6850	-4,4386	http://objetivomalaga.diariosur.es/fotos-NENE307/medusa-gigante-1196107.html
53	July 1, 2012	Aguadulce, Almeria, Alboran Sea	s	36,8176	-2,5458	Present study (photo credit A. Perez, Aquatours Almeria)
54	July 1, 2012	Torremolinos, Malaga, Alboran Sea	b	36,6226	-4,4929	http://www.diariosur.es/v/20120701/malaga/aparece-medusa-proporciones-gigantes-20120701.html
55	July 3, 2012	Malaga, Alboran Sea	b	36,6614	-4,3547	http://www.diariosur.es/20120703/local/malaga/aparecen-medusas-grandes-dimensiones-201207031704.html
56	July 7, 2012	Marina del Este, La Herradura, Granada, Alboran Sea	b	36,7203	-3,7281	Present study (photo credit J. Peralta)
57	July 7, 2012	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7353	-3,7817	Present study (photo credit R. Carrapiso)
58	July 7, 2012	La Adelfa, Marbella, Malaga, Alboran Sea	b	36,4711	-4,9850	http://www.produnas.org/A/Noticias%202013_12.htm
59	July 8, 2012	Playa Rodeito, Marbella, Malaga, Alboran Sea	b	36,4778	-4,9664	https://www.youtube.com/watch?v=eq3t1Vt9mes
60	July 11, 2012	Roquetas de Mar, Almeria, Alboran Sea	b	36,7617	-2,6053	http://www.albamar.net/?p=1269
61	July 30, 2012	Motril, Granada, Alboran Sea	b	36,7181	-3,5356	https://motrildigital.blogia.com/2012/agosto.php
62	August 15, 2012	Almuñécar, Granada, Alboran Sea	b	36,7250	-3,6856	https://motrildigital.blogia.com/2012/agosto.php
63	January 24, 2013	Cortadura, Cadiz, Gulf of Cadiz	b	36,4947	-6,2726	Prieto et al., 2013
64	January 29, 2013	Cortadura, Cadiz, Gulf of Cadiz	b	36,4947	-6,2726	Prieto et al., 2013
65	February 6, 2013	Doñana National Park, Gulf of Cadiz	b	37,0697	-6,6888	Prieto et al., 2013
66	February 7, 2013	Doñana National Park, Gulf of Cadiz	b	36,9825	-6,5299	Prieto et al., 2013
67	March 31, 2013	Sardina, Gran Canaria, NW African Coast	s	28,1503	-15,7042	Present study (photo credit K. Machin)
68	April 1, 2013	Tenerife, NW African Coast	s	28,2444	-16,6075	Present study (photo credit I. Domingues)

Records of *R. luteum* between 1998 to 2015

Record No.	Record date	Location	Obser-vations	Latitude	Longitude	References
69	April 11, 2013	Mogan, Gran Canaria, NW African Coast	s	27,7133	-15,9122	Present study (photo credit A. Telle Thiemann)
70	September 25, 2013	Bahia de Algeciras, Strait of Gibraltar	s	36,1614	-5,3979	Present study (photo credit J. and M. Longman)
71	October, 2013	Maro-Cerro Gordo, Malaga, Alboran Sea	s	36,7367	-3,7772	Present study (photo credit M. A. Marquez Navarrete)
72	October 2, 2013	Almada, Lisbon, Portuguese Coast	s	38,5611	-9,2167	Present study (photo credit L. Quinta)
73	October 12, 2013	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7183	-3,7339	Present study (photo credit E. Sánchez Castillo)
74	October 14, 2013	Playa Galera, Almuñécar, Granada, Alboran Sea	b	36,7456	-3,6572	Present study (photo credit A. Zipse)
75	October 16, 2013	La Herradura, Granada, Alboran Sea	s	36,7375	-3,7539	Present study (photo credit M. O'Gorman)
76	October 17, 2013	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7353	-3,7817	Present study (photo credit A. Zipse)
77	October 21, 2013	Punta de la Mona, La Herradura, Malaga, Alboran Sea	s	36,7183	-3,7339	Present study (photo credit F. J. Nieto Vera)
78	October 25, 2013	La Herradura, Granada, Alboran Sea	s	36,7316	-3,7454	Present study (own observations)
79	November 1, 2013	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7193	-3,7264	Present study (photo credit M. Defat)
80	November 1, 2013	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit M. Defat)
81	November 2, 2013	La Herradura, Granada, Alboran Sea	b	36,7316	-3,7454	Present study (photo credit A. Jimenez)
82	December 5, 2013	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	b	36,7726	-3,7264	Present study (own observations)
83	December 9, 2013	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7193	-3,7264	Present study (photo credit H. Fernández)
84	January 8, 2014	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7726	-3,7264	Present study (own observations)
85	January 12, 2014	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7726	-3,7264	Present study (own observations)
86	January 13, 2014	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7726	-3,7264	Present study (own observations)
87	January 15, 2014	La Herradura, Granada, Alboran Sea	b	36,7316	-3,7454	Present study (photo credit E. Carbajo Pereda)
88	January 24, 2014	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7385	-3,7858	Present study (photo credit A. Zipse)
89	July 8, 2014	Motril, Granada, Alboran Sea	s	36,7181	-3,5356	Present study (photo credit E. Domínguez Díaz)
90	July 21, 2014	Calahonda, Granada, Alboran Sea	s	36,7017	-3,4044	Present study (photo credit M. Bustos Rodriguez)
91	September 5, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)
92	September 9, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit D. Enayati)
93	September 13, 2014	Maro-Cerro Gordo, Granada, Alboran Sea	s	36,7385	-3,7858	Present study (photo credit B. Waddell)
94	September 18, 2014	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7726	-3,7264	Present study (own observations)
95	September 18, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)
95	September 18, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)

Records of *R. luteum* between 1998 to 2015

Record No.	Record date	Location	Obser-vations	Latitude	Longitude	References
96	September 18, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit V. Costa and J. M. Cabrera)
97	September 20, 2014	La Rijana, Maro-Cerro Gordo, Granada, Alboran Sea	s	36,7367	-3,7772	Present study (photo credit ScubAzul)
98	September 20, 2014	Torrox Costa, Malaga, Alboran Sea	b	36,7283	-3,9442	Present study (photo credit A. Paredes Arroyo)
99	September 21, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit P. Parra)
100	September 21, 2014	Calahonda, Granada, Alboran Sea	s	36,7019	-3,4108	Present study (photo credit M. Bustos Rodriguez and F. Lasarte Romero)
101	September 22, 2014	La Herradura, Granada, Alboran Sea	b	36,7375	-3,7539	Present study (own observations)
102	September 23, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)
103	September 23, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit Buceo La Herradura)
104	September 23, 2014	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	b	36,7726	-3,7264	Present study (own observations)
105	September 29, 2014	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7211	-3,7361	Present study (photo credit A. Connolly, Open Water La Herradura)
106	October 1, 2014	Cortadura, Cadiz, Gulf of Cadiz	b	36,4924	-6,2701	Present study (photo credit R. Jiménez Ramos)
107	October 4, 2014	Isla Tarifa, Strait of Gibraltar	s	35,9972	-5,6122	Present study (photo credit F. J. Zambrana)
108	October 4, 2014	Playa Fabriquilla, Cabo de Gata, Almeria, Alboran Sea	s	36,7356	-2,2186	Present study (photo credit J. L. Pérez Campos)
109	October 4, 2014	Playa Cañuelo, Maro- Cerro Gordo, Malaga, Alboran Sea	s	36,7431	-3,7892	Present study (photo credit C. Hita Suarez)
110	October 23, 2014	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7183	-3,7339	Present study (photo credit A. Zipse)
111	October 24, 2014	Maro-Cerro Gordo, Malaga, Alboran Sea	s	36,7385	-3,7858	Present study (photo credit C. Garcia, Open Water La Herradura)
112	October 26, 2014	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7193	-3,7264	Present study (photo credit C. Garcia, Open Water La Herradura)
113	November 13, 2014	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit P. Parra)
114	November 25, 2014	Fosa de San Felipe, Ceuta, Strait of Gibraltar	s	35,8881	-5,3183	Present study (photo credit E. Alvarez Paz)
115	December 18, 2014	Puerto Deportivo, Ceuta, Strait of Gibraltar	s	35,8917	-5,3136	Present study (photo credit E. Alvarez Paz)
116	December 30, 2014	Playa Cañuelo, Nerja, Malaga, Alboran Sea	s	36,7431	-3,7911	Present study (photo credit A. Zipse)
117	January 11, 2015	La Herradura, Granada, Alboran Sea	s	36,7281	-3,7542	Present study (photo credit A. Zipse)
118	January 14, 2015	La Herradura, Granada, Alboran Sea	s	36,7316	-3,7454	Present study (photo credit A. Zipse)
119	January 15, 2015	Bolonia, Gulf of Cadiz	s	35,0689	-5,7550	Present study (photo credit R. Bermejo Lacida)
120	February 15, 2015	Playa Rinconcillo, Algeciras, Strait of Gibraltar	b	36,1556	-5,4333	Present study (photo credit C. Serrano Lopez, Club de buceo CIES Algeciras)
121	April 3, 2015	La Herradura, Granada, Alboran Sea	s	36,7290	-3,7371	Present study (photo credit S. de la Camara)

Records of *R. luteum* between 1998 to 2015

Record No.	Record date	Location	Obser-vations	Latitude	Longitude	References
122	May 3, 2015	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	b	36,7726	-3,7264	Present study (own observations)
122	May 3, 2015	Puerto Deportivo Marina del Este, La Herradura, Granada, Alboran Sea	b	36,7726	-3,7264	Present study (own observations)
123	August 6, 2015	Los Molinos, Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7500	-3,8128	Present study (photo credit M. Carderelli, Buceo Natura La Herradura)
124	August 9, 2015	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7211	-3,7361	Present study (photo credit L. Belladonna, Buceo La Herradura)
125	August 16, 2015	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit P. Rebola)
126	August 16, 2015	Playa Cañuelo, Nerja, Alboran Sea	s	36,7431	-3,7911	Present study (photo credit M. Campillo)
127	August 26, 2015	Playa Calaiza, La Herradura, Granada, Alboran Sea	s	36,7344	-3,7450	Present study (photo credit A. Zipse)
128	August 27, 2015	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7183	-3,7339	Present study (photo credit P. Y. Losco and A. Paredes Arroyo)
129	September 6, 2015	El Palmar, Conil, Gulf of Cadiz	b	36,2320	-6,0748	Present study (photo credit S. Flecha)
130	September 10, 2015	La Herradura, Granada, Alboran Sea	s	36,7290	-3,7371	Present study (photo credit S. Harris)
131	September 10, 2015	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)
132	September 10, 2015	Isla Cristina, Huelva, Gulf of Cadiz	b	37,1922	-7,3150	Present study (photo credit C. Bordehore)
133	September 10, 2015	El Puerto de Santa Maria, Gulf of Cadiz	b	36,5769	-6,2277	Present study (own observations)
134	September 13, 2015	Sabinillas, La Duquesa, Malaga, Alboran Sea	b	36,3626	-5,2250	Present study (photo credit J. Prieto)
135	September 19, 2015	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (photo credit R. Sanchez)
136	September 19, 2015	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7193	-3,7264	Present study (photo credit P. Sierra)
137	September 20, 2015	Chorrillo, Conil, Gulf of Cadiz	b	36,2788	-6,1010	Present study (own observations)
138	September 20, 2015	La Herradura, Granada, Alboran Sea	s	36,7290	-3,7371	Present study (photo credit M. C. Pardial)
139	September 22, 2015	Playa del Puerco, Cadiz, Gulf of Cadiz	b	36,3344	-6,1643	Present study (own observations)
140	September 25, 2015	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7331	-3,7750	Present study (photo credit A. Avile Tapia)
141	September 25, 2015	Los Lances, Tarifa, Strait of Gibraltar	b	36,1807	-5,6308	Present study (photo credit C. Villaescusa)
142	September 26, 2015	Punta de la Mona, La Herradura, Granada, Alboran Sea	s	36,7193	-3,7264	Present study (photo credit M. Campillo)
143	September 28, 2015	Salobreña, Granada, Alboran Sea	s	36,7394	-3,6222	Present study (photo credit V. Vermeulen)
144	October 4, 2015	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7331	-3,7750	Present study (photo credit F. J. Diaz Masna)
145	October 4, 2015	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7331	-3,7750	Present study (photo credit H. Fernández)
146	October 11, 2015	Barbate, Gulf of Cadiz	s	36,1807	-5,9090	Present study (own observations)
147	October 11, 2015	Maro Cerro-Gordo, Malaga, AlboranSea	s	36,7322	-3,7739	Present study (photo credit D. Romero Nuñez)
148	October 11, 2015	La Herradura, Granada, Alboran Sea	s	36,7308	-3,7442	Present study (photo credit D. Romero Nuñez)
149	October 12, 2015	Maro - Cerro Gordo, Malaga, Alboran Sea	s	36,7331	-3,7750	Present study (photo credit M. Cardarelli, Buceo Natura La Herradura)
150	October 12, 2015	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)
151	October 14, 2015	Tarifa, Strait of Gibraltar	b	35,9972	-5,6122	Present study (photo credit C. Gurrea)
152	October 27, 2015	Marina del Este, La Herradura, Granada, Alboran Sea	s	36,7203	-3,7281	Present study (own observations)

Records of *R. luteum* between 1998 to 2015						
Record No.	**Record date**	**Location**	**Obser-vations**	**Latitude**	**Longitude**	**References**
153	November 14, 2015	La Herradura, Granada, Alboran Sea	s	36,7227	-3.7272	Present study (photo credit R. Camacho, Buceo Natura La Herradura)
154	December 8, 2015	La Herradura, Granada, Alboran Sea	s	36,7290	-3.7371	Present study (photo credit A. Zipse)
155	December 26, 2015	La Herradura, Granada, Alboran Sea	s	36,7227	-3.7272	Present study (photo credit K. Funes)

Records of *R. pulmo* between 1993 and 2012						
Record No.	**Record date**	**Location**	**Obser-vations**	**Latitude**	**Longitude**	**References**
156	June 6, 1993	La Herradura, Granada, Alboran Sea	s	36,7300	-3,7631	Present study (photo credit J. C. Moreno Robledo, Red de Vigilantes Marinos)
157	July, 1997	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	Present study (photo credit M. Campillo)
158	August 22, 1998	Maro - Cerro Gordo, Granada, Alboran Sea	s	36,7494	-3,8167	Present study (photo credit J. C. Moreno Robledo, Red de Vigilantes Marinos)
159	August 7, 2011	Calahonda, Granada, Alboran Sea	s	36,7017	-3,4044	Present study (photo credit J. M. Osorio Rodriguez)
160	January 19, 2012	Doñana National Park, Gulf of Cadiz	b	36,8124	-6,3990	Present study (own observations)
161	August 14, 2012	Doñana National Park, Gulf of Cadiz	b	36,9476	-6,4873	Present study (own observations)

Records of *C. tagi* between 2007 and 2015						
Record No.	**Record date**	**Location**	**Obser-vations**	**Latitude**	**Longitude**	**References**
162	November 29, 2007	Port Lisbon, Portuguese Coast	s	38,7117	-9,1183	https://www.flickr.com/photos/luisa/2074898433/in/photolist-qWCQmJ-qDoBnW-pyLt1m-r2rxcD-qJRLtq-qTPua1-qDFRJz-pY6s4t-oj1suA-okQBAY
163	November 23, 2008	Tagus River, Portuguese Coast	s	38,6272	-9,2475	Present study (photo credit A. Gonçalves)
164	August, 2009	La Herradura, Granada, Alboran Sea	s	36,7319	-3,7553	http://www.forobuceo.com/phpBB3/viewtopic.php?f=9&t=64856&view=next
165	December 4, 2013	Tagus River, Portuguese Coast	s	38,6272	-9,2475	Present study (photo credit M. Hilario)
166	January 9, 2014	Aveiro, Portuguese Coast	s	40,6411	-8,6536	Jellywatch http://jellywatch.org/node/4722
167	August 25, 2014	Isla de Tavira, Portuguese Coast	b	37,1000	-7,6125	Present study (photo credit M. Gil Lopez)
168	August 28, 2014	Lisbon, Portuguese Coast	s	38,6953	-9,1858	http://www.perseus-net.eu/en/jellyfish_map/index.html
169	September 1, 2014	Aveiro, Portuguese Coast	s	40,6411	-8,6536	https://www.inaturalist.org/observations/857063
170	October 6, 2014	Isla Tarifa, Strait of Gibraltar	s	36,0017	-5,6042	Present study (photo credit E. Sánchez Castillo)
171	August 21, 2015	Caparica, Lisbon, Portuguese Coast	b	38,6522	-9,2575	Present study (photo credit M. Pola)

Appendix B

Appendix B ST1. Records of *Rhizostoma luteum* from 2016 to March 2019.

Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
February 1, 2016	Fuengirola, Malaga, NW Alboran Sea	36.5469	-4.6133	30-50	Present study (credit K Funes)
February 1, 2016	La Herradura, NE Alboran Sea	36.7372	-3.7519	<30	Present study (credit A Zipse)
February 2, 2016	Marina del Este, La Herradura, NE Alboran Sea	36.7203	-3.7281	16	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	12	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	17	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	12	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	32	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	19	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	10	Kienberger et al. 2018 (own observations)
February 2, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	15	Kienberger et al. 2018 (own observations)
March 5, 2016	Torregorda, Cadiz, Gulf of Cadiz	36.4619	-6.2542	30-50	Present study (credit C Crespo)
March 13, 2016	Bolonia, Strait of Gibraltar	36.0846	-5.7671	30-50	Present study (credit R Bermejo)
March 13, 2016	Cabrera, Balearic Sea	39.1510	2.9335	NA	Present study (credit C Canovas Perez, Observadors del Mar)
March 30, 2016	La Herradura, Granada, NE Alboran Sea	36.7331	-3.7439	30-50	Present study (credit D Enayati)
March 30, 2016	Marina del Este, La Herradura, NE Alboran Sea	36.7203	-3.7281	52	Present study (own observations)
March 30, 2016	Marina del Este, La Herradura, NE Alboran Sea	36.7203	-3.7281	30-50	Present study (own observations)
April 1, 2016	Bahia Algeciras, Strait of Gibraltar	36.1556	-5.4333	30-50	Present study (credit A Villaescusa)
April 2, 2016	La Herradura, Granada, NE Alboran Sea	36.7316	-3.7454	30-50	Present study (credit Celia Garcia, Open Water La Herradura)
April 2, 2016	La Herradura, Granada, NE Alboran Sea	36.7217	-3.7383	NA	Present study (credit A Zipse)
April 2, 2016	La Herradura, Granada, NE Alboran Sea	36.7290	-3.7371	30-50	Present study (credit Luis Maria VG)
April 2, 2016	La Herradura, Granada, NE Alboran Sea	36.7290	-3.7371	30-50	Present study (credit Luis Maria VG)

Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
April 6, 2016	Bahia Algeciras, Strait of Gibraltar	36.1556	-5.4333	>50	Present study (credit A Villaescusa)
April 14, 2016	Marina del Este, La Herradura, NE Alboran Sea	36.7203	-3.7281	55	Present study (own observations)
April 14, 2016	Marina del Este, La Herradura, NE Alboran Sea	36.7203	-3.7281	68	Present study (own observations)
April 14, 2016	Marina del Este, La Herradura, NE Alboran Sea	36.7203	-3.7281	44	Present study (own observations)
April 17, 2016	Torremolinos, Malaga, NW Alboran Sea	36.6161	-4.4969	30-50	Present study (credit E Pereiro Sanchez)
April 18, 2016	Port Marina de Este, La Herradura,NE Alboran Sea	36.7726	-3.7264	34	Present study (own observations)
April 21, 2016	Rincon de la Victoria, Malga, NW Alboran Sea	36.7136	-4.2831	30-50	Present study (credit E Ramirez Romero)
April 21, 2016	Rincon de la Victoria, Malga, NW Alboran Sea	36.7136	-4.2831	30-50	Present study (credit E Ramirez Romero)
April 24, 2016	Benamadena, Malaga, NW Alboran Sea	36.5719	-4.5883	30-50	Present study (credit K Funes)
April 22, 2016	Almunecar, Granada, NE Alboran Sea	36.7456	-3.6603	30-50	Present study (credit A Sousa)
April 25, 2016	La Herradura, Granada, NE Alboran Sea	36.7331	-3.7439	NA	Present study (credit F Romero Aragon)
April 25, 2016	El Ejido, Almeria, NE Alboran Sea	36.6889	-2.6883	30-50	Present study (credit P Toleano)
May 1, 2016	Marbella, Malaga, NW Alboran Sea	36.4964	-4.8050	30-50	https://www.laopiniondemalaga.es
May 1, 2016	Marbella, Malaga, NW Alboran Sea	36.4964	-4.8050	30-50	https://www.laopiniondemalaga.es
May 1, 2016	Marbella, Malaga, NW Alboran Sea	36.4964	-4.8050	30-50	https://www.laopiniondemalaga.es
May 1, 2016	Marbella, NW Malaga, Alboran Sea	36.5067	-4.8972	30-50	Present study (credit anonymous)
May 7, 2016	Fuerteventura, Canary Basin, Eastern Central Atlantic	28.0790	-15.7170	30-50	http://www.redpromar.com (credit A Ubiema)
May 19, 2016	La Herradura, Granada, NW Alboran Sea	36.7217	-3.7383	30-50	Present study (credit K Funes)
May 20, 2016	Agadir, Morocco, Eastern Central Atlantic	30.4309	-9.6519	30-50	Present study (credit M Pigeolet)
June 2, 2016	Rincon de la Victoria, Malaga, Alboran Sea	36.7136	-4.2831	30-50	Present study (credit E Ramirez Romero)
June 18, 2016	Bahia Algeciras, Strait of Gibraltar	36.1556	-5.4333	>50	Present study (credit A Villaescusa)
June 25, 2016	Bahia Algeciras, Strait of Gibraltar	36.1731	-5.4292	>50	http://www.eltempletelosbarrios.com/ya-ha-llegado-el-verano-y-con-el-las-medusas/
July 8, 2016	Balerma, Almeria, NE Alboran Sea	36.7297	-2.8889	30-50	Present study (credit F Lasarte Romero)
July 24, 2016	La Herradura, Granada, NE Alboran Sea	36.7217	-3.7383	60	Present study (own observations)
August 17, 2016	Tarifa, Strait of Gibraltar	35.9768	-5.6040	>50	Present study (credit N Torres)

Record date	Location	Latitude	Longitude	Diameter	References
August 28, 2016	Nerja, Malaga, NW Alboran Sea	36.7433	-3.8972	>50	https://www.facebook.com/auladelmar/
August 29, 2016	Adra, Almeria, NE Alboran Sea	36.7431	-3.0247	>50	Present study (credit T Navarro)
August 29, 2016	La Mamola. Granada, NE Alboran Sea	36.7456	-3.2778	NA	Present study (credit Vanessa)
September 4, 2016	El Ejido, Almeria, NE Alboran Sea	36.7297	-2.8889	>50	Present study (credit F Maldonado)
September 11, 2016	La Herradura, Granada, NE Alboran Sea	36.7417	-3.7944	Na	Present study (credit S Harris)
September 11, 2016	Conil, Gulf of Cadiz	36.2994	-6.0864	30-50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=6182
September 11, 2016	Conil, Gulf of Cadiz	36.2994	-6.0864	30-50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=6182
September 18, 2016	Tarifa, Strait of Gibraltar	36.0339	-5.6281	>50	Present study (credit A Villaescusa)
September 19, 2016	Adra, Almeria, NE Alboran Sea	36.7431	-3.0247	30-50	Present study (credit L Diaz Fernandez)
September 22, 2016	Santander, Bay of Biscay	43.4772	-3.7850	30-50	http://www.eldiariomontanes.es/santander/201609/22/aparecen-medusas-gigantes-sardinero-20160922131406.html
September 22, 2016	Santander, Bay of Biscay	43.4772	-3.7850	30-50	http://www.eldiariomontanes.es/santander/201609/22/aparecen-medusas-gigantes-sardinero-20160922131406.html
September 22, 2016	Santander, Bay of Biscay	43.4772	-3.7850	30-50	http://www.eldiariomontanes.es/santander/201609/22/aparecen-medusas-gigantes-sardinero-20160922131406.html
September 22, 2016	Santander, Bay of Biscay	43.4647	-3.7783	30-50	http://www.eldiariomontanes.es/santander/201609/22/aparecen-medusas-gigantes-sardinero-20160922131406.html
September 22, 2016	Almunecar, Granada, NE Alboran Sea	36.7292	-3.6958	NA	Present study (credit E Lopez Morales)
September 24, 2016	Chambao de Vincente, Bahia La Herradura, NE Alboran Sea	36.7358	-3.7553	30-50	Present study (credit A Sanchez Contreras)
September 25, 2016	El Palo, Malaga, NW Alboran Sea	36.7175	-4.3592	30-50	Present study (credit S Blanco)
September 26, 2016	Almunecar, Granada, NE Alboran Sea	36.7292	-3.6958	NA	Present study (credit E Lopez Morales)
September 30, 2016	Cala Cortina, Catagena, Coast of Murcia	37.5800	-0.9797	NA	Present study (credit JL Alcaide Sanjurjo)
October 2, 2016	Tarifa, Strait of Gibraltar	36.0339	-5.6281	30-50	Present study (credit A Villaescusa)
October 5, 2016	Torremolinos, Malaga, NW Alboran Sea	36.6203	-4.4950	NA	Present study (credit L Felipe Romero)
October 8, 2016	Nerja, Malaga, NW Alboran Sea	36.7494	-3.8664	30-50	Present study (credit I Perez)
October 8, 2016	Morocco, Southern Alboran Sea, S Alboran Sea	35.4392	-2.9864	30-50	Present study (credit J Gongora Amat)

Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
October 8, 2016	La Herradura, Granada,NE Alboran Sea	36.7189	-3.7272	30-50	Present study (anonymous)
October 9, 2016	La Herradura, Granada, NE Alboran Sea	36.7189	-3.7272	30-50	Present study (credit I Orlovic)
October 15, 2016	Almeria, NE Alboran Sea	36.8182	-2.5430	NA	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=6650
October 16, 2016	Tarifa, Strait of Gibralltar	36.0339	-5.6281	30-50	Present study (credit A Villaescusa)
October 17, 2016	Gijón, Bay of Biscay	43.5414	-5.6542	>50	http://www.elcomercio.es/gijon/201610/20/medusas-para-quitar-hipo-20161020003143-v.html
October 17, 2016	Gijón, Bay of Biscay	43.5414	-5.6542	>50	http://www.elcomercio.es/gijon/201610/20/medusas-para-quitar-hipo-20161020003143-v.html
October 20, 2016	Almeria, NE Alboran Sea	36.8180	-2.5431	30-50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=6729
October 20, 2016	Almeria, NE Alboran Sea	36.8180	-2.5431	30-50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=6729
October 21, 2016	La Herradura, Granada, NE Alboran Sea	36.7417	-3.7944	30-50	Present study (credit P Moller)
October 23, 2016	Mazarron, Coast of Murcia	37.5614	-1.3089	>50	Present study (credit Facebook Me encanta Mayarron)
October 28, 2016	Bahia Algeciras, Strait of Gibraltar	36.1556	-5.4333	30-50	Present study (credit J Perez Escribano)
October 30, 2016	San Sebastian, Bay of Biscay	43.3275	-1.9921	30-50	Present study (credit J Manuel)
October 30, 2016	San Sebastian, Bay of Biscay	43.3275	-1.9956	30-50	Present study (credit J Manuel)
November 2, 2016	San Sebastian, Bay of Biscay	43.3156	-1.9892	30-50	Present study (credit J Agudo Perez)
November 2, 2016	Fuenterrabía, Bay of Biscay	43.3758	-1.7939	NA	Present study (credit JC Mailly)
November 2, 2016	Biarritz, France, Bay of Biscay	43.4847	-1.5589	>50	Present study (credit I Darrieumerlou)
November 2, 2016	San Sebastian, Bay of Biscay	43.3219	-1.9900	NA	Present study (credit Facebook San Sebastian Sailing)
November 4, 2016	Los Burros, Cabo de Gata, Almeria, NE Alboran Sea	36.7483	-2.1117	>50	Present study (credit Y Endo)
November 7, 2016	San Sebastian, Bay of Biscay	43.3236	-2.0164	30-50	Present study (credti A Odriozola Fernandez)
November 14, 2016	Bahia Algeciras, Strait of Gibraltar	36.1587	-5.3587	30-50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=9920

Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
November 18, 2016	Almunecar, Granada,NE Alboran Sea	36.7303	-3.6894	30-50	Present study (credit N Persson)
Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
December 5, 2016	Gijón, Bay of Biscay	43.5456	-5.6669	30-50	https://www.lne.es/gijon/2016/12/05/banco-medusas-rhizostoma-llega-costa/2023766.html
December 5, 2016	Gijón, Bay of Biscay	43.5617	-5.6875	30-50	https://www.lne.es/gijon/2016/12/05/banco-medusas-rhizostoma-llega-costa/2023766.html
December 5, 2016	Gijón, Bay of Biscay	43.5617	-5.6875	30-50	https://www.lne.es/gijon/2016/12/05/banco-medusas-rhizostoma-llega-costa/2023766.html
December 8, 2016	Sesimbra, Portuguese Coast	38.4358	-9.1022	NA	Present study (credit M Pereira)
Spring 2017	Brittany, Bay of Biscay	48.3672	-4.4744	NA	Present study (credit N Djeghri)
January 9, 2017	Playa San Cristobal, Almunecar, NE Alboran Sea	36.7308	-3.6986	>50	Present study (credit Ramona Richter)
January 23, 2017	Aguadulce, Almeria, NE Alboran Sea	36.8154	-2.5597	NA	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=7149
February 9, 2017	Playa Poniente, Motril, NE Alboran Sea	36.7181	-3.5422	30-50	Present study (credit S Bernal)
March 28, 2017	Cabo Tiñoso, Coast of Murcia	37.5339	-1.1147	30-50	Present study (credit Juan Gomez)
April 9, 2017	Playa Carchuna, Motril, Granada, Alboran Sea	36.6950	-3.4400	NA	Present study (photo credit Francisco Lasarte Romero
April 12, 2017	Santa Maria del Mar, Gulf of Cadiz	36.5197	-6.2883	30-50	Anonymous
April 25, 2017	San Telmo, Gran Canaria, Eastern Central Atlantic	28.1089	-15.4144	30-50	Present study (credit Facebook Bajio de San Telmo)
May 6, 2017	Fuerteventura, Canary Basin, Eastern Central Atlantic	28.1503	-14.3577	30-50	http://www.redpromar.com (credit A Ubiema)
May 15, 2017	Puerto Mazarrón, Coast of Murcia	37.5636	-1.2559	NA	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=7582
June 5, 2017	Playa El Zapillo, Almeria Capital, NE Alboran Sea	36.8261	-2.4508	>50	Present study (credit R Alcaraz)
July 10, 2017	La Linea, Bahia Algeciras, Strait of Gibraltar	36.1617	-5.3581	NA	Present study (credit E Moreno)
August 5, 2017	Punta de la Mona, La Herradura, Granada, NE Alboran Sea	36.7217	-3.7383	30	Present study (credit V Vermeule)
August 17, 2017	Camposoto, Cadiz, Gulf of Cadiz	36.4421	-6.2382	>50	Present study (credit JM Espigares)
August 18, 2017	Playa Balneario, Tarifa, Strait of Gibraltar	36.0086	-5.6097	40-50	Present study (credit Divecentre Blue Corner)
August 21, 2017	Azohia, Coast of Murcia	37.5522	-1.1733	30-50	Present study (credit J Murcia Requena)
August 31, 2017	Bolonia, Strait of Gibraltar	36.0872	-5.7742	30-50	Present study (credit R Bermejo)

Record date	Location	Latitude	Longitude	Diameter	References
September 5, 2017	La Calita, La Herradura, Granada, NE Alboran Sea	36.7220	-3.7351	30-50	Present study (credit Erika González Sánchez)
September 23, 2017	Cala de Roche, Conil, Gulf of Cadiz	36.3247	-6.1601	30-50	Present study (credit M Bethencour)
September 23, 2017	Cala de Roche, Conil, Gulf of Cadiz	36.3247	-6.1601	30-50	Present study (credit M Bethencour)
September 23, 2017	Harbour, Gibraltar, Strait of Gibraltar	36.1306	-5.3606	30-50	Present study (credit W Warwick)
September 24, 2017	Playa Victoria, Cadiz, Gulf of Cadiz	36.5171	-6.2859	NA	http://andaluciainformacion.es/sociedad/703186/aparecen-varias-medusas-de-gran-tamano-en-la-playa-victoria/
September 24, 2017	Playa Victoria, Cadiz, Gulf of Cadiz	36.5171	-6.2859	NA	http://andaluciainformacion.es/sociedad/703186/aparecen-varias-medusas-de-gran-tamano-en-la-playa-victoria/
September 24, 2017	Playa Victoria, Cadiz, Gulf of Cadiz	36.5171	-6.2859	NA	http://andaluciainformacion.es/sociedad/703186/aparecen-varias-medusas-de-gran-tamano-en-la-playa-victoria/
September 26, 2017	Gibraltar, Strait of Gibraltar	36.1364	-5.3564	30-50	Present study (credit S Warr)
September 28, 2017	Formentera, Balearic Sea	38.6992	1.3867	30-50	Present study (credit Roberto Pillon)
September 30, 2017	Chiclana, Gulf of Cadiz	36.3684	-6.1825	30-50	Present study (credit A Ruiz Leal)
October 9, 2017	El Ejido, Almeria, NE Alboran Sea	36.7172	-2.8772	>50	Present study (credit I Mulero)
October 10, 2017	Palmones, Bahia Algeciras, Strait of Gibraltar	36.1573	-5.4438	>50	https://www.naturamediterraneo.com/forum/topic.asp?TOPIC_ID=292682
October 14, 2017	La Herradura, Granada, NE Alboran Sea	36.7240	-3.7366	>50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=8890
October 14, 2017	El Rinconcillo, Bahia Algeciras, Strait of Gibraltar	36.1565	-5.4443	>50	Present study (credit H Kerrad Conzalez)
October 14, 2017	Playa Marina del Este, La Herradura, Granada, Alboran Sea	36.1565	-5.4443	30-50	Present study (credit H Fernandez)
October 15, 2017	El Rinconcillo, Bahia Algeciras, Strait of Gibraltar	36.1565	-5.4443	NA	Present study (credit A Villaescusa)
October 15, 2017	Faro, Portuguese Coast	37.0050	-7.9910	>50	Present study (credit PM Cabrera)
October 17, 2017	Torrenueva, Granada, NE Alboran Sea	36.7019	-3.3886	NA	Present study (credit S Sanchez)
October 18, 2017	Biarritz, France, Bay of Biscay	43.4844	-1.5708	>50	Present study (credit M Barrere)
October 20, 2017	Aguadulce, Roquetas del Mar, NE Alboran Sea	36.8150	-2.5617	>50	Present study (credit PROMAR)

Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
October 21, 2017	Los Yesos, Granada, NE Alboran Sea	36.7505	-3.2665	NA	Present study (credit A Carmona)
October 22, 2017	Casares, Malaga, NW Alboran Sea	36.3738	-5.2204	>50	Present study (credit A Villaescusa)
October 24, 2017	Albufeira, Faro, Portuguese Coast	37.0884	-8.1753	NA	https://www.inaturalist.org/observations/8549133
October 29, 2017	Los Catamaranes, Sotogrande, Cadiz, Gulf of Cadiz	36.2872	-5.2732	>50	Present study (credit A Villaescusa)
October 30, 2017	Ibiza, Balearic Sea	38.9861	1.2950	30-50	Present study (credit V Nunez)
November 2, 2017	Punta del Boqueron, San Fernando, Cadiz, Gulf of Cadiz	36.3951	-6.2183	NA	Present study (credit D Iguña)
November 5, 2017	La Herradura, Granada, NE Alboran Sea	36.7220	-3.7351	>50	http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=3&id=8720
November 10, 2017	Tanger, Morroco, Strait of Gibraltar	35.7467	-5.9444	>50	Present study (credit N Tamsouri)
December 9, 2017	Pardor de Mazagon, Gulf of Cadiz	37.1103	-6.7722	30-50	Present study (credit F Bravo Guerrero)
December 10, 2017	El Rinconcillo, Bahia Algeciras, Strait of Gibraltar	36.1565	-5.4443	>50	Present study (credit A Villaescusa)
January 20, 2018	Aguadulce, Almeria, Alboran Sea	36.8097	-2.5694	>50	Present study (credit ML Rodriguez Murillo)
March 13, 2018	Playa Marina del Este, La Herradura, Granada, Alboran Sea	36.7231	-3.7278	41	Present study (own observations)
March 20 2018	Aguadulce, Almeria, Alboran Sea	36.8097	-2.5694	>50	Present study (credit L Fernandez)
March 28, 2018	Aguadulce, Almeria, Alboran Sea	36.8097	-2.5694	NA	Present study (credit L Avi)
August 30, 2018	Playa Velilla, Almunecar, Granada, Alboran Sea	36.7292	-3.6958	>50	Present study (credit A Aybar Ramirez)
September 9, 2018	Famara, Lanzarote, Eastern Central Atlantic	29.1194	-13.5647	>50	http://www.lancelotdigital.com/lanzarote/aparece-una-aguaviva-de-17-kilos-en-famara
September 12, 2018	Playa Getares, Bahia Algeciras, Strait of Gibraltar	36.1664	-5.4374	>50	https://www.europasur.es/comarca/Medusas-gigantes-Getares_0_1282671905.html
September 18, 2018	Chipiona, Gulf of Cadiz	36.7467	-6.5489	>50	Present study (credit Jose Blanco)
September 21, 2018	Playa de la Calzada, Sanlucar, Gulf of Cadiz	36.7891	-6.3589	30-50	Present study (credit A Villaescusa)
September 21, 2018	Playa de la Calzada, Sanlucar, Gulf of Cadiz	36.7891	-6.3589	30-50	Present study (credit A Villaescusa)

Record date	Location	Latitude	Longitude	Diameter	References
September 21, 2018	Playa de la Calzada, Sanlucar, Gulf of Cadiz	36.7891	-6.3589	30-50	Present study (credit A Villaescusa)
September 23, 2018	Ondarreta, San Sebastian, Bay of Biscay	43.3173	-2.0020	30-50	https://www.diariovasco.com/san-sebastian/aparecen-medusas-orilla-20180923010314-ntvo.html
September 23, 2018	Ondarreta, San Sebastian, Bay of Biscay	43.3173	-2.0020	30-50	https://www.diariovasco.com/san-sebastian/aparecen-medusas-orilla-20180923010314-ntvo.html
September 23, 2018	Chiclana, Gulf of Cadiz	36.3344	-6.1643	>50	Present study (credit A Ortega)
September 29, 2018	Palmar, Gulf of Cadiz	36.2320	-6.0748	>50	Psesent study (credit I Vendoiro)
September 30, 2018	Gibraltar, Strait of Gibraltar	36.1190	-5.3513	NA	Present study (credit The Nautilus Project-Gibraltar)
September 30, 2018	Sesimbra, Portuguese Coast	38.4833	-9.1920	30-50	Present study (credit R Germano)
October 6, 2018	El Rinconcillo, Algeciras, Strait of Gibraltar	36.1565	-5.4443	NA	Present study (credit A Villaescusa)
October 6, 2018	El Puerto de Santa Maria, Gulf of Cadiz	36.5212	-6.2871	NA	Present study (credit N Las Flores)
October 13, 2018	Conil, Gulf of Cadiz	36.2922	-6.1121	>50	Present study (credit A Villaescusa)
October 15, 2018	Biarritz, France, Bay of Biscay	43.4831	-1.5586	30-50	Present study (credit T Masse)
October 20, 2018	Playa de La Alcaidesa, San Roque, NW Alboran Sea	36.2329	-5.3139	30-50	Present study (credit A Villaescusa)
October 24, 2018	Alvor, Lagos, Portuguese Coast	37.1314	-8.6128	30-50	https://www.inaturalist.org/observations/17819461
Octobre 26, 2018	Sesimbra, Portuguese Coast	38.4833	-9.1920	30-50	Present study (credit R Germano)
December 23, 2018	Maro-Cerro Gordo, NE Alboran Sea	36.7385	-3.7858	NA	Present study (credit J Ortiz Duarte)
January 17, 2019	Illa de Arousa, Pontevedra	42.5561	-8.8683	>50	Present stuedy (credit P Diosramos)
January 17, 2019	Gibraltar, Strait of Gibraltar	36.1393	-5.3573	30-50	Present study (credit The Nautilus Project-Gibraltar)
January 17, 2019	Gibraltar, Strait of Gibraltar	36.1393	-5.3573	30-50	Present study (credit The Nautilus Project-Gibraltar)
January 17, 2019	Gibraltar, Strait of Gibraltar	36.1393	-5.3573	30-50	Present study (credit The Nautilus Project-Gibraltar)
January 18, 2019	Playa de la Misericordia, Malaga, NW Alboran Sea	36.6914	-4.4400	30-50	https://www.facebook.com/search/str/aula+del+mar+málaga+rhizostoma/keywords_search?epa=SEARCH_BOX
January 18, 2019	Playa de la Misericordia, Malaga, NW Alboran Sea	36.6914	-4.4400	30-50	https://www.facebook.com/search/str/aula+del+mar+málaga+rhizostoma/keywords_search?epa=SEARCH_BOX

Record date	**Location**	**Latitude**	**Longitude**	**Diameter**	**References**
January 18, 2019	Playa Rinconcillo Algeciras, NW Alboran Sea	36.1565	-5.4443	NA	Present study (credit A Villaescusa)
January 19, 2019	Gibraltar, Strait of Gibraltar	36.1217	-5.3527	30-50	Present study (credit The Nautilus Project-Gibraltar)
January 21, 2019	Gibraltar, Strait of Gibraltar	36.1286	-5.3585	NA	Present study (credit The Nautilus Project-Gibraltar)
February 8, 2019	Gibraltar, Strait of Gibraltar	36.1286	-5.3585	30-50	Present study (credit The Nautilus Project-Gibraltar)
February 12, 2019	Rota, Gulf of Cadiz	36.695573	-6.425878	>50	Present study (credit anonymous)
February 15, 2019	Dakar, Senegal, Eastern Central Africa	14.7167	-17.4675	>50	Present study (credit F Linardon)
March 7, 2019	La Herradura, Granada,NE Alboran Sea	36.7189	-3.7272	30-50	Present study (credit M Cardarelli, Buceo Natura)

Appendix C

Appendix C S1 Dataset. Settlement preferences of *Rhizostoma luteum* planulae. *BOTT* bottom of the glass flasks: *GS* glass slides; *LAT* sides of glass flasks.

Flask N°	Location	N° Scyphistoma
1	BOTT	3
1	LAT	2
1	LAT	1
1	LAT	0
2	BOTT	0
2	BOTT	0
2	BOTT	1
2	LAT	3
2	LAT	0
2	LAT	1
2	GS	0
2	BOTT	2
3	LAT	10
3	LAT	4
3	GS	1
4	BOTT	2
4	BOTT	2
4	BOTT	1
4	GS	0
4	BOTT	1

Appendix C S2 Dataset. *Rhizostoma luteum* scyphistoma, podocyst and ephyra count. Variable list: Initial (before temperature drop), Time (Weeks 8, 11 and 16).

Flask N°	**#1**			**#2**			**#3**			**#4**		
	Scyphistoma	Podocyst	Ephyra	Scyphistoma	Podocyst	Ephyra	Scyphistoma	Podocyst	Ephyra	Scyphistoma	Podocyst	Ephyra
Initial	9			3			8			11		
8 weeks	3	32	0	8	34	3	11	72	11	4	10	1
11 weeks	5	38	1	9	37	6	13	52	19	5	13	1
16 weeks	6	45	4	7	36	12	15	na	30	6	16	3

Control	**#1**			**#2**			**#3**		
	Scyphistoma	Podocyst	Ephyra	Scyphistoma	Podocyst	Ephyra	Scyphistoma	Podocyst	Ephyra
Initial	4			2			2		
8 weeks	7	0	0	7	0	0	3	0	0
11 weeks	7	2	0	9	8	1	5	4	1
16 weeks	6	8	6	7	8	5	6	8	5

Appendix C S3 Dataset. Morphometric measures of *Rhizostoma luteum* ephyra**.** *TBD* total body diameter, *CDD* central disc diameter, *TMLL* total marginal lappet length, *ML* manubrium length.

Days	TBD	CDD	TMLL	ML
1	4436	2461	1165	1045
1	3195	1758	777	na
1	2897	1707	609	489
1	3893	1927	1081	na
1	4640	2360	1133	766
1	4447	2423	1226	548
1	3573	2143	808	714
2	5950	3243	1374	1176
2	3661	2107	868	na
2	3380	1952	881	523
2	5452	2841	1417	933
2	5093	2651	1308	853
2	5308	2985	1317	648
2	5187	3218	1277	1056
3	5975	3604	1293	1196
3	4563	2656	1074	593
3	4556	2865	823	885
3	5493	3622	1111	1253
3	5987	3503	1500	1233
3	6164	3869	1207	1300
3	5734	3517	1195	924
4	6750	4120	1212	1391
4	4859	2911	1028	1302
4	4889	3654	1121	1119
4	6682	4466	1083	1188
4	6852	4367	1526	1663
4	7011	4490	1439	1491
4	5828	3991	1107	na
5	7360	5057	1316	1908
5	5193	3447	930	1737
5	5848	3989	na	1324
5	7469	5117	1119	1791
5	7170	4582	1468	1257
5	8129	5407	1391	na
5	6721	4502	1271	na
6	7530	5469	1113	1965
6	5780	4051	1355	2534
6	6437	4365	1174	1565
6	8162	5760	na	2262
6	7197	4707	1425	2449
6	7779	5174	1314	2247
6	7506	5511	1393	2062

7	7728	6159	1096	3131
7	5693	4107	855	2304
7	6513	4492	1150	2130
7	7885	6268	797	2124
7	7438	5788	1060	2745
7	8000	6784	na	2479
7	8074	5802	1516	2434
8	8800	6644	na	2337
8	6367	4443	1441	2451
8	7344	5386	1234	2108
8	9000	7100	1334	1933
8	8049	5765	1309	2520
8	9000	7217	1212	na
8	9000	6794	1174	2819
9	11000	8000	1484	3251
9	7583	5674	1160	2953
9	6784	5347	na	na
9	9500	7500	1151	na
9	8385	6158	1260	2931
9	9400	7085	na	na
9	9100	7000	1250	na
10	11500	9000	1484	3401
10	8000	6129	na	na
10	7537	5499	1027	2418
10	10500	10000		na
10	9500	7000	1533	3470
10	10500	9500	1392	na
11	12500	10500	1250	na
11	8000	6200	1224	3717
11	12000	12500	1179	3518
11	9500	7000	1178	3555
11	11500	10000	1253	na
11	12000	11500	na	na
12	13000	11500	1188	3419
12	9000	7500	1115	3374
12	12500	11500	802	
12	10000	8500	1250	3652
12	12500	11000	na	na
13	14000	12000	1020	na
13	11000	9000	1083	na
13	13000	12000	na	na
13	12000	9000	1355	na
13	13000	11000	na	na
14	11500	10000	1279	4133
14	14000	13000	1085	3850
14	11500	10000	1214	3532

14	13500	12000
15	12000	9000
15	16000	13500
15	14000	12000
15	13500	13000
16	13000	11500
16	11000	10000
16	16500	15000
16	14000	12000
16	14000	12500
17	13500	12000
17	12500	11500
17	16500	15000
17	15000	13000
17	15000	15500
18	14000	13000
18	12500	12000
18	16500	16000
18	15000	13000
18	16500	16000
18	17000	18000
19	15000	14000
19	14000	13000
19	18000	17500
19	15000	13000
19	18500	16000
19	19000	19000
20	15500	14500
20	14500	14000
20	18000	18000
20	15000	14000
20	20000	20000
21	15500	14500
21	14000	14000
21	20500	20000
21	18000	16500
21	20000	20000
21	20000	20000

Appendix C S4 Dataset. Morphological measures of young medusa *Rhizostoma luteum*. *TOAL* total oral arm length, *UOAL* unfrilled oral arms length, *FOAL* frilled oral arms length.

Days	Wet Weight (g)	Bell size (cm)	TOAL (mm)	UOAL (mm)	FOAL (mm)
56	58.9	9.9	45	33	11
56	36.2	8.2	31	24	14.83
56	43.1	8.5	31	17	11.2
56	47.9	8.6	35	24	16.5
56	34.7	8.1	29	20	12.4
103	270.7	16.3	84	54	32
103	133.2	10.6	67	50	37.2
103	177.2	12.6	76	66	39.75
109	241.8	16.4	na	na	45
109	153.2	11.4	72	56	38.33
109	147.2	12.5	78	66	34.75
109	147.1	13.1	75	64	26.6

Bell size	2 month	3 month
mean	8.66	13.27
max	9.90	16.40
min	8.10	10.60
SD	0.72	2.26

Wet Weight	2 month	3 month
mean	44.16	181.49
max	58.90	270.70
min	34.70	133.20
SD	9.81	53.39

Appendix D

Appendix D ESM 1 *Rhizostoma luteum* early life stages and statistical results.

ANOVA tested for differences among temperature (T), salinity (S), and their interaction (T x S). Statistical tests were two way ANOVA (F statistic) or Kruskal-Wallis one way ANOVA on Ranks (H statistic) on early life stages. Different letters (a, b, c) indicate significantly different groups determined by multiple comparison procedure (Tukey's HSD), Bonferroni pair-wise differences are indicated by uppercase letters (A-C) for temperature and by lowercase letters (a-c) for salinity. *n* = 6, 12, 9 and 9 replicates at 14, 18, 23 and 28°C, respectively.

Salinity	Temperature				Test statistic	*P* value
	14°C	18°C	23°C	28°C		
Planula mean settlement time (weeks)						
24	4.67±0.57 c	3.00±0.00 b	1.00±0.00 a	1.33±0.57 a	T $F_{3,32}$=129.939	**T *P*<0.0001**
30	5.33±0.57 c	3.33±0.57 b	2.33±0.57 ab	1.66±0.57 a	S $F_{3,32}$=23.758	**S *P*<0.0001**
36.5	4.33±0.57 c	3.00±0.00 b	1.33±0.57 a	2.00±0.00 ab	TxS $F_{9,32}$=2.263	**TxS *P*=0.0433**
38	6.67±0.57 b	3.67±0.57 a	3.00±0.00 a	2.67±0.57 a		
Mean survival of scyphistomae						
24	6.67±15.11 ab	19.00±27.68 b	13.56±12.24 b	-4.00±3.32 a	T $F_{3,128}$=5.334	**T *P*=0.0017**
30	0.67±2.07 a	3.17±4.47 a	3.33±2.01 a	-3.33±2.45 a	S $F_{3,128}$=9.588	**S *P*<0.0001**
36.5	2.01±6.57 a	1.33±3.23 a	2.44±2.96 a	-1.11±1.45 a	TxS $F_{9,128}$=2.176	**TxS *P*=0.0277**
38	-0.33±1.97 a	-0.67±2.99 a	-3.56±4.45 a	-1.78±2.11 a		
Mean size of fully grown scyphistomae (mm)						
24	0.96±0.02 B	1.40±0.19 B	1.67±0.36 A	1.51±0.28 AB	T H_3=18.775	**T *P*=0.0003**
30	NA	1.25±0.05 B	1.55±0.18 A	NA	S H_3=3.828	S NS
36.5	1.21±0.22 B	1.32±0.28 B	1.52±0.49 A	NA		
38	NA	1.19±0.21 B	1.40±0.24 A	NA		
Mean total no. of podocysts produced at the end of the experiment						
24^{a}	0±0 B	2.50±3.68 AB	3.78±3.87 A	0.78±1.39 B	T H_3=23.307	**T *P*<0.0001**
30^{ab}	0±0 B	0.75±1.14 AB	3.33±6.54 A	0.67±2.00 B	S H_3=10.892	**S *P*=0.0123**
36.5^{ab}	1.5±1.97 B	1.50±2.47 AB	1.44±1.24 A	0.11±0.33 B		
38^{b}	0±0 B	0.33±0.78 AB	2.33±4.31 A	0±0 B		
Mean total no. of ephyrae produced						
24^{a}	0±0 B	0.50±0.90 AB	2.11±1.90 A	0±0 B	T H_3=19.341	**T *P*=0.0002**
30^{ab}	0±0 B	0.33±0.65 AB	0.44±0.73 A	0±0 B	S H_3=13.599	**S *P*=0.0035**
36.5^{b}	0±0 B	0.17±0.39 AB	0.11±0.33 A	0±0 B		
38^{b}	0±0 B	0.08±0.29 AB	0±0 A	0±0 B		
Mean size ephyrae (mm) new released						
24		4.08±0.29	3.82±0.49		T $F_{1,25}$=1.616	T NS
30		4.01±0.01	3.67±0.50		S $F_{2,25}$=1.220	S NS
36.5		4.36±0.33	4.43±0.01		TxS $F_{2,25}$=0.179	TxS NS
38						

Statistical test for 28°C had low power because of small sample sizes (high mortality with the consequent lost of replicates per treatment) and results should be interpreted cautiously. *P*<0.05 is considered significantly different, bold *P* values are significant, *NA* not applicable, *NS* not significant difference (*P*>0.05).

Appendix D ESM 2 *Rhizostoma luteum* early life stages and statistical results.

Kruskal-Wallis one way ANOVA on Ranks (H statistic) tested for differences among temperature (T), salinity (S), and (L) location. Bonferroni pair-wise differences are indicated by uppercase letters (A-C) for temperature and by different lowercase letters (a-c) for salinity. *n* = 6, 12, 9 and 9 replicates at 14, 18, 23 and 28°C, respectively.

Salinity	Temperature				Test statistic	*P* value
	14°C	18°C	23°C	28°C		
Mean no. of scyphistomae per location						
BOTT a	3.14±2.32 AB	6.06±5.12 A	3.57±2.89 B	1.57±0.79 B	T H_3=13.103	**T *P*=0.0044**
GS ab	13.50±16.26AB	6.17±7.72 A	2.00±1.89 B	NA	S H_3=18.469	**S *P*=0.0003**
LAT b	NA	3.43±4.79 A	1.78±1.30 B	1.50±0.71 B	L H_2=8.4431	**L *P*=0.0147**
	BOTT	**GS**	**LAT**			
24^{a}	6.45±5.25	9.00±9.42	3.44±4.13			
30^{ab}	4.00±2.92	2.57±2.51	1.60±1.34			
36.5^{b}	2.78±2.48	1.25±0.50	1.00±0.01			
38^{b}	2.0±1.15	1.8±0.84	NA			

Statistical test for 28°C had low power because of small sample sizes (high mortality with the consequent lost of replicates per treatment) and results should be interpreted cautiously. $P<0.05$ is considered significantly different, bold *P* values are significant, *BOTT* bottom of glass flask, *GS* glass slide, *LAT* sides of glass flask, *NA* not applicable, *NS* not significant difference ($P>0.05$).

Appendix ESM 3 *Rhizostoma luteum* early life stages and statistical results.

ANOVA tested for differences among temperature (T), salinity (S), and their interaction (T x S). Statistical tests were two way ANOVA (F statistic) or Kruskal-Wallis one way ANOVA on Ranks (H statistic) on early life stages. Different letters (a, b, c) indicate significantly different groups determined by multiple comparison procedure (Tukey's HSD), Bonferroni pair-wise differences are indicated by uppercase letters (A-C) for temperature and by lowercase letters (a-c) for salinity. *n* = 5 replicates.

Salinity	Temperature				Test statistic	*P* value
	14°C	18°C	23°C	28°C		
7-days post-liberation						
			Mean development stage			
24	2.0±0.0 a	NA	4.6±0.5 b	2.0±0 a	T $F_{3,37}$=82.930	**T *P*<0.0001**
33	1.0±0.0 a	3.2±0.5 c	4.6±0.5 d	2.4±0.9 b	S $F_{2,37}$=37.316	**S *P*<0.0001**
38	0.6±0.5 a	1.0±0.0 a	NA	1.0±0.0 a	TxS $F_{4,37}$=6.229	**TxS *P*=0.0006**
			Mean TBD (mm)			
24	8.16±0.14 b	NA	8,25±0.74 b	5.34±0.62 a	T $F_{3,38}$=42.948	**T *P*<0.0001**
33	5.93±0.61 a	7.55±0.66 b	8.47±0.76 b	5.15±0.70 a	S $F_{2,38}$=53.120	**S *P*<0.0001**
38	4.46±0.41 ab	5.24±0.34 b	NA	3.80±0.90 a	TxS $F_{4,38}$=7.060	**TxS *P*=0.0002**
14-days post-liberation						
			Mean development stage			
24	4.0±0.0 a	NA	5.6±0.5 b	4.5±0.6 ab	T $F_{3,36}$=23.484	**T *P*<0.0001**
33	2.4±0.9 a	4.6±0.5 bc	5.6±0.5 c	3.8±1.6 b	S $F_{2,36}$=42.972	**S *P*<0.0001**
38	0.8±0.5 a	3.7±0.6 b	NA	0.5±1.0 a	TxS $F_{4,36}$=3.654	**TxS *P*=0.0134**
			Mean TBD (mm)			
24	10.50±0.50 b	NA	11.26±2.12 b	7.06±0.67 a	T $F_{3,33}$=42.231	**T *P*<0.0001**
33	8.45±0.96 b	10.30±0.76 b	13.70±1.79 c	6.08±1.41 a	S $F_{2,33}$=19.867	**S *P*<0.0001**
38	5.87±0.28 ab	8.00±0.50 b	NA	3.87±3.87 a	TxS $F_{4,33}$=5.014	**TxS *P*=0.0029**
21-days post-liberation						
			Mean development stage			
24	6.0±0.0 a	NA	6.8±0.4 b	NA	T $F_{2,26}$=59.161	**T *P*<0.0001**
33	4.4±0.9 a	5.0±0.0 a	6.8±0.4 b	NA	S $F_{2,26}$=81.376	**S *P*<0.0001**
38	1.2±0.4 a	5.3±0.6 b	NA	NA	TxS $F_{2,26}$=42.971	**TxS *P*<0.0001**
			Mean TBD (mm)			
24	13.60±1.19 a	NA	15.80±4.13 a	NA	T $F_{2,26}$=20.251	**T *P*<0.0001**
33	11.5±1.50 a	14.00±0.93 a	19.90±2.33 b	NA	S $F_{2,26}$=17.346	**S *P*<0.0001**
38	6.28±0.27 a	10.50±0.87 b	NA	NA	TxS $F_{2,26}$=7.106	**TxS *P*=0.0034**
Mean days until metaephyra stage						
24	32.2±1.6 A	NA	18.4±2.5 C	NA	T H_2=19.436	**T *P*<0.0001**
33	35.7±1.5 A	29.6±1.3 B	20.2±1.8 C	NA	S H_1=1.005	S NS
38	NA	NA	NA	NA		

TBD total body diameter, *NS* non-significant difference (*P*>0.05), bold *P* values are significant, *NA* not applicable