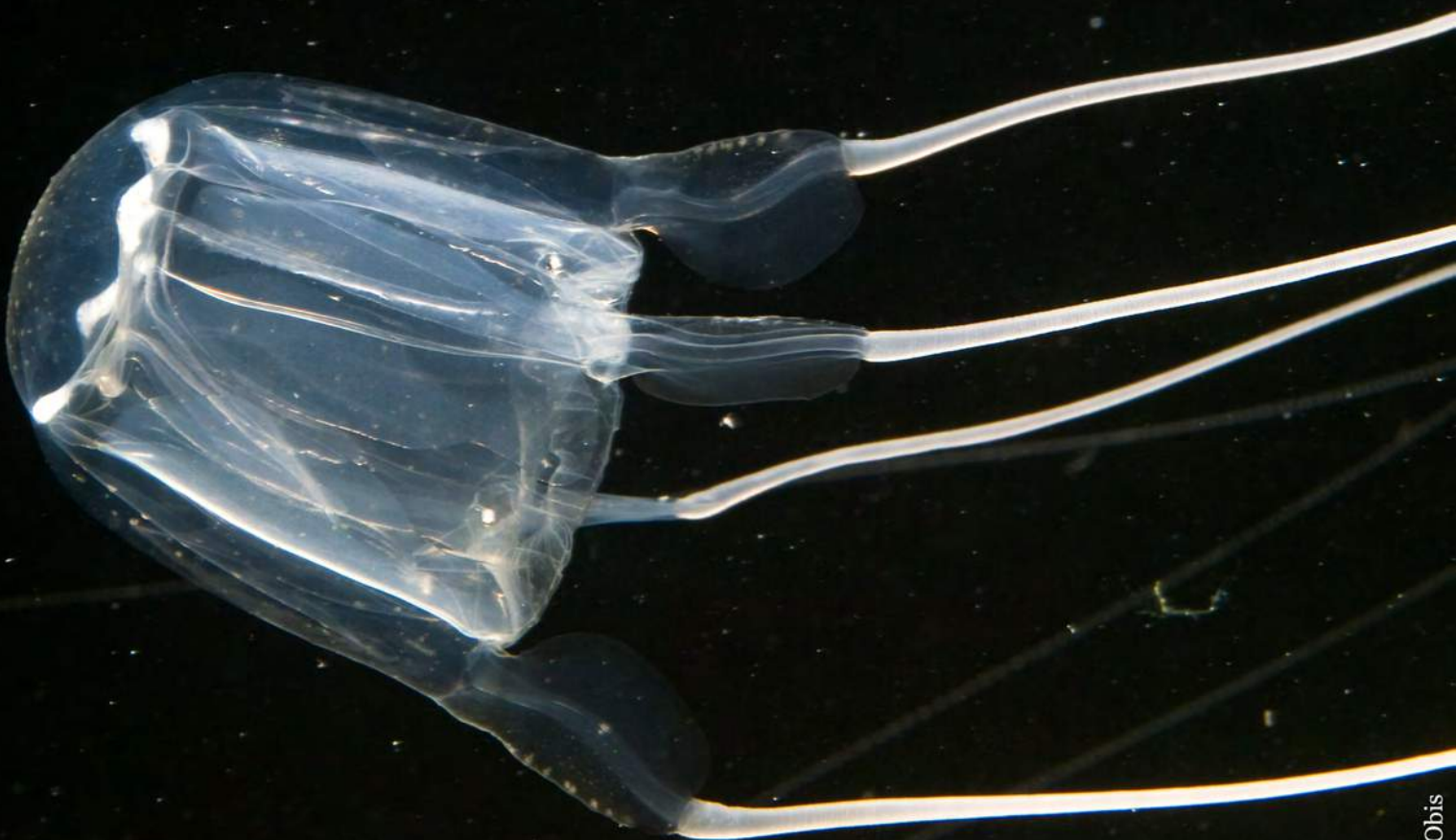


LIFE+NAT ES0064 “CUBOMED”

«Development and demonstration of eradication and control methods for an invasive species: *Carybdea marsupialis* (Cubozoa), Mediterranean»



Eduardo Obis

Figura 1. *Carybdea marsupialis*. Foto: E. Obis

What is LIFE CUBOMED project?

CUBOMED (LIFE+08 NAT/ES/0064) is an environmental and nature conservation project belonging to the EU LIFE+ program. This project deals with the study of the box jellyfish *Carybdea marsupialis* (Figure 1) on the Spanish Mediterranean coast, mainly in those places where its populations show a high density (Figure 2), analyzing the effects of its proliferations both from an environmental and from a public health point of view. The presence of *Carybdea marsupialis* in bathing waters may generate problems for beach users as it is a very stinging species (Figures 3 and 4), and in addition, an overabundance of them may result in an imbalance in the ecosystem due to its high predatory potential (Figure 5).

This project also studies other gelatinous organisms present on our coasts (e.g. other species of jellyfish and ctenophores).

The beneficiaries of the CUBOMED Project are Instituto de Ciencias del Mar (Barcelona) del Consejo Superior de Investigaciones Científicas (CSIC) (<http://www.icm.csic.es/>), and Universidad de Alicante, through its Instituto Multidisciplinar para el Estudio del Medio "Ramón Margalef" (<https://imem.ua.es/>). This project started on January 1, 2010 and ended on December 31, 2016 and has been co-financed by:

- European Commission (LIFE+ Program) : 48%
- Ministerio de Medio Ambiente, y Medio Rural y Marino -DG Sostenibilidad de la Costa y del Mar- y Fundación Biodiversidad: 44%
- Consellería de Medio Ambiente de la Generalitat Valenciana (DG Agua): 6%
- Universidad de Alicante e Instituto de Ciencias del Mar (CSIC): 2%

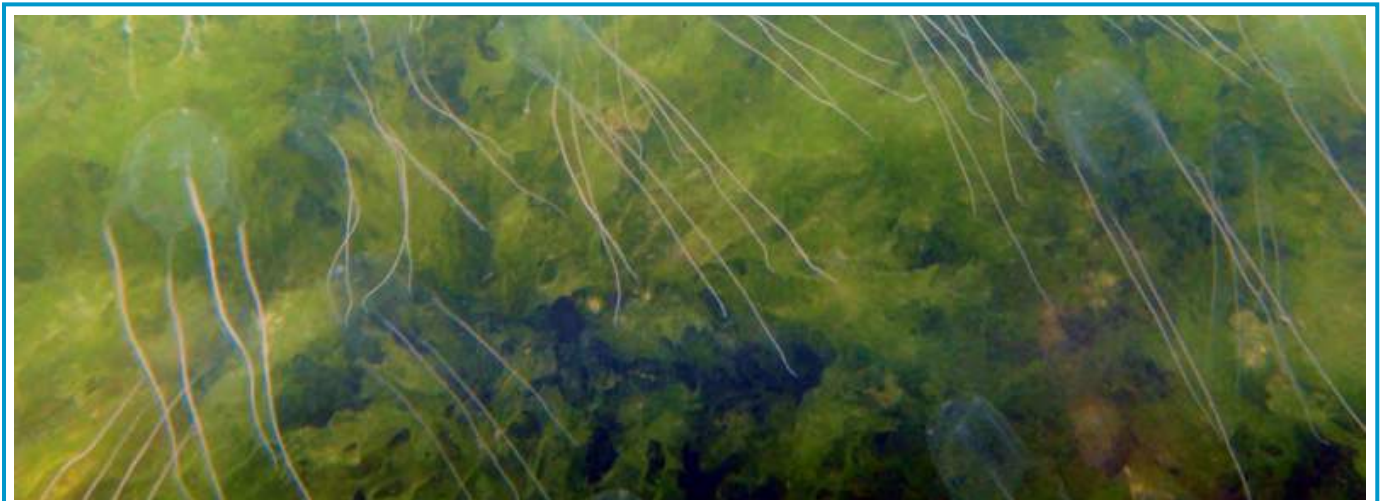


Figure 2. *Carybdea marsupialis* proliferations. Adriatic Sea. Photo: F. Boero



Figure 3. *Carybdea marsupialis* sting scars. The image on the right shows the effect after 5 days.

Carybdea marsupialis, a hardly-known species

Before the implementation of this project, the knowledge about the biology and ecology of *Carybdea marsupialis* was scarce. Aspects such as distribution, life cycle, diet, rate of growth, behavior, dispersion, effects of its sting, or which stinging treatment was the most effective were poorly understood and usually based on inconclusive observations. It was also unknown which methods of capture and quantification should be used for each phase of its development, from juveniles of 0.4 mm to adults up to 4 cm in diameter.



Figure 4. The image on the left is a tentacle of *Carybdea marsupialis* that has not been triggered. On the right, it may be observed the cnidocytes (harpoon-like structures full of venom) shot. When a jellyfish contacts its prey (or sting a bather), thousands of microscopic filaments are shot, injecting different types of toxins.



Figure 5. *Carybdea marsupialis* capturing zooplankton.

LIFE CUBOMED project objectives:

- a) Develop a method and a technique for the detection and quantification of *Carybdea marsupialis*.
- b) Clarify what are the environmental and ecological characteristics that could promote the exponential growth of *Carybdea marsupialis* populations, paying special attention to nutrients and primary and secondary production increments (**Figure 6**).
- c) Description of the diet of *Carybdea marsupialis* (**Figure 7**).
- d) Propose study guidelines, and action and mitigation measures against *C. marsupialis* massive proliferation episodes.
- e) Study the consequences of the massive proliferation of *C. marsupialis* for the ecosystem equilibrium, as well as the ecological impact of other gelatinous organisms such as the ctenophore *Mnemiopsis leidyi* (**Figure 9**) or other jellyfish species.
- f) Propose study guidelines and restoration plans for ecosystems affected by jellyfish proliferations.
- g) Recognize and treat *C. marsupialis* stings, and propose guidelines for managing the information received from Spanish lifeguard and First-Aid services.

These studies have been carried out both in the field (in several locations on the Spanish Mediterranean coast) and in the laboratory, mainly in the ZAE (Experimental Aquarium Zone) at ICM-CSIC. We have also collaborated with research teams from Italy, Malta and Tunisia analyzing the local populations of *Carybdea marsupialis* present in those countries.



Figure 6. Water sampling using an oceanographic “Niskin” bottle.



Figure 7. Prey consumption experiment (zooplankton).

***Carybdea marsupialis*, an endemic species of the Mediterranean**

Carybdea marsupialis belongs to the Cubozoa class, which together with corals and other jellyfish, conform the phylum Cnidaria (from Greek “cnida”=nettle). This term is used to reference a common characteristic of cnidarians, which is the possession of stinging cells called cnidocytes distributed over the body surface (mainly in tentacles). These cells have defensive and capture purposes.

Jellyfish belonging to the Cubozoa class are named “cubomedusae” or “box jellyfish” due to the cubic form of this umbrella (Figure 8). Although they constitute a small fraction within Cnidaria, the number of described species of cubozoans has increased over recent years and currently, fifty species form part of this group.

Cubozoans can be found in all oceans, typically in tropical and subtropical regions, in shallow coastal areas. *Carybdea* species are largely distributed in the tropical waters of the Atlantic and large populations are found in the Caribbean Sea.

Carybdea marsupialis was described for the first time in the Mediterranean Sea by Linneo in 1758 (Linnaeus 1758) and a century later, in 1878, it was detected in the Adriatic by Claus (Di Camillo et al. 2006). At the end of the 20th century its sightings increased (probably because of a population increment) and it was observed in different locations: central Adriatic, near Fano in 1985 (Boero & Minelli 1986); Gulf of Venice during summer 1992 (Mizzan 1993); Gulf of Trieste in October 1998 (Bettoso 2002); different localities along the Emilia Romagna (ARPA, 2001, 2004) and in Riviera de Conera, Ancona, also in the Adriatic Sea (Di Camillo et al. 2006).

To date, there was some confusion with the identification of *Carybdea marsupialis* since this species had been cited not only in the Mediterranean but also elsewhere in the world (Caribbean, Red Sea). In the framework of the LIFE Cubomed project, we have carried out studies on the genetics of the genus *Carybdea* and its worldwide distribution (Acevedo 2016) concluding that *Carybdea marsupialis* is not an exotic species as it was initially considered, but a Mediterranean native species with high invasive capacity.



Figure 8. *Carybdea marsupialis* adult specimen. Approx. 30 mm in diameter.

Mnemiopsis leidyi is a filtering and non-stinging gelatinous organism originally native to the western Atlantic coastal waters. It belongs to the phylum Ctenophora (therefore it is not a cnidarian). It was first recorded in Spanish waters in 2009 (Fuentes et al. 2010, Marambio et al. 2013) and since 2010 some of its populations have been monitored –especially in the Ebro River Delta- thanks to the sampling campaigns conducted by the LIFE CUBOMED project.

M. leidyi is one of the “100 World’s Worst Invaders” listed by the International Union for Conservation of Nature –IUCN- (Lowe et al. 2000), as it causes significant ecological problems in the invaded areas due to its intense predation on plankton and fish larvae. *M. leidyi* usually appears in large aggregations and its gelatinous appearance may confuse beach users, causing alarm and rejection in the same way as jellyfish do.



Figure 9. LIFE Cubomed contributes to the knowledge on the invasive ctenophore *Mnemiopsis leidyi*

Where has this species been detected?

Carybdea marsupialis has a wide range of distribution and can be present in all coastal areas of the Mediterranean, so its mere presence is not a rarity nor an indication of any alteration of the ecological balance. However, in certain places, abundances of more than 5 adult individuals per cubic meter have been observed (Figure 10). This high density can lead to hundreds of stung bathers, as well as to alterations of the trophic networks. Since the 1980s, there has been an increase in the abundance of *C. marsupialis* in the Adriatic Sea, and since 2008 the same happened in some areas of the Spanish Mediterranean.



Figure 10. Sampling and measuring *Carybdea marsupialis* on the beach.



Water quality: How do anthropogenic activities influence *Carybdea marsupialis* populations?

Over-fertilization of coastal waters with phosphorus and nitrogen can be caused by the proximity of urban agglomerations (phosphorus comes mainly from sewage) or intensive agriculture (nitrogen discharges in the form of nitrates from fertilizers). Both nutrients can reach the sea via run-off, aquifer submarine discharges or sewage outlet pipes.

If the discharge of these nutrients occurs in sensitive areas (such as rivers, estuaries or relatively sheltered coastal waters or with little hydrodynamics), a phytoplankton-zooplankton production cascade may be produced. Although wastewater treatment plants can remove more than 95% of pollutants in the incoming waste (mainly phosphorous), sometimes the 5% remaining can be enough to generate disturbances in sensitive aquatic ecosystems.

As *Carybdea marsupialis* feeds on plankton, an induced over-fertilization that rises plankton abundance would lead to an increase in jellyfish populations. In certain places where *C. marsupialis* has been found to be highly abundant or other species of gelatinous organisms are usually present, measures should be taken to reduce nutrient inputs, focusing on nitrogen from agriculture and phosphorus from wastewater (Figure 11 y Figure 12).

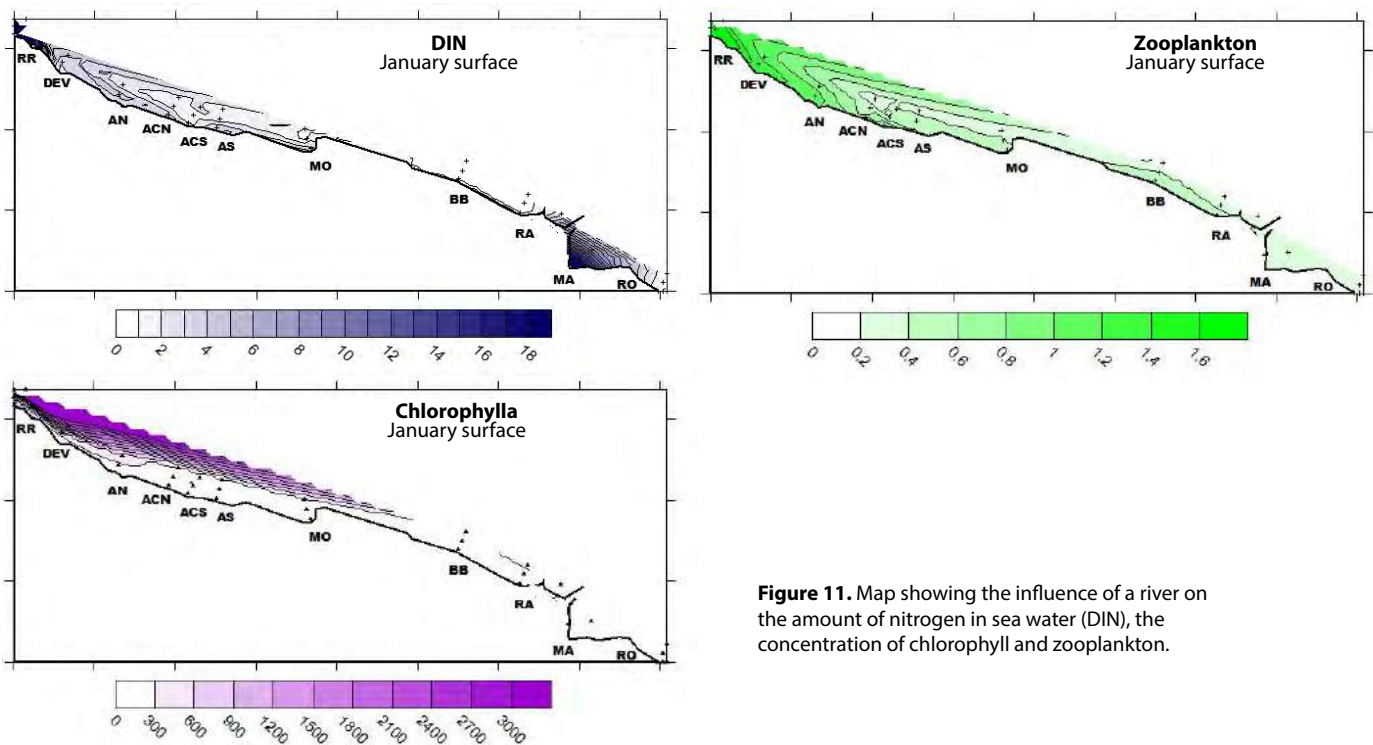


Figure 11. Map showing the influence of a river on the amount of nitrogen in sea water (DIN), the concentration of chlorophyll and zooplankton.

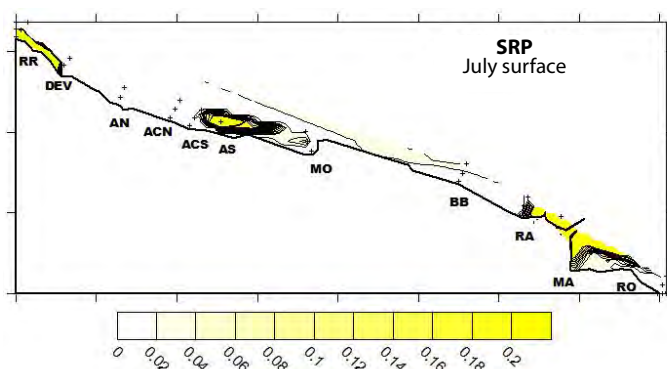


Figure 12. Map showing the increase of the phosphorus concentration (SRP) associated with the discharge of treated wastewaters.

What is the life cycle of the species?

One of the objectives achieved in the LIFE Cubomed Project has been the monitoring of life cycle and seasonality of *C. marsupialis* in the Mediterranean. Cubozoan life cycle comprises a benthic polyp phase with asexual reproduction, and a swimming medusa phase with sexual reproduction. In the Spanish coasts, juveniles of *C. marsupialis* (0.4 mm in diameter) are released through the metamorphosis of the polyp mainly between May and July. These juveniles grow up to adult sizes (greater than 15 mm in diameter) by mid-August. Reproductive adults, up to 40 mm in diameter, appear between the end of August and the end of October. At the beginning of November, cubomedusae disappear, leaving the population reduced to the polyps, which remain latent until the next spring. These polyps are generated by the settlement of the larvae (Figure 13) after reproduction episodes.

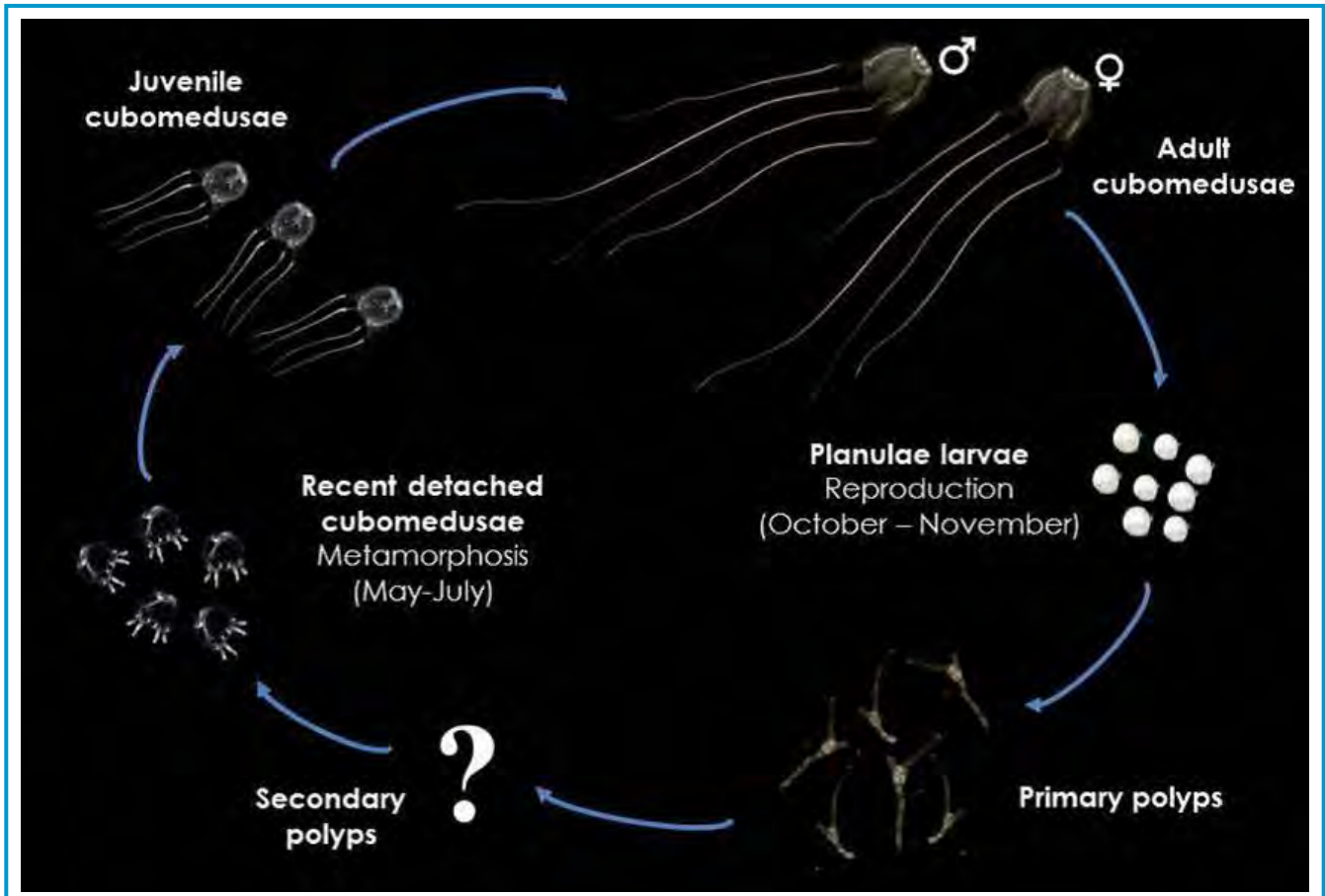


Figure 13. . Life cycle of *Carybdea marsupialis* (Acevedo 2016).



Figure 14. Experimental aquariums with polyps of *Carybdea marsupialis*.

CUBOMED

REPORTA TUS AVISTAMIENTOS



REPORT YOUR JELLYFISH SIGHTINGS!

The LIFE Cubomed Project has generated a database of sightings of *Carybdea marsupialis* and other jellyfish species through the project and Citizen Science surveys. The database provides information on the distribution of this species as well as other gelatinous organisms (www.cubomed.eu).

CITIZEN SCIENCE

Do you want to collaborate by providing sightings of gelatinous organisms?

Download the jellyfish identification guide in <https://goo.gl/jhp18A>
And send an email to medusa@icm.csic.es

Guía de identificación y tratamiento de picaduras de medusas y otros organismos gelatinosos 

cubomed
LIFE08 NAT ES 0064

 <i>Carybdea marsupialis</i>	 <i>Pelagia noctiluca</i>	 <i>Rhizostoma pulmo</i>	 <i>Cotylorhiza tuberculata</i>	 <i>Aurelia sp.</i>
 <i>Chrysaora hysoscella</i>	 <i>Discomedusa lobata</i>	 <i>Rhizostoma luteum</i>	 <i>Aequorea forskalea</i>	 <i>Velella velella</i> <i>Porpita porpita</i>
 <i>Olindias, Gonionemus, Pandae</i>	 <i>Physalia physalis</i>	 <i>Otros gelatinosos</i>	 <i>Especies invasoras</i>	 <i>Otras medusas del Mediterráneo</i>

Most relevant objectives achieved in LIFE Cubomed

- a) Design and development of several methods of quantification and capture of all life stages of the species, from juveniles of 0.4 mm to adults up to 40 mm in umbrella diameter.
- b) Description of the annual cycle, with information on both temporal and spatial variability, as well as swimming velocities of each size categories. From about 15 mm in diameter, *C. marsupialis* can overcome the coastal currents and therefore, select an appropriate habitat.
- c) Identification of environmental factors that affect its growth and distribution, being food availability a key factor.
- d) Description of diet and energy requirements of *C. marsupialis*, as well as quantification of the impact on the planktonic community. Dietary overlap is high when compared to fish (>85%), especially larvae and juveniles, so competition with them can be significant.
- e) Development of prediction models at the Mediterranean level and simulation of their life cycle with the possibility of simulating scenarios, where the reduction of food availability is shown as the most effective strategy to reduce the species population.
- f) Through genetic techniques *Carybdea marsupialis* has been described as a native Mediterranean species, clarifying the confusion found in the scientific literature so far, where *C. marsupialis* was cited as present on the Caribbean Sea.
- g) Development of several aquarium prototypes for the different life stages, as well as the techniques for their feeding and maintenance.
- h) Socioeconomically, the collected data show that jellyfish stings (considering all species) account for 60% of lifeguard assistances at Spanish Mediterranean beaches. In the framework of LIFE Cubomed, different measures are proposed to use the information derived from beach attendances as indicators of the presence of jellyfish proliferations in the coast.
- i) LIFE Cubomed project has been disseminated to all the municipalities of the Spanish Mediterranean coast (234 municipalities), as well as to more than 5000 students from different grades and through traveling exhibitions, to the general public.

Scientific production

In the framework of the LIFE Cubomed project, the study of the biology and ecology of cubozoans has been promoted significantly, generating more than 10 scientific publications and 25 communications in congresses, highlighting the organization of a special session on cubozoans and the LIFE Cubomed project at the 5th International Congress of Jellyfish Blooms Symposium, held in Barcelona from May 30 to June 3, 2016 (www.jellyfishbloom1016.com) with more than 250 attendees from all over the world. To see the updated list of publications visit www.cubomed.eu.



Figure 15. . Installation of "inverse traps" in order to find polyps.

Training and dissemination

Dissemination and training activities have been constant all over the project. We have been working with the general public as well as with students, and specific stakeholders such as scuba divers and the staff of the Spanish lifeguard and first-aid Services.



Figure 16. On the left, dissemination activities for elementary school students carried out in Dénia. On the right, a divulgation event addressed to the general public held at ICM-CSIC facilities in Barcelona.

In the framework of LIFE Cubomed, information on the identification on jellyfish and the most appropriate treatments for their stings has been provided to all coastal municipalities of the Spanish Mediterranean. We have also directly trained more than 500 lifeguards in various seminars throughout the study area.



Figure 17. Training seminar for lifeguards, held at Balearia facilities in Dénia

Acknowledgements

The LIFE Cubomed Project (besides the direct co-financing by the European Commission, Ministerio de Medio Ambiente, y Medio Rural y Marina, Fundación Biodiversidad and Generalitat Valenciana) has also been supported and co-financed by other entities and institutions:

- Parques Nacionales
- Agencia Catalana de l'Aigua
- Fundación Baleària
- Ayuntamiento de Dénia
- Asociación ACIF Marina Alta
- El Portet de Dénia
- Marina de Dénia
- Club Náutico Dénia

What is a LIFE project?

LIFE is the EU's financial instrument supporting environmental, nature conservation and climate action projects throughout the EU. It was created in 1992 and to date has co-financed more than 4300 projects.



DO YOU WANT TO KNOW MORE?
<http://ec.europa.eu/environment/life/index.htm>



Figura 18. Experimental Aquarium Zone at ICM-CSIC.

Bibliography

Acevedo MJ (2016) Biology, ecology and ecophysiology of the box jellyfish *Carybdea marsupialis* (Cnidaria: cubozoa). PhD thesis. Universitat Politècnica de Catalunya

Bettoso N (2002) First record of *Carybdea marsupialis* (L., 1758) (Cnidaria, Cubozoa) in the Gulf of Trieste. *Period Biol* 104:233

Boero F, Minelli A (1986) First record of *Carybdea marsupialis* new record cnidaria cubozoa from the adriatic sea. *Boll del Mus Civ di Stor Nat di Venezia* 35:179–180

Camillo C Di, Bo M, Puce S, Tazioli S, Bavestrello G (2006) The cnidome of *Carybdea marsupialis* (Cnidaria: Cubomedusae) from the Adriatic Sea. *J Mar Biol Ass uK* 86:705–709

Fuentes VL, Angel DL, Bayha KM, Atienza D, Edelist D, Bordehore C, Gili JM, Purcell JE (2010) Blooms of the invasive ctenophore, *Mnemiopsis leidyi*, span the Mediterranean Sea in 2009. *Hydrobiologia* 645:23–37

Linnaeus C (1758) *Systema naturae per regna tria naturae: secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata, Holmiae, Impensis L. Salvii.* :824

Lowe S, Browne M, Boudjelas S, Poorter M De (2000) 100 of the world's worst invasive alien species. A selection from the Global Invasive Species Database. *Invasive Species Spec Gr a Spec Gr Species Surviv Comm World Conserv Union*:12

Marambio M, Franco I, Purcell JE, Canepa A, Guerrero E, Fuentes V (2013) Aggregations of the invasive ctenophore *Mnemiopsis leidyi* in a hypersaline environment, the Mar Menor lagoon (NW Mediterranean). *Aquat Invasions* 8:243–248

Mizzan L (1993) Prima segnalazione di *Carybdea marsupialis* L., 1758 Cnidaria, Cubozoa in Adriatico settentrionale Golfo di Venezia. *Soc Veneziana di Sci Nat Lav* 18:321–322