Hydrobiologia
July 2022, Volume 849, Pages 2933-2948
https://doi.org/10.1007/s10750-022-04903-y
https://archimer.ifremer.fr/doc/00775/88704/

Ontogenetic dietary shifts of the medusa *Rhizostoma pulmo* (Cnidaria: Scyphozoa)

Leoni Valentina [1, *], Molinero Juan-Carlos [2], Crochemore Sandrine [1], Meffre Marie [1], Bonnet Delphine [1]

[1] MARBEC, Univ. Montpellier, CNRS, Ifremer, IRD, Montpellier, France
[2] MARBEC, IRD, CNRS, Ifremer, Univ. Montpellier, Sète, France

* Corresponding author : Valentina Leoni, email address : valentina.leoni@umontpellier.fr

Abstract :

Identifying ontogenetic changes in jellyfish diet is fundamental to understand trophic interactions during their life cycle. Scyphomedusae blooms exert major predation pressure on plankton communities, although their role in ecosystems has long been misrepresented. This study assesses seasonal and ontogenetic changes in the diet of the scyphomedusa Rhizostoma pulmo, one of the largest yet overlooked Mediterranean jellyfish. Medusae gut contents (n = 127) were collected during one year in Bages Sigean lagoon, southern France. Results show that the diet composition differs from the availability of prey in the environment with contrasting preferences along ontogeny. Calanoid (70%) and harpacticoid (45.8%) copepods were the most frequent prey and the major carbon contributors for small medusae (bell diameter < 15 cm). In contrast, ciliates (43.5%) were the most frequent prey for large organisms (> 15 cm), which obtain most of their carbon intake from ciliates and fish eggs (20.9%). The overall impact on micro and mesozooplankton showed that small medusae consume 5% of the copepods daily standing stock, while large medusae consumed 8% of ciliates daily standing stock. Our results stress that R. pulmo display different trophic pathways along its life cycle, firstly interacting with the classical food web, and shifting afterwards to a greater interaction with the microbial loop.

Keywords : Gut content, Barrel jellyfish, Mediterranean Sea, Developmental stages

Introduction

Assessing the trophic role of species, i.e., what they eat and in what proportions, is fundamental to understand energy fluxes and ecosystem functioning (Cury et al., 2008). In marine ecosystems, gelatinous zooplankton are increasingly recognized as conspicuous prey (Hays et al., 2018), consumers (Robinson & Graham, 2014), and prominent players in biogeochemical cycles (Lebrato et al., 2019). Cnidarians represent approximately 92% of the total global biomass of gelatinous zooplankton (Lucas et al., 2014), with scyphomedusae species developing the largest individual body weight and forming spectacular blooms (Dawson & Hamner, 2009). Due to their voracity on lower trophic levels (Purcell, 1992), scyphomedusae blooms impact the whole food web structure in coastal marine ecosystems, thereby shaping the ecosystem functioning and services (West et al., 2009).

Despite the central role of scyphomedusae and their impact on local economies (e.g., Bosch-Belmar et al., 2021), the trophic ecology of this taxon is often misrepresented, as the large majority of studies have focused on two genera, *Aurelia* and *Chrysaora*. The remaining 85% of scyphomedusae species are overlooked. Consequently, ecosystem models have historically oversimplified their role as predators (Pauly et al., 2009), although recent evidence has shown a wide variation in their trophic role which changes not only across species, but also throughout their ontogeny (Fleming et al., 2015).

During their life cycle, scyphozoans display a wide range of sizes, from few millimeters up to 1 meter. The few studies that have addressed their ontogenetic dietary shifts have identified diverse responses. For instance, benthic and pelagic stages trophic niches of some species widely overlap (*Aurelia coerulea* von Lendenfeld, 1884: Marques et al., 2021), others shift their trophic level with size (*Lychnorhiza lucerna* Haeckel, 1880: Nagata et al., 2015 and *Cyanea nozakii* Kishinouye, 1891: Wang et al., 2020), or modify prey preferences and diversity (*Stomolophus meleagris* Agassiz, 1860 and *Aurelia aurita* (Linnaeus, 1758): Larson, 1991; Graham & Kroutil, 2001; Álvarez-Tello et al., 2016). Such diet changes have been associated with shifts in mouth size, prey encounter probability, or small-scale currents generated by bell pulsations (Costello & Colin, 1995; Nagata et al., 2016).

Among the methods used to determine the trophic role, four have been implemented on scyphozoans' diet analyses (Pitt et al., 2009): i.e., gut content, grazing experiments, stable isotope (SI) and fatty acid (FA) analyses. SI and FA analyses provide the signal of the assimilated food, but require a previous knowledge of the prey's signal in the environment (Pitt et al., 2009). In turn, gut content analysis provides information on recently ingested food, although it does not allow detecting the assimilated one, and therefore underestimate preys that are digested rapidly (e.g., Purcell, 1997; Båmstedt & Martinussen, 2000). Notwithstanding this limitation, it remains the most direct method for prey identification (Purcell,

2018). A protocol to standardize the assessment of scyphomedusae gut content has been only recently provided (Nagata & Morandini, 2018). To date, however, gut content analyses on jellyfish have generally based their conclusions on a small sample size, and overlooked the predators and prey seasonality, which are not only crucial to define dietary niches but are also essential to incorporate this group in food web models.

By virtue of their relative shallowness and protection from the open sea, semi-enclosed ecosystems, such as coastal lagoons, are exceptional places to study jellyfish trophic interactions in natural environments (Marques et al., 2021). Bages Sigean (France) (43°05'12.72" N; 3°00'35.3" E) is a small Mediterranean coastal lagoon (38 km^2, mean depth of 2 m) located in a protected area (Cesmat et al., 2012). The lagoon is a shelter for migrant birds and fishes, and an important biodiversity reservoir (PNRNM, 2018), where a perennial population of the barrel jellyfish *Rhizostoma pulmo* (Macri, 1778) has been detected since 2014.

R. pulmo is a native Rhizostomeae scyphomedusa from the Mediterranean and Black Seas (Leoni et al., 2021a). This scyphomedusa can reach biomasses of 300 tons km^{-2} during bloom events (Basso et al., 2019), with dramatic effects on fisheries (Nastav et al., 2013) and potentially on the food web structure (e.g., Brodeur et al., 2002). In Bages Sigean, summer blooms of *R. pulmo* (Leoni et al., 2021b) are a major issue for artisanal fishing due to net clogging, spoiling the commercial value of captured fish and because of the risk of stings (S. Marin pers. comm.). Similar problems have been reported in other ecosystems inhabited by this species, such as Mar Menor lagoon (Spain) (Fuentes et al., 2011).

The feeding mechanism of *R. pulmo* is peculiar. Instead of a central mouth, such as in *Aurelia* and *Chrysaora*, Rhizostomeae medusae have eight specialized oral arms covered with millimetric mouths where the food is ingested due to micro-currents generated by bell pulsation (Nagata et al., 2016). However, little is known about the diet of this species (Pérez-Ruzafa et al., 2002; Dönmez & Bat, 2019).

In this study, we benefited from the large size range of individuals present across the year in Bages Sigean lagoon (Leoni et al., 2021b) to assess *R. pulmo* diet throughout the pelagic stages. Owing to the medusa mouths size (ca. 3,000 µm), we hypothesized (H1) a diet mainly composed by a dominance of small prey. In addition, as it is expected that prey encounter rate and micro-currents generated by bell pulsations increase along medusa ontogeny (Nagata et al., 2016), we hypothesized an increase of prey richness (H2) and mobile prey (H3) in the diet along with medusa growth. By means of gut content analysis, we (1) describe prey diversity and size range during ontogeny, (2) quantify prey relative abundance, (3) examine potential prey-selectivity patterns of the species, and (4) identify the main sources of carbon supply during ontogeny, as well as its predatory impact in the lagoon.

Materials and Methods

Biological samples were gathered biweekly between February and November 2019 in Bages Sigean lagoon (see Figure 1 in Leoni et al., 2021b) between 09:00 and 12:00 AM. The abundances of *R. pulmo* pelagic stages were estimated using two complementary sampling procedures. Young individuals were caught with a 700-µm mesh size plankton net fitted with a flowmeter, which was towed horizontally at the sub-surface due to the shallowness of the lagoon. In turn, the abundance of large organisms was estimated with a non-intrusive method of visual counting from the boat (Leoni et al., 2021b). Abundance estimations were expressed as the number of medusae per 100 cubic meters.

Jellyfish gut content analysis

R. pulmo medusae size classes were defined based on the absence (juveniles) or presence (adults) of gonads. We used 15 cm as the bell diameter (BD) threshold to distinguish juveniles from adults' specimens, as gonads were present in all individuals with BD >15 cm.

The methodology applied to assess the medusae gut content was based on Nagata & Morandini (2018). Medusa specimens were carefully collected by dip nets from the boat. Only unharmed and active individuals were considered. BD (±0.05 cm) and wet weight (WW ±0.05 kg) of the specimens were recorded. Each medusa was stored individually in a plastic jar with formalin 10%. In the laboratory, medusae were singly laid down on a tray and methylene blue was injected into the specimens' gut to facilitate prey visualization (Nagata & Morandini, 2018). Later, oral arms were removed by scissors and the cruciform gut was opened. The cavity was rinsed with 0.2 µm filtered seawater and the washed content was retained on a 63-µm sieve. Consequently, 63-µm was the lower size threshold of prey identification. The gut walls were also examined under a Leica stereomicroscope to collect prey eventually retained therein. Both, prey items removed by rinsing and those attached to the gastric cirri were conserved in formalin 4% and included in counts.

Prey items were identified and counted in a Bogorov chamber under a stereomicroscope. When possible, specimens were identified at species or genus level. Otherwise, identification was carried out to the greatest detail possible. Phytoplankton was counted but was not considered for the analyses as it was probably underestimated due to the minimal size of prey identification. Individual prey measurements were determined from pictures with a camera integrated into the stereomicroscope, using the software Leica Application Suite X (LAS X). Schematic representations of how measurements were taken are presented in Figure S1, mainly following Uye (1982).

Characterization of food availability

To characterize the zooplankton community and to evaluate *R. pulmo* prey selectivity patterns, zooplankton and medusae collections were performed simultaneously. Micro-, meso- and macrozooplankton were collected with 63-µm (0.40 m diameter x 1.00 m length), 200-µm (0.54 m diameter x 2.50 m length) and 700-µm (0.78 m diameter x 2.00 m length) mesh size plankton nets, respectively, each fitted with a flowmeter and towed horizontally in the sub-surface layer. Samples were fixed in formalin 4%. Two samples were taken for meso- and macrozooplankton in the study area at each sampling date, while only one sample was collected for microzooplankton at each date.

Microzooplankton was analyzed with a FlowCam imaging system (Fluid Imaging Inc.; Sieracki et al., 1998) at the Villefranche Oceanography Laboratory, LOV, while the posterior classification was performed in EcoTaxa. Data are available online (https://ecotaxa.obs-vlfr.fr/prj/3279). Meso- and macrozooplankton organisms were counted and identified under the stereomicroscope using taxonomic guides (Trégouboff & Rose, 1978; Hecq et al., 2014). Zooplankton abundances were estimated from subsamples and expressed as individuals per cubic meter.

Statistical analysis

To characterize and quantify the diet of *R. pulmo*, each medusa was treated as a sampling unit. In addition, to allow future comparative analyses, we used standard equations and nomenclature employed in trophic indices assessments, as summarized in da Silveira et al. (2020).

Assessing the minimum sample size

A cumulative curve of the number of medusae analyzed against the number of prey taxa in the guts was used to determine adequate sample size, i.e., the minimum number of samples (*MNS*) to represent the total richness. We used the Chao1 non-parametric estimator (S_{Chao1}):

$$S_{Chao1} = S_{obs} + \frac{{n_1}^2}{2n_2}$$

where S_{obs} is the observed richness, n_1 is the number of species found once, and n_2 is the number of species found twice (Chao et al., 2009). The sampling effort was considered sufficient when S_{obs} was equal to 80% of S_{Chao1} (Jiménez-Valverde & Hortal, 2003). This analysis was performed in the software R using the *rarc* function of the `rich´ package (Rossi, 2011).

Objective 1. Prey's richness and frequency of occurrence during R. pulmo ontogeny

To evaluate whether changes during ontogeny were related to changes in prey's richness, the number of prey taxa found in juvenile and adult medusae was compared using a Kruskal-Wallis test. A significance level of 5% was applied. In addition, the proportion (%) of empty guts (G_e) was estimated by month per stage of development.

The frequency of occurrence (*FO* in %) of a prey category (*i*) in the gut content of both juvenile and adult medusae was estimated as the number of guts with this food item (G_i) over the total number of guts containing food (G_f) (da Silveira et al., 2020).

Objective 2. Relative abundance of prey on the diet of R. pulmo pelagic life stages

The percentage of the relative abundance (*N*) of prey was calculated for each prey taxa for all medusae containing food. The average relative abundance was determined by month for both stages of development. *N* (%) was estimated as the number of individuals of a prey category (*Ni*) over the total number of prey items found in the medusa gut ($\sum N_i$) (Hyslop, 1980):

$$\%N = \left(\frac{N_i}{\sum_{x=1}^{i} N_i}\right) * 100$$

Trophic niche determination

The trophic niche breath (*NB*) was estimated for juveniles and adults medusae using Levin's standardized index (Krebs, 1999):

$$NB_k = \frac{1}{n - (\frac{1}{\sum p_{ik}^2} - 1)}$$

where NB_k is Levin's standardized index for a predator *k*; p_{ik} is the proportion of a prey *i* in the diet of the predator *k*, and *n* is the number of prey categories. This index ranges from 0 to 1, where low values represent a narrow niche breadth or a specialized diet, whilst higher values suggest a generalist diet with a wide niche breadth.

Lastly, we assessed the niche overlap between juveniles and adults when they coexist. This metric was expressed as the percentage of similarity between juveniles' (*j*) and adults' (*a*) diets, and estimated with the simplified Morista's index of similarity (*NO*) (Krebs, 1999):

$$NO = 2 * \frac{\sum(P_{ij} * P_{ia})}{\sum P_{ij}^2 + \sum P_{ia}^2}$$

Here, P_{ij} and P_{ia} are the proportions of the resource *i* used respectively by juveniles and adults in relation to the total resources. The values ranged from 0 to 100%, with 0% representing no diet overlap, and 100%

identical diets. We followed the criteria of Langton (1982), and determined three categories of niche overlap: low (<29%), medium (30-60%) and high (>60%). This index is sensitive to the taxonomic resolution of preys identified. Here, differences at the species level, if they exist, were not considered in the niche overlap evaluation.

Objective 3. Prey selectivity patterns during R. pulmo ontogeny

To account for differences in the relative abundance (*N*) of a prey type (*i*) in the gut (G_i) *vs* prey in the environment (E_i), we used the linear food selection index (*LFSI*) (Strauss, 1979). 'Positive selection' was considered when the G_i was higher than E_i, and 'negative selection' when G_i was lower than E_i. *LFSI* was estimated per medusa. Values of *LFSI* range from -1 to 1, reflecting the magnitude of prey selection. Null values indicate no selection, positive values suggest some degree of positive selection and negative values have a probable disability to consume the prey, i.e., negative selection. To evaluate whether seasonal and/or ontogenetic changes were related to changes in prey selectivity patterns, a heatmap of *LFSI* was performed by month and by medusae size range (BD in cm) for each type of prey. Size classes of 5 cm were set up with juveniles including the classes 0-5, 5-10 and 10-15 cm, and adults the classes 15-20, 20-25, 25-30 and >30 cm.

Objective 4: Daily Carbon Ration (DCR) assessment and predatory impact

The daily ration (*DR*) was estimated as the number of prey consumed (P_c) by medusa per day as:

$$DR = P_c * 24 * DT^{-1}$$

using the digestion time (*DT*, in hours) for each prey provided in Larson (1991) (Table S1) and corrected by temperature as follows:

$$DT_t = 1*(DT*Q_{10}^{(t-29)/10})^{-1}$$

where *t* is the temperature at each sampling date and Q_{10} was assumed equal to 2.08 (Purcell, 2009). Lastly, *DR* was converted to daily carbon ration (*DCR*) by multiplying P_c by the carbon content (C in mg) estimated for each prey using specific equations (Table S1). *DCR* was estimated for prey items found in more than 5% of samples. We used a biometric conversion factor estimating C as a percentage of *R. pulmo*'s WW: C%WW=0.34 (Purcell et al., 2010) to estimate the C content of each medusa (WW range: 0.25 to 2761g). Quantile regressions (5^{th}, 50^{th} and 95^{th}) were used to evaluate possible changes in carbon sources with medusae size (mgC) (Cade & Noon, 2003).

Based on medusae abundances, the predatory impact of *R. pulmo* in the lagoon was estimated as $PI=DR*(D_{pred}*D_{prey}^{-1})*100$, where *PI* is the percentage of the prey standing stock consumed by the

246 medusae population per day, D_{pred} is the abundance of medusae (ind m^{-3}) and D_{prey} the abundance of the
247 prey in the environment (ind m^{-3}) (Nagata & Morandini, 2018). PI was estimated for juveniles and adults
248 by prey taxa.
249
250 All the analyses were performed using the open source software R 3.6.0 (R Core Team, 2020)
251 and plots were created with the `ggplot2´ package (Wickham, 2016).

Results

Jellyfish population dynamics

In total, 16 samplings were performed. Ephyrae and small medusae appeared for the first time in April and the last medusae were observed in October. The seasonal dynamics of *R. pulmo* pelagic stages showed a maximum abundance in April and two smaller peaks in June and July (Fig. 1a). Ephyrae occurred from April to June, juveniles from April to September, and adults from May to October. Maximum abundances of ephyrae, juveniles and adults medusae were observed in June (6.1 ind 100 m^{-3}), April (17.5 ind 100 m^{-3}) and June (1.0 ind 100 m^{-3}), respectively. Due to overlapped cohorts, in some months, more than one developmental stage was present. The first cohort developed from April to September, the second cohort from May to October, and the third cohort from June to October. However, as there were not enough individuals collected from the third cohort, only specimens from the two first cohorts were considered for gut content analysis (Fig. 1b, c). In total, 127 individuals were collected (Fig. 1b), from May to October, with a BD ranging from 0.95 to 34.00 cm (Fig. 1c) and a WW from 0.25 to 2761.00 g.

Prey richness

A total of 16,239 prey items were counted in the gut contents, with an average prey concentration of 20 ±22 prey $medusa^{-1}$ in juveniles and 253 ±338 prey $medusa^{-1}$ in adults. After screening, 14 taxa were assigned into three different prey categories based on size criteria: (i) microzooplankton (63-200 µm) containing: foraminifers (phylum Foraminifera), ciliates (phylum Ciliophora), bivalve and gastropod veligers (phylum Mollusca), ostracods and copepods' nauplii (phylum Arthropoda), (ii) mesozooplankton (200-700 µm) composed by: cirripeds' nauplii and harpacticoid, cyclopoid and calanoid copepods (phylum Arthropoda), and (iii) macrozooplankton (>700 µm) including: hydromedusae (phylum Cnidaria), fish eggs (phylum Chordata), crustacean malacostraceans (phylum Arthropoda) and polychaetes (phylum Annelida). Micro- and mesozooplankton specimens were the most recurrent prey, while the predominant group by category was the ciliates (20.8%) and the calanoid copepods (22.8%), respectively. Ciliates were mostly represented by the genus *Codonella*, *Favella*, and *Tintinnopsis*, whilst calanoid copepods were mostly represented by three species: *Acartia clausi* Giesbrecht, 1889, *Paracalanus parvus* (Claus, 1863) and *Pseudocalanus elongatus* (Brady,1865).

Our sampling size was representative of the prey richness in *R. pulmo*'s guts. Indeed, the Chao1 non-parametric estimator indicated that the sampling effort can be considered sufficient when analyzing 84 specimens (Fig. 2a), when S_{obs} (observed richness = 35) is 80% of the S_{Chao1} (total richness estimated

= 39). The number of taxa identified (14 taxa) showed a significant difference between juveniles and adults (df=1, p=0.007), with more taxa in adults (4.2±2.4 taxa medusa^{-1}) compared to juveniles (2.8±1.9 taxa medusa^{-1}) (Fig. 2b).

Among the total specimens analyzed, 13% of the medusae guts were judged empty (17 specimens out of 127). Of them, 10 guts had only items of vegetal origin and the remaining 7 were empty. Vegetal structures, including *Pinus* pollen grains and stellate hair of vascular plants (i.e., *Elaeagnus* sp.) were observed in 61.2% of the guts analyzed, but were removed from the analyses as they were considered as terrestrial contamination. Thus, only 110 individuals were retained for the analyses: 62 juveniles and 48 adults. G_e (%) displayed a marked temporal variability (Fig. 2c). When *R. pulmo* appeared, more than 30% of juveniles presented empty guts, although no empty guts were observed at the beginning of summer (June), and only a few (~10%) until September. Conversely, all adults presented food in their guts from May to July. From July, an increase in the number of empty guts was noted, up to a maximum in October (G_e = 50%).

Objective 1. Frequency of occurrence of prey during R. pulmo ontogeny

The ingested prey covered a size range from 45 to 9,000 µm (Fig. 3a), with most of them (80%) figuring between 165-240 µm. Prey size varied significantly (Kruskal-Wallis, df=1, p<0.001) between juvenile and adult medusae, with juvenile medusae feeding on larger sizes (385±190 µm) than adult medusae (136±60 µm).

Calanoid and harpacticoid copepods were, together with ciliates, the most frequent prey (Fig. 3b). Of them, calanoid copepods doubled their occurrence in juveniles compared to adults, reaching a *FO*=70% in juveniles. Conversely, ciliates, copepods' nauplii and malacostraceans were slightly more frequent in adults compared to juveniles (Fig. 3b). In general, the biggest prey as malacostraceans, were observed with a frequency of occurrence (*FO*) lower than 20%. The less consumed items, i.e., ostracods (ca. 130 µm), hydromedusae (ca. 500 µm) and polychaetes (ca. 9000 µm), present in less than 5% of the specimens, were removed for posterior analyses.

Objective 2. Relative abundance of prey in the diet of R. pulmo pelagic life stages

The relative abundance of prey in the gut contents of the medusae did not follow their seasonal abundance in the environment (Fig. 4). From May to July, the diet of juveniles was composed mainly of calanoid copepods (*N*>25% each month). In August and September, this composition changed to a predominance of foraminifera (*N*>25%), matching with the abundance peak of this group (Fig. 5), and in lower

proportion to ciliates and harpacticoid copepods. The predation on fish eggs was noticeable during May (ca. 20%) and August (8%). In contrast, adults showed a different diet composition, with a predominance of ciliates, mainly in June (ca. 40%), August (*N*>80%) and September (ca. 40%), followed by calanoid and harpacticoid copepods, and bivalve veligers.

Trophic niche breadth suggested a specialist feeding behavior for juvenile and adult medusae with low values in both cases ($NB_{juveniles}$=0.07, NB_{adults}=0.08). Most of the time, when juveniles and adults of different cohorts coexist, the niche overlap was low (13% in June and September, and 22% in May), but increased to medium or high in August (54%) and July (68%), respectively (Fig. 4). The highest overlap observed in July was caused by the predominance of bivalve veligers and calanoid copepods in the diet of both juveniles and adults.

Objective 3. Prey selectivity patterns during R. pulmo ontogeny

The prey selectivity showed a heterogeneous pattern (Fig. 5). Copepods' nauplii and gastropod veligers showed a negative selection most of the time, except for a positive selection of gastropod veligers by the biggest medusae (>30 cm). Foraminifers, calanoid copepods and fish eggs were positively selected by juvenile medusae during some months. It is worth noticing that calanoid copepods were mostly ingested by the smallest sizes of *R. pulmo* (<10 cm), while ciliates were selected by adult medusae (>15 cm) (Fig. 5).

Objective 4: Daily Carbon Ration assessment and predatory impact

The daily carbon ration (*DCR*, mgC consumed per medusa per day) consumed by the medusae differed between prey types during *R. pumo* growth (Fig. 6). At the smallest sizes of *R. pulmo* (<34 mgC or <6 cm of BD), copepods were the most consumed prey, with a *DCR* between ca. 0.01 to 1 mgC medusa^{-1} day^{-1}, decreasing their maximum consumption (95th quantile) on biggest sizes (Fig. 6). Along with medusae growth (from 10 to 2,761g WW, or 34 to 9,387mgC), prey richness increased. Indeed, 95th quantile regression highlighted significant patterns for ciliates, bivalve veligers, cirripeds' nauplii and fish eggs (Fig. 6). For all of them, the *DCR* increased from juvenile to adult medusae. Ciliates *DCR* showed a marked increase in the 95th quantile, from ca. 0.01 to 10 mgC medusa^{-1} day^{-1}. Among mollusks, bivalve veligers contributed significantly more to adult medusae than to juvenile stages *DCR*, while gastropods were an important food resource only for the adults. Finally, fish eggs showed an increase in the mean *DCR* values (50th quantile) from ca. 1 mgC medusa^{-1} day^{-1} in juvenile medusae to 4 mgC medusa^{-1} day^{-1} in adults, and with maxima (95th quantile) ranging from ca. 2 to 30 in juvenile and adult

348 medusae, respectively. For *R. pulmo*, the prey that mostly contributed to the *DCR* were copepods for
349 juveniles, and fish eggs and ciliates for adult medusae.
350 The predatory impact of *R. pulmo* differs during ontogeny. Juveniles fed on a maximum of 5% of
351 the copepods' standing stock per day, whilst adults showed a consumption of 8% of the ciliates' standing
352 stock per day. Fish eggs (mean diameter 1170±126 µm) were consumed with high frequency for juveniles
353 (40%) and adults (20%) with a consumption of approximately 5% of the fish eggs daily standing stock
354 in the lagoon. For the other taxa, the ingestion was lower than 1% of the daily standing stock.
355

Discussion

We have assessed the diet composition of *R. pulmo* pelagic stages and identified ontogenetic dietary shifts. In addition, the analysis of two different cohorts allows evaluating the degree of trophic niche breath overlapping between juveniles and adults. The present study, therefore, provides a baseline to understand the trophic role of *R. pulmo* in plankton food webs.

Rhizostoma pulmo diet composition

R. pulmo fed on different trophic levels, including ciliates, copepods and larger taxa such as fish eggs. These observations are in agreement with the diet reported for this species in the Black Sea (Dönmez & Bat 2019). However, our results contrast with Pérez-Ruzafa et al. (2002) who suggested that *R. pulmo* feed mainly on prey with no active escape ability (e.g., phytoplankton). Such statement is likely biased by the small sample size used (n=5). Our results indicate that *R. pulmo* is a selective filter-feeder, although a wide prey species richness is observed, as for other Rhizostomeae (e.g., Kikinger, 1992; Álvarez-Tello et al., 2016; Nagata & Morandini, 2018; Syazwan et al., 2021) that also include phytoplankton, as detected in this study (e.g., diatoms *Bacillaria paxillifer*) and previously documented for *R. pulmo* (Pérez-Ruzafa et al., 2002; Dönmez & Bat, 2019) and *R. octopus* (Gmelin, 1791) (Hays et al., 2012).

R. pulmo prey size spectrum

Using the only available study to date (Pérez-Ruzafa et al. 2002), Lilley et al. (2009) determined that *R. pulmo* mostly consume (>80%) small prey (<200 µm). Our results show a wide prey size spectrum, from 45 to 9,000 µm, with 80% of prey belonging to the 165-240 µm window. This size range is expected for the Rhizostomeae diet due to their small mouths size (<3,000 µm). In Semaeostomeae species such as *Aurelia* and *Chrysaora*, more than 50% of prey are commonly larger than 800 µm (Lilley et al., 2009). Here, the smaller prey size identified for *R. pulmo* suggests a probable niche partitioning and different trophic roles between these jellyfish.

Consequences of ontogenetic dietary shifts on jellyfish

Ontogenetic dietary shifts are widespread in the animal kingdom (Sánchez-Hernández et al., 2019), and there is evidence that jellyfish can modify their diet over their life span (e.g., Graham & Kroutil, 2001). Here, gut content analysis revealed that *R. pulmo* raised prey richness with increasing size of medusae (supporting our H1), but also fed on specific trophic levels during ontogeny. Juvenile stages of *R. pulmo* showed a marked preference for calanoid copepods (*FO*>60%), whilst the feeding pressure on this taxon

388 decreased when medusae grew. In contrast, an increasing consumption, i.e., daily carbon ration, was
389 observed mainly on ciliates with medusae growth (see Fig. 6). These different prey preferences during
390 ontogeny and the observed niche partitioning of *R. pulmo*, can affect medusae growth and survival,
391 probably with evolutionary advantages for multicohort species. For instance, a niche partitioning
392 between juvenile and adult medusae could reduce competition for food between cohorts, enabling their
393 coexistence in the lagoon.
394 From a food web perspective, this ontogenetic diet shift might modify the impact and pressure
395 across the plankton food web. In shallow areas of the ocean, lakes or lagoons, the classical food web
396 (large phytoplankton-mesozooplankton-fish) is commonly connected with the microbial food web
397 (bacteria-heterotrophic flagellates-protists) by mesozooplankton species such as copepods (Moore et al.,
398 2019). However, mesocosm experiments have shown that gelatinous zooplankton has a top-down control
399 on both the microbial loop (Turk et al., 2008) and the classic food web (Granéli & Turner, 2002). Our
400 results support these observations and further quantify the impact on standing stocks of copepods and
401 ciliates. Juvenile medusae of *R. pulmo* consumed a maximum of 5% of the copepods' daily standing
402 stock, whilst adults showed a consumption of 8% of the ciliates' daily standing stock. These results are
403 close to the reported values for the Rhizostomeae *L. lucerna*, for which a consumption of 6 to 12% of
404 the copepods' daily standing stock was estimated in the southwestern Atlantic coast (Nagata & Morandini,
405 2018), but smaller than the estimations for the giant Japanese Rhizostomeae *Nemopilema nomurai*
406 Kishinouye, 1922 (24% of mesozooplankton daily standing stocks) (Uye, 2008). Estimations on
407 Semaeostomeae species present a wider interstudy variability, ranging from <3% of the crustaceans' daily
408 standing stocks consumed by *Aurelia labiata* Chamisso & Eysenhardt, 1821 and *Cyanea capillata*
409 (Linnaeus, 1758) in Alaska (Purcell, 2003), to 21% of copepods' daily standing stocks consumed by
410 *Aurelia solida* Browne, 1905 in Bizerte lagoon (France) (Gueroun et al., 2020). Despite these differences,
411 we observed that large medusae of *R. pulmo* consumed small percentages of copepods' standing stocks
412 when compared to younger stages, as described for *C. capillata* (Purcell 2003). These results highlight
413 that the predatory impact by some jellyfish species on the copepods' community could be relevant only
414 during the first pelagic stages.
415
416 <u>Potential driving mechanisms of ontogenetic diet changes</u>
417 The high prey richness we detected in large medusae agrees with reports for other scyphomedusae species
418 (Graham & Kroutil, 2001; Padilla-Serrato et al., 2013; Dönmez & Bat, 2019), and might be related to an
419 increasing encounter rate of unabundant taxa with the predator. This support our hypothesis H2 regarding

a diet diversification on adult stages. In contrast, we do reject our hypothesis H3 about an increase of mobile prey, e.g., copepods, in medusae guts along with medusa growth.

To understand these results, we discuss potential feeding mechanisms below. The food selection of filter-feeder medusae depends on the pre-encounter and post-encounter feeding mechanisms. Pre-encounter rates are dependent on current speeds generated by the medusae, escape capacity of the prey, and encounter rate related to predator-prey abundance and size (Costello & Colin, 1994). In this line, our results do not follow the proposed functional model for prey selection of *A. aurita* (Costello & Colin 1995), which hypothesized that the largest medusae might capture more efficiently big mobile prey than juveniles. This explanation is based on the marginal flow speed created by bell pulsation and the increase of encounter rate, which scale with medusae BD (Costello & Colin, 1994), as observed also in Rhizostomeae species (Nagata et al., 2016; Graham et al., 2003). Nevertheless, deviations from this pattern have been already reported. The high presence of copepods in the youngest medusae stages of *Rhopilema nomadica* Galil, Spanier & Ferguson, 1990 (Kuplik & Angel, 2020), *S. meleagris* (Álvarez-Tello et al. 2016), *Pelagia noctiluca* (Forsskål, 1775) (Rosa et al., 2013), and also in *A. aurita* (Costello & Colin, 1994; Graham & Kroutil, 2001), suggests that other mechanisms, besides size, could take place. For instance, small medusae may not be detected by copepods, which could explain their capture. Conversely, copepods' ability to detect marginal flow speeds created by large medusae (BD >10 cm) would result in a low consumption by medusae in adult stage, limiting their trophic impact (Wagner et al., 2020). Beyond that, a faster metabolism and growth rates observed in the youngest specimens compared to adult stage is a probable explanation for *R. pulmo* (Leoni et al., 2021b), as previously suggested for *P. noctiluca* (Rosa et al., 2013).

Once the contact is established, the ingestion of the prey depend on the physical constrains of the feeding structures (Kuplik & Angel, 2020), as well as on the type and amount of nematocysts (Purcell, 1984, 1997, 2003), which suggest that ontogenetic differences partly depend on nematocysts composition (Purcell & Mills, 1988; Carr & Pitt, 2008; Regula et al., 2009). Broadly, the efficiency of the nematocysts type varies between 'soft-' vs 'hard-bodied' prey (Purcell, 2003) and has been widely described on hydromedusae and siphonophores (e.g., Purcell, 1984; Purcell & Mills, 1988; Damian-Serrano et al., 2021). Also, interspecific variations in the feeding ecology of scyphomedusae with similar bell marginal flow have been correlated with differences in nematocysts composition (Purcell, 2003), which have been also observed between stages of development (e.g., planulae, scyphystomeae, ephyrae and medusae) in some Rhizostomeae and Semaeostomae species (reviewed in Calder, 1983). However, a shift in nematocysts composition or proportion during medusae ontogeny have been only identified in cubozoan

452 species (*Chironex fleckeri* Southcott, 1956 and *Chiropsalmus quadrigatus* Haeckel, 1880) and related
453 with prey consumption (Carrette et al., 2002; Oba et al., 2004). In both cases, ontogenetic shifts from a
454 crustacean dominant diet ('hard-bodied' prey) to a fish diet ('soft-bodied' prey) were observed. To our
455 knowledge, only one recent study has analyzed those changes on scyphomedusae (*P. noctiluca*;
456 Ballesteros et al., 2021), while ontogenetic shifts in nematocysts composition remains unknown for *R.*
457 *pulmo*. In our study, the observations could be related to shifts in hydrodynamics and encounter rates
458 (Costello & Colin, 1994), predator-prey behavior (Graham & Kroutil, 2001), or to ontogenetic changes
459 of nematocysts composition.
460
461 Recommendations for future jellyfish gut content analysis
462 The gut content analysis of a wide range of medusae sizes has provided novel information on the trophic
463 ecology of *R. pulmo* medusae stages. Our suggestion is that jellyfish diet and their influence on the food
464 web should consider all stages of development. Moreover, to identify all potential prey, the use of
465 biochemical markers must be implemented (e.g., Marques et al., 2021). This is particularly important on
466 small prey as phytoplankton, microplankton smaller than 63 µm and detritus, already identified as
467 important food items for some jellyfish species. Despite the limitations of direct gut content analysis, this
468 technique provides a baseline for posterior SI analyses (Pitt & Lucas, 2013) and it is the unique approach
469 for which the size of the prey can be measured.
470 Previous studies have analyzed a limited medusae size range or have only focused on one
471 developmental stage (Costello & Colin, 1994; Álvarez-Tello et al., 2016; Milisenda et al., 2018). Because
472 of this, interspecific comparisons on trophic changes, diet compositions, and selectivity patterns should
473 be made with caution, as a wide variety of methodologies and medusae sizes have been used. We stress
474 the need for a standardization of methods (e.g., Nagata & Morandini, 2018) to assess trophic dietary
475 niches of jellyfish that will allow to identify feeding patterns or latitudinal changes, which are not
476 achievable with current knowledge.

Conclusions

Rhizostomeae jellyfish are an important component of the coastal ecosystems, but the dietary niche of most of the species (ca. 85%) remains unknown and therefore their trophic role in food webs. Here, we identified that medusae of *R. pulmo* do not feed on the same prey during ontogeny, providing evidence that this jellyfish modifies its trophodynamics, as other marine animals such as bony fish (Olson, 1996; Costalago et al., 2012), sharks (Bethea et al., 2007), sea turtles (Vélez-Rubio et al., 2016) and marine mammals (Vales et al., 2014). Since *R. pulmo* appear seasonally on most Mediterranean coasts for approximately 7 months (Leoni et al., 2021a), its predatory impact on the planktonic community should be temporally restricted and variable depending on the stage of development, abundances, and prey densities. Here, we provide the first *in situ* estimations of their predatory impact in natural ecosystems. The inclusion of indirect techniques as SI and FA analyses will help to elucidate if some other prey items are neglected by the visual analysis method. Metabarcoding techniques have also proved to be useful in diet composition identification for marine animals (Berry et al., 2015; Bergmann et al., 2021), and should be considered in future analyses of the diet of Rhizostomeae. Our study provides a baseline for understanding the trophic role of this species, and therefore for its better representation in food web models.

Figures

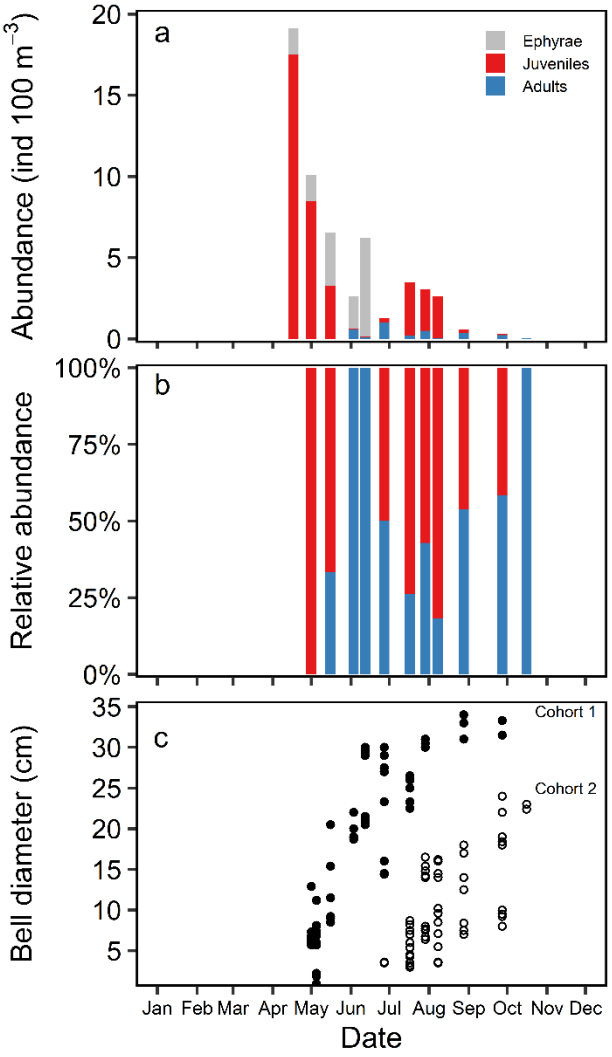
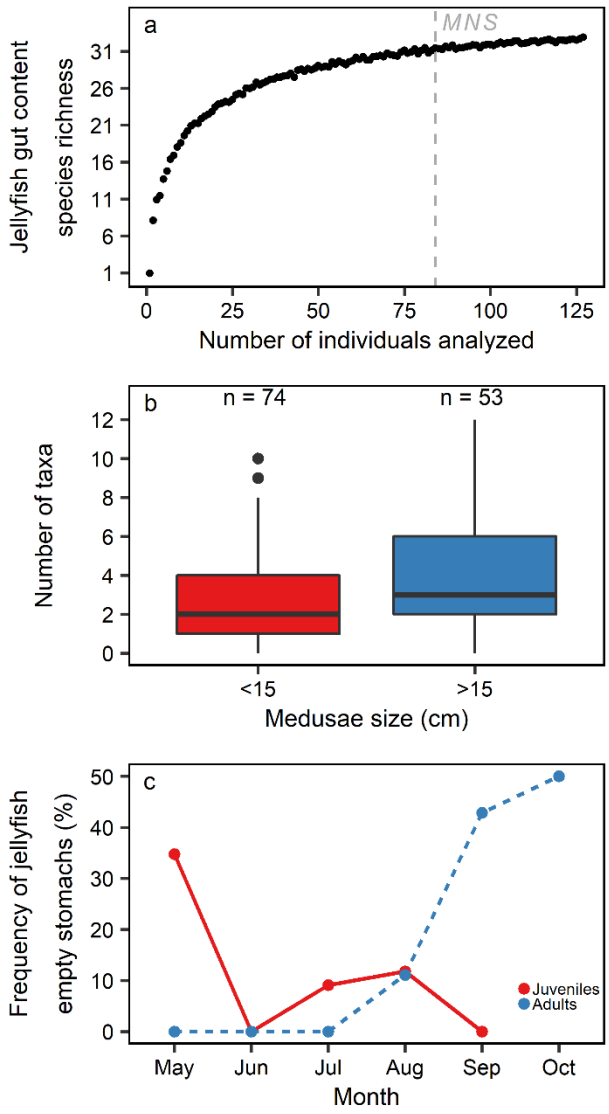
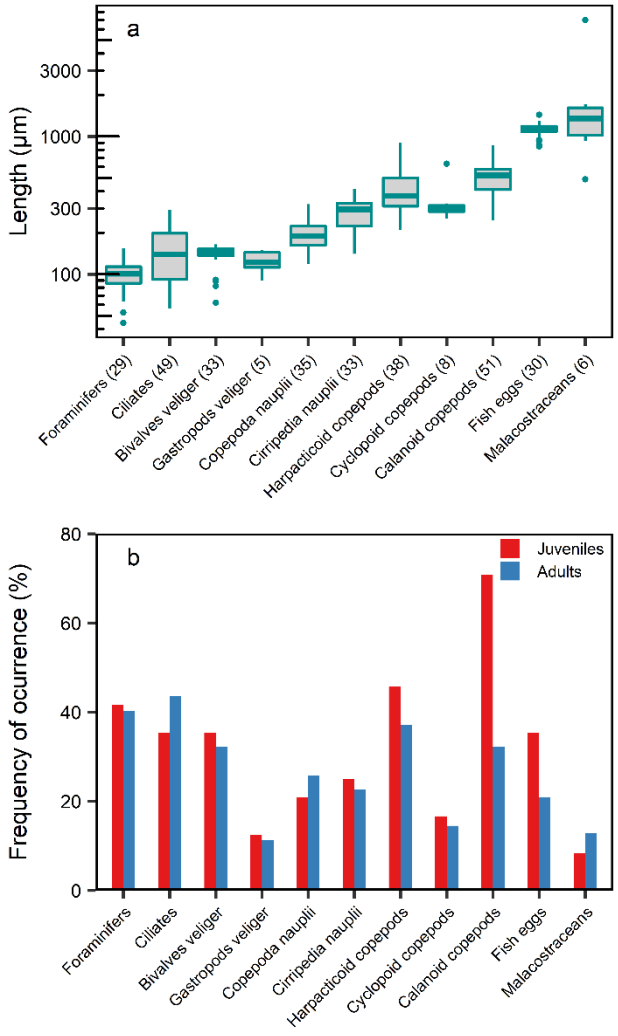


Figure 1. a. Total abundance (ind 100 m^{-3}) of the pelagic stage of *Rhizostoma pulmo* estimated during 2019 in Bages Sigean lagoon (France). Abundance for each developmental stage is indicated in colors: ephyrae in grey, juveniles (bell diameter <15 cm) in red and adults (bell diameter >15 cm) in blue. **b.** Relative abundance (%) of juveniles (red) and adults (blue) stages of *R. pulmo* collected for gut content analysis, and **c.** bell diameter (cm) of medusae collected for gut content analysis by sampling date. Full dots are the individuals from the first cohort and empty dots are the individuals from the second cohort.



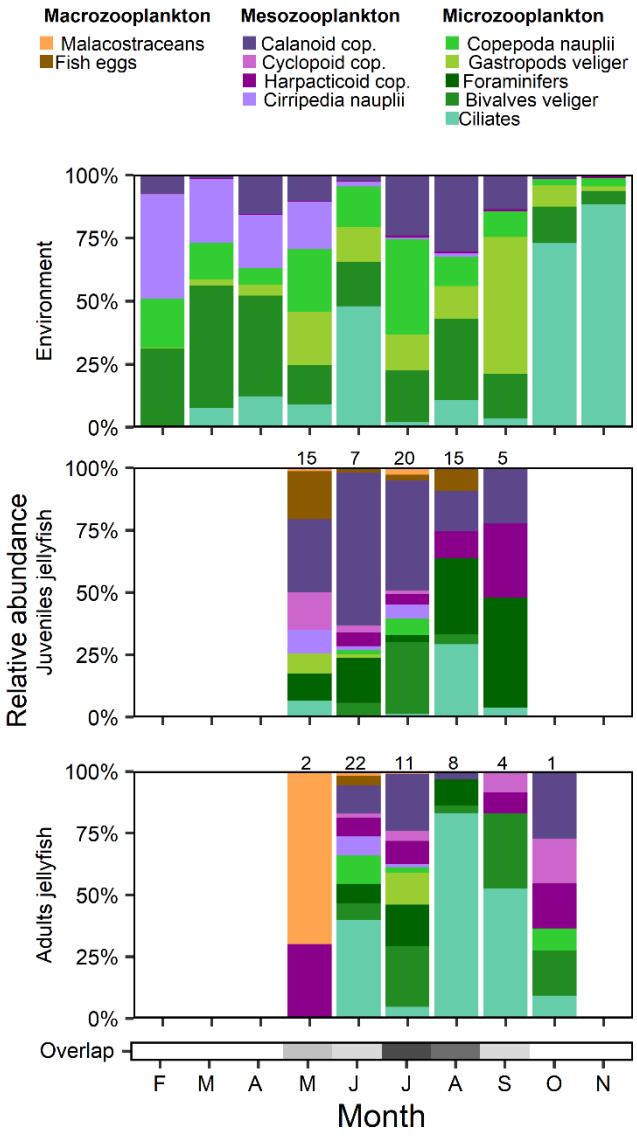
503

504 **Figure 2. a.** Rarefaction curve for prey species richness indicating the required sampling effort to avoid unbiased
505 *Rhizostoma pulmo* medusa diet determination. In the present study, the minimum number of guts (*MNS*) to
506 represent prey richness was 84 (grey dashed line). The cumulative curve was calculated using the content of each
507 medusa gut as a sampling unit (n=127). **b.** Number of taxa observed in the gut content of juveniles (bell diameter
508 <15 cm) and adults (bell diameter >15 cm) specimens of *R. pulmo* (Kruskal-Wallis chi^2=7.38, df=1, p=0.007). **c.**
509 Frequency of medusae empty guts (%) by stage of development (juveniles, represented by a red continuous line,
510 and adults represented by a blue dotted line).
511



512

513 **Figure 3. a.** Size (µm) of the different zooplankton prey items in the gut content of *Rhizostoma pulmo* (n=110)
514 during 2019 in Bages Sigean lagoon (France). Numbers in parenthesis indicate the prey specimens measured. Note
515 that the 'Y' axis is represented in a logarithmic scale. **b.** Frequency of occurrence of the different prey items in the
516 gut content of juvenile (bell diameter <15 cm, in red) and adult (bell diameter >15 cm, in blue) medusae of *R.*
517 *pulmo*.
518



519

520 **Figure 4.** Monthly relative abundance (%) of each taxon observed in Bages Sigean lagoon (France) (top) from
521 February to November 2019, and in the gut content of *Rhizostoma pulmo* medusae juveniles (center) and adults
522 (bottom) from May to October 2019 for the three size categories: Macrozooplankton (>700 µm, in orange scale:
523 malacostraceans and fish eggs), mesozooplankton (200-700 µm, in purple scale: Calanoid, cyclopoid and
524 harpacticoid copepods, and Cirripedia nauplii) and microzooplankton (63-200 µm, in green scale: Copepoda
525 nauplii, gastropods and bivalves veliger, foraminifers and ciliates). Note the temporal variability in the number of
526 medusae analyzed by month (numbers on top). Niche overlap between juveniles and adults is represented at the
527 bottom, only for months with juveniles and adults coexisting (from May to September). Scale from 0 to 100%,
528 with 0% (white) representing no diet overlap, and 100% (black) identical diets.
529

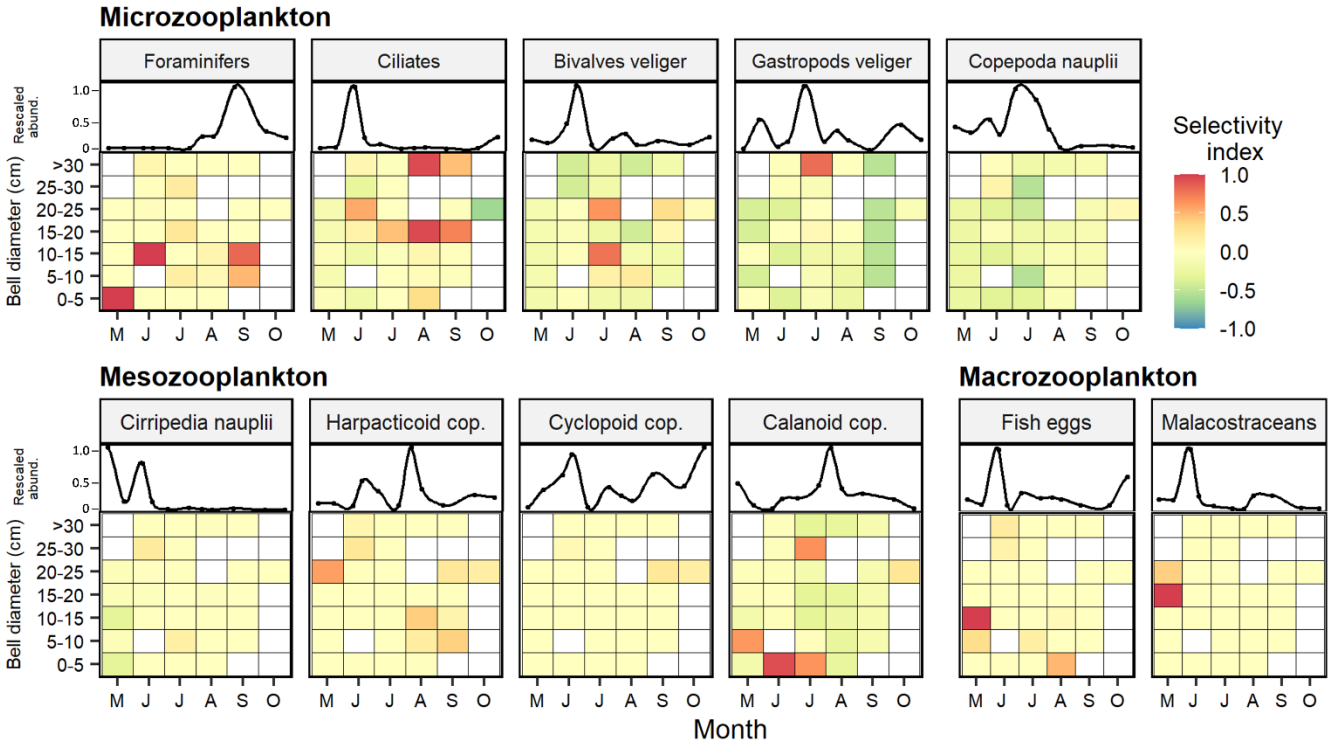
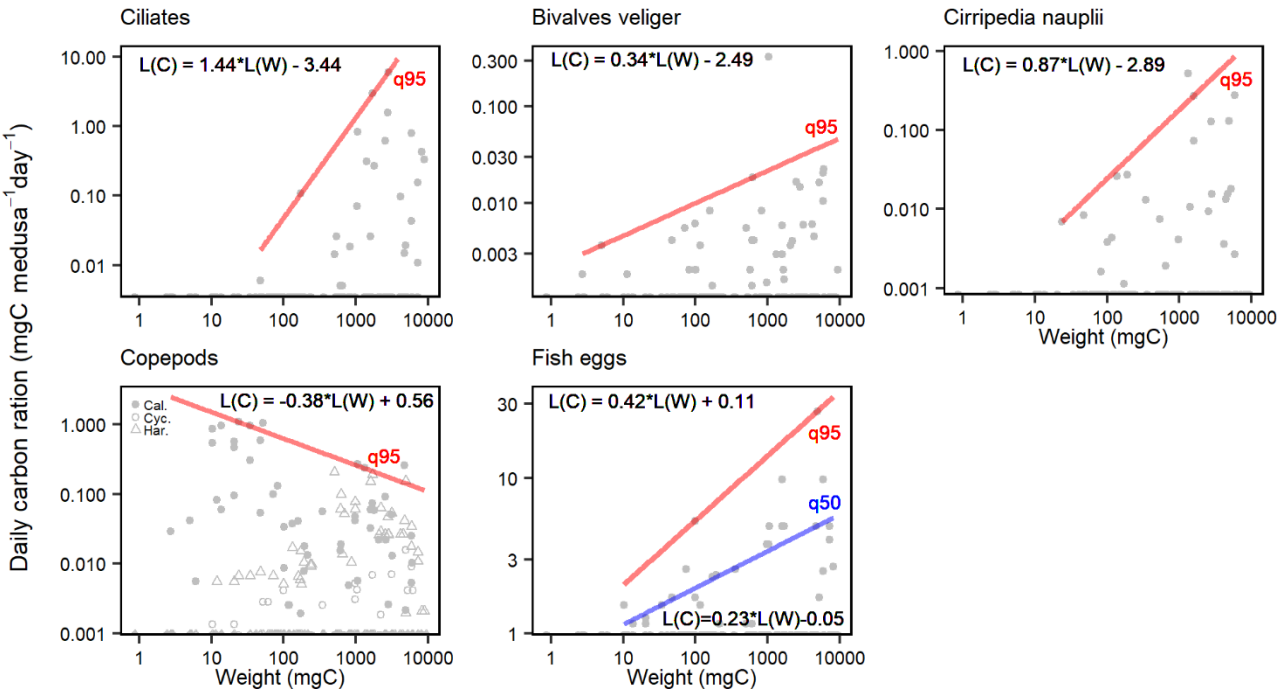


Figure 5. Selectivity index (*LFSI*) for zooplankton prey per month according to *Rhizostoma pulmo* medusa size. Prey taxa are grouped by size on microzooplankton (63-200 µm), mesozooplankton (200-700 µm) and macrozooplankton (>700 µm). Positive (orange-red) or negative (green-blue) index values indicate selectivity at a rate above or below environmental concentration, respectively. Yellow indicates that dietary composition was similar to the proportional abundances in the surrounding zooplankton. White areas denote no data. Prey abundance seasonal patterns during 2019 in Bages Sigean lagoon (France), rescaled to 0-1, are shown in the top panel of each plot.



538

539 **Figure 6.** Daily Carbon Ration (mgC consumed medusa^{-1} day^{-1}) from *Rhizostoma pulmo* gut content in relation
540 with the medusae weight (in mgC). Each point represents one medusa and regression lines the quantile 50% (blue)
541 and 95% (red). Only the statistical significative regressions ($p<0.05$) are represented. Equations correspond to the
542 quantile regressions between log10 Daily Carbon Ration (L(C) in mgC medusa^{-1} day^{-1}) and the log10 medusae
543 weight (L(W) in mgC). 'X' and 'Y' axis are represented in logarithmic scale. Note that the 'Y' axis scale differs
544 among taxa. Cal.=Calanoid, Cyc=Cyclopoid, Har=Harpacticoid.

Acknowledgements

This study was conducted as part of the Ph.D. dissertation of V.L. and was supported by the National Agency for Research and Innovation, ANII, Uruguay (code POS_CFRA_2017_1_147109). Part of the analyses were financed by EMBRC-France, a program managed by the ANR within the “Investing for the future program” under the reference ANR-10-INBS-02. The authors acknowledge the funding support of the French ANR project CIGOEF (grant ANR-17-CE32-0008-01), and the support of LabEx CeMEB, an ANR "Investissements d'avenir" program (ANR‐10‐LABX‐04‐01). We acknowledge Sun Hee Lee, Ruben Tournier and Raquel Marques for their assistance during fieldwork. We greatly appreciated the advice from Renato M. Nagata for laboratory manipulations and the advice from Alejandro Ariza for graphic designs. We are grateful to the kitesurf center ‘Narbonne Kite Passion’ and local fishermen for the logistic support, in particular to the skipper Stéphane Marin, for his support on board and for sharing his expertise on the lagoon. We would like to thank the reviewers and the associated editor of the previous version of this manuscript, whose comments greatly improved the quality of the present manuscript.

559 **References**

560 Álvarez-Tello, F. J., J. López-Martínez, & D. B. Lluch-Cota, 2016. Trophic spectrum and feeding
561 pattern of cannonball jellyfish *Stomolophus meleagris* (Agassiz, 1862) from central Gulf of California.
562 Journal of the Marine Biological Association of the United Kingdom 96: 1217–1227.

563 Ballesteros, A., C. Östman, A. Santín, M. Marambio, M. Narda, & J.-M. Gili, 2021. Cnidome and
564 morphological features of *Pelagia noctiluca* (Cnidaria: Scyphozoa) throughout the different life cycle
565 stages. Frontiers in Marine Science 8: 714503.

566 Båmstedt, U., & M. B. Martinussen, 2000. Estimating digestion rate and the problem of individual
567 variability, exemplified by a scyphozoan jellyfish. Journal of Experimental Marine Biology and
568 Ecology 251: 1–15.

569 Basso, L., L. Rizzo, M. Marzano, M. Intranuovo, B. Fosso, G. Pesole, S. Piraino, & L. Stabili, 2019.
570 Jellyfish summer outbreaks as bacterial vectors and potential hazards for marine animals and humans
571 health? The case of *Rhizostoma pulmo* (Scyphozoa, Cnidaria). Science of The Total Environment 692:
572 305–318.

573 Bergmann, M. P. M. van Z., B. D. Postaire, K. Gastrich, M. R. Heithaus, L. A. Hoopes, K. Lyons, Y. P.
574 Papastamatiou, E. V. C. Schneider, B. A. Strickland, B. S. Talwar, D. D. Chapman, & J. Bakker, 2021.
575 Elucidating shark diets with DNA metabarcoding from cloacal swabs. Molecular Ecology Resources
576 21: 1056–1067.

577 Berry, O., C. Bulman, M. Bunce, M. Coghlan, D. C. Murray, & R. D. Ward, 2015. Comparison of
578 morphological and DNA metabarcoding analyses of diets in exploited marine fishes. Marine Ecology
579 Progress Series 540: 167–181.

580 Bethea, D. M., L. Hale, J. K. Carlson, E. Cortés, C. A. Manire, & J. Gelsleichter, 2007. Geographic and
581 ontogenetic variation in the diet and daily ration of the bonnethead shark, *Sphyrna tiburo*, from the
582 eastern Gulf of Mexico. Marine Biology 152: 1009–1020.

583 Bonnet, D., 2013. Etude du plancton gélatineux sur la façade Méditerranéenne- GELAMED-
584 Programme Liteau (189)- rapport d'activités. , http://docplayer.fr/106255915-Gelamed-etude-du-
585 plancton-gelatineux-sur-la-facade-mediterraneenne.html.

586 Bosch-Belmar, M., G. Milisenda, L. Basso, T. K. Doyle, A. Leone, & S. Piraino, 2021. Jellyfish
587 impacts on marine aquaculture and fisheries. Reviews in Fisheries Science & Aquaculture 29: 242–259.

588 Brodeur, R., H. Sugisaki, & G. Hunt, 2002. Increases in jellyfish biomass in the Bering Sea:
589 implications for the ecosystem. Marine Ecology Progress Series 233: 89–103.

590 Cade, B. S., & B. R. Noon, 2003. A gentle introduction to quantile regression for ecologists. Frontiers
591 in Ecology and the Environment 1: 412–420.

592 Calder, D. R., 1983. Nematocysts of stages in the life cycle of *Stomotophus meleagris*, with keys to
593 scyphistomae and ephyrae of some western Atlantic Scyphozoa. Canadian Journal of Zoology 61:
594 1185–1192.

595 Carr, E. F., & K. A. Pitt, 2008. Behavioural responses of zooplankton to the presence of predatory
596 jellyfish. Journal of Experimental Marine Biology and Ecology 354: 101–110.

597 Carrette, T., P. Alderslade, & J. Seymour, 2002. Nematocyst ratio and prey in two Australian
598 cubomedusans, *Chironex fleckeri* and *Chiropsalmus* sp. Toxicon 40: 1547–1551.

599 Cesmat, L., K. Dusserre, A. Fiandrino, & L. Benau, 2012. Etude hydrologique de l'étang de Bages-
600 Sigean. Impact de différents scénarii d'aménagement et de gestion sur les variations de salinité. Agence
601 de l'eau, Conservatoire des Espaces Naturels du Languedoc-Roussillon, Parc Naturel Régional de la
602 Narbonnaise en Méditerranée, Ifremer, France: 84.

603 Chao, A., R. K. Colwell, C.-W. Lin, & N. J. Gotelli, 2009. Sufficient sampling for asymptotic minimum
604 species richness estimators. Ecology 90: 1125–1133.

605 Costalago, D., J. Navarro, I. Álvarez-Calleja, & I. Palomera, 2012. Ontogenetic and seasonal changes
606 in the feeding habits and trophic levels of two small pelagic fish species. Marine Ecology Progress
607 Series 460: 169–181.

608 Costello, J. H., & S. P. Colin, 1994. Morphology, fluid motion and predation by the scyphomedusa
609 *Aurelia aurita*. Marine Biology 121: 327–334.

610 Costello, J. H., & S. P. Colin, 1995. Flow and feeding by swimming scyphomedusae. Marine Biology
611 124: 399–406.

612 Cury, P., Y. Shin, B. Planque, J. Durant, J. Fromentin, S. Kramerschadt, N. Stenseth, M. Travers, & V.
613 Grimm, 2008. Ecosystem oceanography for global change in fisheries. Trends in Ecology & Evolution
614 23: 338–346.

615 da Silveira, E. L., N. Semmar, J. E. Cartes, V. M. Tuset, A. Lombarte, E. L. C. Ballester, & A. M. Vaz-
616 dos-Santos, 2020. Methods for trophic ecology assessment in fishes: A critical review of stomach
617 analyses. Reviews in Fisheries Science & Aquaculture 28: 71–106.

618 Damian-Serrano, A., S. H. D. Haddock, & C. W. Dunn, 2021. The evolution of siphonophore tentilla
619 for specialized prey capture in the open ocean. Proceedings of the National Academy of Sciences
620 National Academy of Sciences 118: 1–9.

621 Dawson, M. N., & W. M. Hamner, 2009. A character-based analysis of the evolution of jellyfish
622 blooms: adaptation and exaptation. Hydrobiologia 616: 193–215.

623 Dönmez, M. A., & L. Bat, 2019. Detection of feeding dietary *Rhizostoma pulmo* (Macri, 1778) in
624 Samsun coasts of the Black Sea, Turkey. Ege Journal of Fisheries and Aquatic Sciences 36: 135–144.

625 Fleming, N. E. C., C. Harrod, J. Newton, & J. D. R. Houghton, 2015. Not all jellyfish are equal:
626 isotopic evidence for inter- and intraspecific variation in jellyfish trophic ecology. PeerJ 3: 1–21.

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

631 Graham, W. M., & R. M. Kroutil, 2001. Size-based prey selectivity and dietary shifts in the jellyfish,
632 *Aurelia aurita*. Journal of Plankton Research 23: 67–74.

633 Graham, W. M., D. L. Martin, D. L. Felder, V. L. Asper, & H. M. Perry, 2003. Ecological and economic
634 implications of a tropical jellyfish invader in the Gulf of Mexico. Marine Bioinvasions: Patterns,
635 Processes and Perspectives 53–69.

636 Granéli, E., & J. Turner, 2002. Top-down regulation in ctenophore-copepod-ciliate-diatom-
637 phytoflagellate communities in coastal waters: a mesocosm study. Marine Ecology Progress Series 239:
638 57–68.

639 Gueroun, S. K. M., J. C. Molinero, S. Piraino, & M. N. D. Yahia, 2020. Population dynamics and
640 predatory impact of the alien jellyfish *Aurelia solida* (Cnidaria, Scyphozoa) in the Bizerte Lagoon
641 (southwestern Mediterranean Sea). Mediterranean Marine Science 21: 22–35.

642 Hays, G. C., T. Bastian, T. K. Doyle, S. Fossette, A. C. Gleiss, M. B. Gravenor, V. J. Hobson, N. E.
643 Humphries, M. K. S. Lilley, N. G. Pade, & D. W. Sims, 2012. High activity and Levy searches:
644 jellyfish can search the water column like fish. Proceedings of the Royal Society B: Biological
645 Sciences 279: 465–473.

646 Hays, G. C., T. K. Doyle, & J. D. R. Houghton, 2018. A paradigm shift in the trophic importance of
647 jellyfish?. Trends in Ecology & Evolution 33: 874–884.

648 Hecq, J. H., A. Collignon, & A. Goffart, 2014. Atlas du zooplancton des eaux côtières corses. Travail
649 de synthèse réalisé à la demande de l'Agence de l'Eau RMC, France,
650 http://orbi.ulg.ac.be/handle/2268/168.

651 Hyslop, J. F., 1980. Stomach content analysis-a review on methods and their application. Journal of
652 Fish Biology 17: 411–429.

653 Jiménez-Valverde, A., & J. Hortal, 2003. Las curvas de acumulación de especies y la necesidad de
654 evaluar la calidad de los inventarios biológicos. Revista Ibérica de Aracnología 8: 151–161.

655 Kikinger, R., 1992. *Cotylorhiza tuberculata* (Cnidaria: Scyphozoa) - Life history of a stationary
656 population. Marine Ecology 13: 333–362.

657 Krebs, C. J., 1999. Ecological methodology. Benjamin Cummings, New York.

658 Kuplik, Z., & D. L. Angel, 2020. Diet composition and some observations on the feeding ecology of
659 the rhizostome *Rhopilema nomadica* in Israeli coastal waters. Journal of the Marine Biological
660 Association of the United Kingdom 100: 681–689.

661 Langton, R. W., 1982. Diet overlap between Atlantic cod, *Gadus morhua*, silver hake, *Merluccius*
662 *bilinearis*, and fifteen other northwest Atlantic finfish. Fishery Bulletin 80: 16.

663 Larson, R. J., 1991. Diet, prey selection and daily ration of *Stomolophus meleagris*, a filter-feeding
664 scyphomedusa from the NE Gulf of Mexico. Estuarine, Coastal and Shelf Science 32: 511–525.

Lebrato, M., M. Pahlow, J. R. Frost, M. Küter, P. Jesus Mendes, J. Molinero, & A. Oschlies, 2019. Sinking of gelatinous zooplankton biomass increases deep carbon transfer efficiency globally. Global Biogeochemical Cycles 33: 1764–1783.

Leoni, V., D. Bonnet, E. Ramírez‐Romero, & J. C. Molinero, 2021a. Biogeography and phenology of the jellyfish *Rhizostoma pulmo* (Cnidaria: Scyphozoa) in southern European seas. Global Ecology and Biogeography 30: 622–639.

Leoni, V., J. C. Molinero, M. Meffre, & D. Bonnet, 2021b. Variability of growth rates and thermohaline niches of *Rhizostoma pulmo*'s pelagic stages (Cnidaria: Scyphozoa). Marine Biology 168: 107.

Lilley, M. K. S., J. D. R. Houghton, & G. C. Hays, 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: 39.

Lucas, C. H., D. O. B. Jones, C. J. Hollyhead, R. H. Condon, C. M. Duarte, W. M. Graham, K. L. Robinson, K. A. Pitt, M. Schildhauer, & J. Regetz, 2014. Gelatinous zooplankton biomass in the global oceans: geographic variation and environmental drivers. Global Ecology and Biogeography 23: 701–714.

Marques, R., D. Bonnet, C. Carré, C. Roques, & A. M. Darnaude, 2021. Trophic ecology of a blooming jellyfish (*Aurelia coerulea*) in a Mediterranean coastal lagoon. Limnology and Oceanography 66: 141–157.

Milisenda, G., S. Rossi, S. Vizzini, V. L. Fuentes, J. E. Purcell, U. Tilves, & S. Piraino, 2018. Seasonal variability of diet and trophic level of the gelatinous predator *Pelagia noctiluca* (Scyphozoa). Scientific Reports 8: 12140.

Moore, M. V., B. T. De Stasio, K. N. Huizenga, & E. A. Silow, 2019. Trophic coupling of the microbial and the classical food web in Lake Baikal, Siberia. Freshwater Biology 64: 138–151.

Nagata, R. M., & A. C. Morandini, 2018. Diet, prey selection, and individual feeding rates of the jellyfish *Lychnorhiza lucerna* (Scyphozoa, Rhizostomeae). Marine Biology 165: 187.

Nagata, R., A. Morandini, S. Colin, A. Migotto, & J. Costello, 2016. Transitions in morphologies, fluid regimes, and feeding mechanisms during development of the medusa *Lychnorhiza lucerna*. Marine Ecology Progress Series 557: 145–159.

Nagata, R., M. Moreira, C. Pimentel, & A. Morandini, 2015. Food web characterization based on $\delta^{15}N$ and $\delta^{13}C$ reveals isotopic niche partitioning between fish and jellyfish in a relatively pristine ecosystem. Marine Ecology Progress Series 519: 13–27.

Nastav, B., M. Malej, A. Malej Jr., & A. Malej, 2013. Is it possible to determine the economic impact of jellyfish outbreaks on fisheries? A case study – Slovenia. Mediterranean Marine Science 14: 214.

Oba, A., M. Hidaka, & S. Iwanaga, 2004. Nematocyst composition of the cubomedusan *Chiropsalmus quadrigatus* changes with growth. Hydrobiologia 530/531: 173–177.

Olson, M. H., 1996. Ontogenetic niche shifts in Largemouth Bass: Variability and consequences for first-year growth. Ecology 77: 179–190.

Padilla-Serrato, J. G., J. López-Martínez, A. Acevedo-Cervantes, E. Alcántara-Razo, & C. H. Rábago-Quiroz, 2013. Feeding of the scyphomedusa *Stomolophus meleagris* in the coastal lagoon Las Guásimas, northwest Mexico. 23: 9.

Pauly, D., W. M. Graham, S. Libralato, L. Morissette, & M. L. D. Palomares (eds), 2009. Jellyfish in ecosystems, online databases, and ecosystem models. Hydrobiologia 616: 67–85.

Pérez-Ruzafa, A., J. Gilabert, J. M. Gutiérrez, A. I. Fernández, C. Marcos, & S. Sabah, 2002. Evidence of a planktonic food web response to changes in nutrient input dynamics in the Mar Menor coastal lagoon, Spain. Hydrobiologia 359–369.

Pitt, K. A., R. M. Connolly, & J. E. Purcell, 2009. Stable isotope and fatty acid tracers in energy and nutrient studies of jellyfish: a review. 616: 119–132.

Pitt, K. A., & C. H. Lucas (eds), 2013. Jellyfish blooms. Springer, Dordrecht.

PNRNM, 2018. Réhabilitation et valorisation de l'étang de Bages-Sigean. Les Zones Humides. , http://www.zones-humides.org/agir/retours-experiences/rehabilitation-et-valorisation-de-l039etang-de-bages-sigean.

Purcell, J., 1992. Effects of predation by the scyphomedusan *Chrysaora quinquecirrha* on zooplankton populations in Chesapeake Bay, USA. Marine Ecology Progress Series 87: 65–76.

Purcell, J. E., 1984. The functions of nematocysts in prey capture by epipelagic siphonophores (Coelenterata, Hydrozoa). The Biological Bulletin 166: 310–327.

Purcell, J. E., 1997. Pelagic cnidarians and ctenophores as predators: Selective predation, feeding rates, and effects on prey populations. Annales de l'Institut océanographique 73: 125–137.

Purcell, J. E., 2003. Predation on zooplankton by large jellyfish, *Aurelia labiata*, *Cyanea capillata* and *Aequorea aequorea*, in Prince William Sound, Alaska. Marine Ecological Progress Series 16.

Purcell, J. E., 2009. Extension of methods for jellyfish and ctenophore trophic ecology to large-scale research. Hydrobiologia 616: 23–50.

Purcell, J. E., 2018. Of jellyfish, fish, and humans. ICES Journal of Marine Science 75: 1235–1244.

Purcell, J. E., V. Fuentes, D. Atienza, U. Tilves, D. Astorga, M. Kawahara, & G. C. Hays, 2010. Use of respiration rates of scyphozoan jellyfish to estimate their effects on the food web. Hydrobiologia 645: 135–152.

Purcell, J. E., & C. E. Mills, 1988. The correlation between nematocyst types and diets in pelagic Hydrozoa In Hessinger, D. A., & H. M. Lenhoff (eds), The Biology of Nematocysts. Academic Press: 464–483.

R Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/.

Regula, C., S. Colin, J. Costello, & H. Kordula, 2009. Prey selection mechanism of ambush-foraging hydromedusae. Marine Ecology Progress Series 374: 135–144.

737 Robinson, K., & W. Graham, 2014. Warming of subtropical coastal waters accelerates *Mnemiopsis*
738 *leidyi* growth and alters timing of spring ctenophore blooms. Marine Ecology Progress Series 502:
739 105–115.

740 Rosa, S., M. Pansera, A. Granata, & L. Guglielmo, 2013. Interannual variability, growth, reproduction
741 and feeding of *Pelagia noctiluca* (Cnidaria: Scyphozoa) in the Straits of Messina (Central
742 Mediterranean Sea): Linkages with temperature and diet. Journal of Marine Systems 111–112: 97–107.

743 Rossi, J. P., 2011. rich: an R package to analyse species richness. Diversity 3: 112–120.

744 Sánchez-Hernández, J., A. D. Nunn, C. E. Adams, & P.-A. Amundsen, 2019. Causes and consequences
745 of ontogenetic dietary shifts: a global synthesis using fish models: Ontogenetic dietary shifts.
746 Biological Reviews 94: 539–554.

747 Sieracki, C., M. Sieracki, & C. Yentsch, 1998. An imaging-in-flow system for automated analysis of
748 marine microplankton. Marine Ecology Progress Series 168: 285–296.

749 Strauss, R. E., 1979. Reliability estimates for Ivlev's electivity index, the forage ratio, and a proposed
750 linear index of food selection. Transactions of the American Fisheries Society 108: 344–352.

751 Syazwan, W. M., A. Y.-H. Then, V. C. Chong, & M. Rizman-Idid, 2021. Trophic ecology of a tropical
752 scyphozoan community in coastal waters: Insights from stomach content and stable isotope analyses.
753 Continental Shelf Research 225: 104481.

754 Trégouboff, G., & M. Rose, 1978. Manuel de planctonologie méditerranéenne. France.

755 Turk, V., D. Lučić, V. Flander-Putrle, & A. Malej, 2008. Feeding of *Aurelia* sp. (Scyphozoa) and links
756 to the microbial food web. Marine Ecology 29: 495–505.

757 Uye, S., 1982. Length-weight relationships of important zooplankton from the Inland Sea of Japan.
758 Journal of the Oceanographical Society of Japan 38: 149–158.

759 Uye, S., 2008. Blooms of the giant jellyfish *Nemopilema nomurai*: a threat to the fisheries
760 sustainability of the East Asian Marginal Seas. Plankton and Benthos Research 3: 125–131.

761 Vales, D. G., F. Saporiti, L. Cardona, L. R. De Oliveira, R. A. Dos Santos, E. R. Secchi, A. Aguilar, &
762 E. A. Crespo, 2014. Intensive fishing has not forced dietary change in the South American fur seal
763 *Arctophoca* (=*Arctocephalus*) *australis* off Río de la Plata and adjoining areas. Aquatic Conservation:
764 Marine and Freshwater Ecosystems 24: 745–759.

765 Vélez-Rubio, G. M., L. Cardona, M. López-Mendilaharsu, G. Martínez Souza, A. Carranza, D.
766 González-Paredes, & J. Tomás, 2016. Ontogenetic dietary changes of green turtles (*Chelonia mydas*) in
767 the temperate southwestern Atlantic. Marine Biology 163: 57.

768 Wagner, Z., J. H. Costello, & S. P. Colin, 2020. Fluid and Predator-Prey Interactions of Scyphomedusae
769 Fed Calanoid Copepods. Fluids 5: 60.

770 Wang, P., F. Zhang, M. Liu, S. Sun, & H. Xian, 2020. Isotopic evidence for size-based dietary shifts in
771 the jellyfish *Cyanea nozakii* in the northern East China Sea. Journal of Plankton Research 1–13.

West, E. J., K. A. Pitt, D. T. Welsh, K. Koop, & D. Rissik, 2009. Top-down and bottom-up influences of jellyfish on primary productivity and planktonic assemblages. Limnology and Oceanography 54: 2058–2071.

Wickham, H., 2016. ggplot2: Elegant Graphics for Data Analysis. Springer International Publishing, https://www.springer.com/gp/book/9783319242750.