Estuarine, Coastal and Shelf Science 201 (2018) 29–39

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

journal homepage: www.elsevier.com/locate/ecss

Reproductive and bloom patterns of *Pelagia noctiluca* in the Strait of Messina, Italy

G. Milisenda [a, b, c, *], A. Martinez-Quintana [d], V.L. Fuentes [d], M. Bosch-Belmar [a], G. Aglieri [a, c], F. Boero [a, c], S. Piraino [a, c, **]

[a] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), University of Salento, Lecce, Italy
[b] Dipartimento Terra e Ambiente, CNR-IAMC, sezione di Mazara del Vallo, Via Luigi Vaccara 61, 91026 Mazara del Vallo, Italy
[c] Consorzio Nazionale Interuniversitario per le Scienze del Mare (CoNISMa), Rome, Italy
[d] Institut de Ciencies del Mar, ICMCSIC, Passeig Marıtim de la Barceloneta 37-49, Barcelona 08003, Spain

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form
25 November 2015
Accepted 1 January 2016
Available online 13 January 2016

Keywords:
Gonadosomatic index
Biochemical composition
Spawning
Reproductive cycle
Scyphozoa

ABSTRACT

Investigations on sexual reproduction of jellyfish are essential to understanding mechanisms and patterns of outbreaks formation. *Pelagia noctiluca* (Forskål, 1775) (Scyphozoa) is known as the predominant jellyfish species with direct development in Western and Central Mediterranean Sea. In this paper we used integrated morphometric, histological, and biochemical approaches to investigate the annual reproductive biology of *P. noctiluca* from the Strait of Messina (South Thyrrenian Sea), a key proliferation area for this species due to favourable temperatures and high productivity. From November 2011 to September 2012, *P. noctiluca* sexual reproduction occurred throughout the year, with two seasonal peaks (autumn, spring) of spawning and embryonic development. Gonads of female *P. noctiluca* were characterized by a large amount of mature eggs of small size (diameter < 200 μm) during high food availability, whereas fewer, larger eggs (diameter > 200 μm) were detected during low availability of prey. Two morphometric indexes were applied: the Gonad-Somatic Index (GSI, gonadal/somatic tissue dry weight ratio) and Fecundity Index (FI, n° eggs mm^{-2} * gonadal dry weight). The FI showed longer spawning periods than the GSI, providing a better causal-mechanistic explanation for the year-round occurrence of *P. noctiluca* in the Strait of Messina. Protein contents of the gonads changed seasonally, with the highest concentrations during the pre-spawning periods. We suggest that investigations on jellyfish sexual reproduction can provide biological information relevant for understanding mechanisms of jellyfish blooms as well as for the management of coastal zones affected by outbreaks of gelatinous species.

1. Introduction

In recent years, jellyfish blooms have attracted considerable scientific interest for their potential impacts on human activities and ecosystem functioning (Graham et al., 2014), with special attention to jellyfish as predators and gelatinous biomass as a carbon sink. The ability to form blooms is a natural feature of jellyfish, which often occurs seasonally, sometime predictably, and yet seemingly abruptly each spring and summer (Hamner and Dawson, 2009).

Pelagia noctiluca (Forskål, 1775) (Scyphozoa, Semaeostomeae, Pelagiidae) is the most abundant jellyfish in the Mediterranean Sea (Russell, 1970), perhaps because of its short generation times. Fertilized eggs develop into planulae in three days (Delap, 1906), which undergo metamorphosis in 92 h into the planktonic ephyrae (Sandrini and Avian, 1983). At 8 mm in diameter, a juvenile stage is characterized by the development of oral arms and marginal tentacles (Russell, 1970), whereas adult specimens have usually a bell diameter greater than 35 mm (Sandrini and Avian, 1991). This is an outbreak-forming species (Arai, 2001, 1997; Malej, 1989; Mills, 2001; Purcell, 2005), often appearing in persistent large swarms with densities over 100 medusae m^{-3} for periods of days to weeks (Malej, 1989; Zavodnik, 1987). Massive populations of *P. noctiluca* in

* Corresponding author. Dipartimento Terra e Ambiente, CNR-IAMC, sezione di Mazara del Vallo, Via Luigi Vaccara 61, 91026 Mazara del Vallo, Italy.
** Corresponding author. Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali (DiSTeBA), University of Salento, Lecce, Italy.
E-mail addresses: giacomo.milisenda@gmail.com (G. Milisenda), stefano.piraino@unisalento.it (S. Piraino).

http://dx.doi.org/10.1016/j.ecss.2016.01.002
0272-7714/

the Mediterranean Sea are commonly found from March to June (Mariottini et al., 2008; Rosa et al., 2013). These medusae represent an interfering plague for coastal human activities, with dramatic socio-economic implications, and a serious problem for the ecosystem because of their overall high predatory pressure in the epipelagic food web (Canepa et al., 2014a; Rosa et al., 2013). They also are important predators of fish eggs and larvae and potential competitors with zooplanktivorous fishes (Purcell et al., 2014a,b; Sabatés et al., 2010). Thus, clarifying the mechanisms that determine the sexual reproduction of *P. noctiluca* is fundamental to predict its sudden outbreaks and to implement appropriate management and mitigation strategies.

The swarms of *P. noctiluca*, a voracious zooplanktivore, are directly influenced by food availability and favourable environmental conditions (Sandrini and Avian, 1991). Local-scale factors related to high primary production may lead to increased abundance of herbivorous crustacean prey and to higher *P. noctiluca* individual growth and reproduction (Kogovšek et al., 2010) and population blooms (Boero, 2013). Sexual reproduction requires a large investment of energy for the development of gonadal tissues and reproductive success directly depends on the amount of ingested food or on previously stored reserves (Fernández and Camacho, 2005). Climatic drivers also contribute to seasonal patterns in feeding, growth, reproduction and abundance of all marine organisms (Gori et al., 2013), including jellyfish (Molinero et al., 2005). Water temperature directly affects the life cycle and metabolism of most aquatic organisms. In *P. noctiluca*, feeding and metabolism are directly proportional to sea water temperature (Malej et al., 1986; Morand et al., 1987). Lower temperatures lead to slower swimming, reduced foraging ability, and higher prey digestion times (Sandrini and Avian, 1989). In contrast, higher temperatures result in faster metabolism and increased food requirement (Lilley et al., 2014a). Thus, water temperature affects oocyte development both by inducing metabolic changes and by influencing the amount of ingested food (Avian et al., 1991). By affecting the storage cycle, water temperature plays a key role in gonadal development, growth, and gamete differentiation (Ansell, 1972; Carrasco et al., 2006).

Gonad maturation can be specifically monitored by the biochemical changes occurring throughout gametogenesis, which involves active mobilisation and synthesis of organic molecules (Giese, 1959; Pillay and Nair, 1973). In particular, large amounts of nucleic acids are needed to produce many sperm heads, whereas proteins and especially lipids are highly mobilised and stored in oocytes (Pillay and Nair, 1973). Indeed, lipids can store higher energy content per unit volume than proteins or carbohydrates (Torreiro et al., 1998). Consequently, diet has a major effect on the development of gonads and fecundity. For instance, lipids affect the composition of fish eggs most among the dietary constituents of brood stock (Watanabe et al., 1984). The quantity and quality of brood stock nutritional reserves determine the fecundity of the spawners and the quality of the gametes (Huang et al., 2010).

The spawning period of *P. noctiluca* is not clearly defined, due to inconsistencies among different geographic areas. It occurs in autumn in the Bay of Biscay (Atlantic Ocean) and the western side of the English Channel (Kramp, 1924; Russell, 1970). Conversely, in the Gulf of Naples, mature individuals were reported throughout the year, whereas different larval stages were observed from November to March (Lo Bianco, 1909). Along the Mediterranean coast of France (Franqueville, 1971; Lilley et al., 2014a; Morand et al., 1992) described a summer-autumn reproductive period. However, both Lo Bianco (1909) and Kramp (1924) hypothesized that *P. noctiluca* might have a longer reproductive period in the Mediterranean Sea than in the Atlantic ocean (Kramp, 1924; Lo Bianco, 1909). A longer period was found also by in the North Adriatic, where *P. noctiluca* gonad maturity was observed more or less all year round (Sandrini and Avian, 1991).

Here, for the first time, we examined the spawning periods of *P. noctiluca* over one year by combining different approaches: a) histological analysis of mature female gonads to assess differences in the number of oocytes and their size class distributions, b) evaluation of gonad-somatic and fecundity indices among female *P. noctiluca* specimens, c) analysis of the biochemical variations in contents of organic matter, proteins, carbohydrates, and lipids.

2. Materials and methods

2.1. Study area

The study was carried out in the Strait of Messina (Sicily, Italy). This site is strongly influenced by the peculiar hydrodynamic regime of the strait, characterized by a six-hour alternation of northward (from the Ionian to Tyrrhenian seas) and southward (from the Tyrrhenian to Ionian seas) tidal currents, with upwelling and down-welling water masses reaching up to 200 cm s^{-1} speed (for further details see (Rosa et al., 2013)). The hydrodynamic complexity of the strait ecosystem has a major influence on the spatial and vertical distribution of the organisms, especially on zooplankton communities (Zagami et al., 1996). The regular alternation of water masses prevents stratification of the water column and the formation of a summer thermocline. For this reason, the Strait of Messina is characterized by higher productivity than other Mediterranean coastal areas (Azzaro et al., 2007). Probably due to the particular current regime, *P. noctiluca* can be sampled in this area all year round.

2.2. Sampling

Sea surface water temperature (SST) was measured monthly with a bucket thermometer and supplemented with daily time-series sea surface temperature downloaded from www.mareografico.it (ISPRA).

Thirty to fifty specimens of *P. noctiluca* were collected every month from November 2011 to September 2012 with a 1-cm mesh hand net from a boat, two hours after the sunrise. In January and March 2012, the sampling was not performed due to adverse weather conditions and the near absence of specimens in surface waters. On board, all specimens were kept in a 50-L tank with a continuous flow of seawater. Immediately after sampling, jellyfish were brought into the laboratory, where were measured their bell diameters (exumbrellar side up on a flat surface) to the nearest millimetre with a calliper, and their genders were identified by visual analysis (Milisenda et al., 2014). Male gonads were dark purple and composed of series of small cylindrical follicles stacked together. Female gonads were pink to red, with easily distinguishable eggs. For specimens whose gender determination was uncertain visually, a small piece of gonad tissue was removed and examined with a stereomicroscope. For each sampling event, ten female jellyfish (diameter range 6–8 cm) were randomly selected for histological analysis to confirm their level of maturation and for subsequent analyses.

2.3. Cohort identification

Diameter-frequency histograms were constructed from 336 jellyfish sampled in different months. Potential cohorts were identified from the histograms by use of the modal progression routine of FiSAT II (Food and Agriculture Organization–International Center for Living Aquatic Resources Management stock assessment tools (Gayanilo et al., 2002)). This program uses

Bhattacharya's (Bhattacharya, 1967) method to fit normal components to mode means in the diameter-frequency histograms and then employs NORMSEP (Hasselblad, 1966) to refine parameter estimates. The latter method applies an iterative process of the maximum likelihood concept to decompose complex size-frequency distributions into a series of normal curves representing each cohort within the data set. Modes were accepted as distinct cohorts only when differentiated by a separation index above the critical value of 2 (Gayanilo et al., 2002).

2.4. Gonad-somatic index

To assess the seasonality of the reproductive cycle, the Gonad-Somatic Index (GSI) (Byrne, 1990) was calculated for five of the ten collected jellyfish. Gonads of *P. noctiluca* are situated on the floor of the gastric cavity peripheral to the gastric cirri. To avoid loss of gonadal tissue or accidental inclusion of subumbrellar or exumbrellar tissues, gonads were carefully dissected with micro-scissors with magnification of a stereomicroscope. Gonads and the remaining part of the body (*soma*) were frozen in liquid nitrogen, temporarily stored at −20 °C, and transferred to −80 °C one hour before lyophilisation to facilitate the freeze-drying process (48 h). Once dried, the components were weighed and GSI (mean ± standard errors) of 45 specimens was determined as follows (Byrne, 1990):

$$GSI = \frac{gonad\ dry\ wt}{Total\ dry\ body\ wt} \times 100$$

2.5. Histological analysis

Female gonadal tissues (5 from each month sampled) were fixed in 10% formaldehyde/water solution and preserved prior to histological examination for study of gametogenesis and to establish the maturity stages of female gonads during the annual cycle. To prevent damage to the delicate jellyfish gonadal material and minimize tissue shrinkage, samples were prepared according to a special methodology (Johansen, 1940). Selected portions of gonadal tissue were dehydrated in an ascending series of tertiary butyl alcohol (TBA)/ethanol (Johansen series) and embedded in paraffin wax Paraplast® Plus (SIGMA). Histological sections of 7 μm thickness were mounted and stained following Ramón y Cajal's Triple Stain (Gabe, 1968). Sections were examined with a Leica DM IRB light microscope at 50x magnification and two different parts of each gonad were photographed for each of 50 adjacent sections with a Nikon Coolpix 990 digital camera. Each photograph presented an area of 3.65 mm^2 of ovary, which was examined and the diameter (longest axis) of each oocyte measured using an image analyser (ImageJ, http://rsb.info.nih.gov/ij/). The diameters of 200 oocytes, together with the widest space left to the mesoglea surrounding each egg, were measured. This allowed us to estimate an average shrinkage of 5%, which was used as a correction factor for all subsequent oocyte size measurements.

Egg diameters were grouped in 4 size classes (Sandrini and Avian, 1991): 1) <20 μm (differentiation of the oogonia in the germinal tissue), 2) 21–50 μm (oocytes beginning exogenous vitellogenesis), 3) 51–200 μm (oocytes with endogenous vitellogenesis), 4) >201 μm (final stage of oocyte maturation).

2.6. Fecundity index

Some mature oocytes can be found in the ovaria of *P. noctiluca* females throughout the year (Sandrini and Avian, 1991). Because the quantity of gonadal tissue and the percentage of mature oocytes change during the year, to compare jellyfish sampled in different months, we calculated the Fecundity Index (FI) (mean ± standard errors) of 45 *P. noctiluca* specimens according to the following formula:

$$FI = E{*}GDW$$

where FI is the fecundity index, E is the number of mature eggs mm^{-2} and GDW is the total gonad dry weight.

2.7. Biochemical analysis

The content of gonadal organic matter, expressed as the percentage of organic matter of total tissue dry weight (DW), was assessed using monthly samples of 5 female gonads of which approximately 12 mg (±0.01 mg) of dry tissue was reduced to ash for 4 h at 500 °C in a muffle furnace (BICASA B.E. 34). The weight of organic matter (OM) was calculated as the difference between the gonad DW and the ash weight (Slattery and McClintock, 1995).

The biochemical composition of female *P. noctiluca* gonads was analysed to detect monthly changes in protein, carbohydrate, total lipid, and fatty acid compositions. For each month, 5 specimens with umbrella diameter >3.5 cm (first size at sexual maturity (Sandrini and Avian, 1991)) were analysed. Gonadal tissue was frozen in liquid nitrogen, temporarily stored at −20 °C, and briefly transferred one hour before lyophilisation to −80 °C to facilitate freeze-drying (48 h).

Quantification of carbohydrates, proteins and lipids was carried out by colorimetric determination at 480 nm, 750 nm, and 520 nm, respectively. To determine the content of carbohydrates in the gonadal tissue, approximately 7 mg (±0.1 mg) of each lyophilized sample was homogenized in 3 ml of double distilled water (Dubois et al., 1956) with glucose as a standard. To determine the content of proteins, approximately 7 mg (±0.1 mg) of each lyophilized tissue sample was homogenized in 2 ml of 1N NaOH (Lowry, 1951), with albumin as a standard. Finally, approximately 10 mg (±0.1 mg) of each lyophilized tissue sample was homogenized in 3 ml of chloroform-methanol (2:1) for total lipids determination (Barnes and Blackstock, 1973), with cholesterol as a standard. Quantities were expressed as μg mg^{-1} of OM.

2.8. Statistical analysis

One-way permutation univariate analysis of variance (PERMANOVA) was used to analyse monthly GSI, monthly differences in total number of eggs, differences in monthly size distribution of eggs and monthly Fecundity Index (FI) (Anderson, 2001) after ensuring homogeneity of variances by means of Cochran's C tests. Pair-wise tests were used to determine which months differed significantly within each ANOVA for the GSI and the FI. In addition, cluster analyses based on Euclidean distances of GSI values were performed to describe the similarity in the GSI and in the FI of jellyfish species among months. Correlations between FI and GSI were examined by estimating the Pearson Product–Moment Correlation Coefficient (PPMCC). A canonical analysis of principal coordinates (CAP) was performed to relate egg size distribution in jellyfish gonads by month (Anderson and Willis, 2003; Laegdsgaard et al., 1991).

To detect monthly differences in biochemical composition and in the content of organic and inorganic matter, data were analysed using one-way PERMANOVA. The analysis was based on Euclidean distances, so the F-ratios used for tests in PERMANOVA were equivalent to those of traditional ANOVA. P-values were obtained using a permutation procedure, with 999 permutation units. A CAP

was performed to relate the biochemical composition in jellyfish gonads by month (Anderson and Willis, 2003; Laegdsgaard et al., 1991).

In some cases, variances proved to be heterogeneous and could not be stabilised by any suitable transformation. Nevertheless, analysis of variance (ANOVA) is sufficiently robust to departures from the assumption of homogeneous variances, particularly with balanced designs and many independent estimates of sample variance, as in our case. Thus, the untransformed data were used and results were interpreted with the more conservative probability level of 0.01 (Underwood, 1997).

3. Results

3.1. Cohort identification

The size class distribution of 336 *P. noctiluca* medusae collected in this study revealed the occurrence of an intermediate cohort of jellyfish 6–8 cm in diameter throughout the year (Fig. 1). The analysis of diameter-frequency distributions identified the smallest (≈25 mm) and the largest (>120 mm) jellyfish cohorts from February to May. Normal curves that were fitted to diameter-frequency histograms identified a bimodal distribution in February, June and July, thus indicating the presence of two distinct cohorts with Separation Indices (SI) of 3.94; 4.07; 3.68, respectively, while there were three distinct cohorts in April (SI 5.09; 3.92) and May (SI 3.91; 3.58).

Table 1
Results of One-way PERMANOVA comparing the mean gonad-somatic index (GSI), total egg number, egg size class distribution, fecundity index, biochemical composition and organic matter for *Pelagia noctiluca* medusae by month in the Strait of Messina. Significant p-values are indicated by one (p-value < 0.05), two (p-value < 0.01), or three asterisks (p-value < 0.001).

	df	MS	F	p
GSI				
Time	8	0.107	19.586	***
Residuals	36	5.47E-03		
Egg number mm^{-2}				
Time	8	8287.4	7.009	***
Residuals	81	1182.3		
Egg size class				
Time	8	1378	2.555	**
Residuals	81	539.18		
Fecundity index				
Time	8	5.320	3.857	***
Residuals	81	1.379		
Biochemical analysis				
Time	8	4692.3	2.527	**
Residuals	36	1856.2		
Organic matter				
Time	8	153.34	10.072	***
Residuals	36	15.224		

3.2. Gonad-somatic index

GSI values showed statistically significant fluctuations throughout the year (Table 1, Fig. 2). The highest values in the GSI

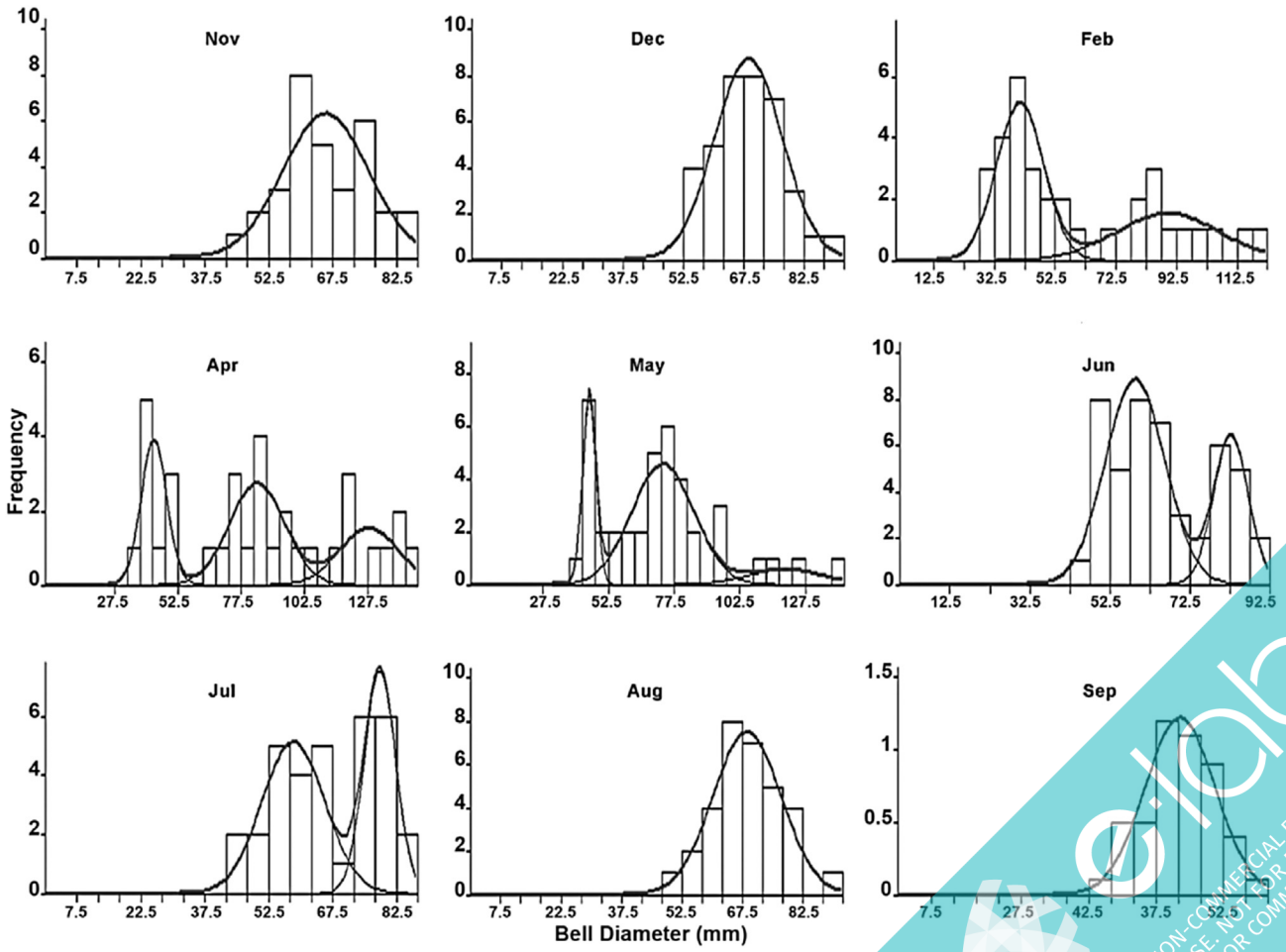

Fig. 1. Monthly size classes distribution of *Pelagia noctiluca* medusae in the Strait of Messina (November 2011–September 2012).

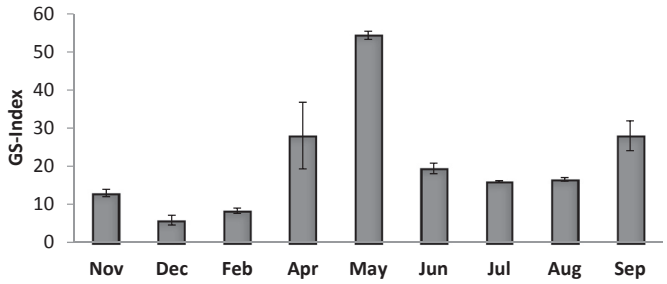

Fig. 2. Monthly changes (mean ± standard errors) of gonad-somatic index (GSI) for *Pelagia noctiluca* medusae in the Strait of Messina (November 2011–September 2012).

were in May (54%), followed by an abrupt decrease between May and June (from 54% to 19%). During summer months (June, July and August), GSI remained constant (~17%) and increased between August and September (from 17% to 28%), coupled with a decrease in surface sea water temperature. Due to the lack of samples in October, we could not determine when the autumn peak occurred. The GSI were lowest (~7%) in the winter months (December, January and February), increased at the beginning of the spring (March) and decreased again in May.

The cluster analysis of GSI values (Fig. 1, Supplementary material) highlighted differences among four main clusters: two groups with high GSI values (mainly the May and September samples) and two groups characterized by low GSI values in mid-summer and mid-winter months.

3.3. Histological analysis

3.3.1. Oogenesis

Each gonad ribbon contained oogonia and basophilic previtellogenic oocytes (<20 μm diameter, pink stained). At this stage, oocytes emerged only in the proximal area of the secondary endoderm and they were characterized by a centrally located nucleus (Fig. 3C). As expected, the topographical pattern of gonad maturation proceeded according to an inner-outer gradient of increasing oocyte diameter (Fig. 3A, C). Oocytes entering exogenous vitellogenesis (21–50 μm) were characterized by pink-stained ooplasm, a centrally located nucleus with an acidophilic germinal vesicle (dyed blue) and a visible nucleolus (bright red). As oocytes developed, the number of yolk-containing vesicles in the ooplasm increased, modifying their stain affinity from basophilic to acidophilic (Fig. 3). Thus, medium-sized oocytes (approx. >100 μm in diameter) progressively changed their cytoplasmic pink colouration to become mauve-purple at their final maturation stage (>200 μm). The ooplasm was completely filled by yolk granules with the nucleus laterally shifted towards the cell periphery in contact with the endoderm. In parallel, the paraovular body (POB) (Avian and Sandrini, 1991; Hertwig and Hertwig, 1979) became progressively thicker (Fig. 3C). Throughout this study, all examined gonads had differentiated oocytes at all the different stages of oogenesis. Most mature oocytes had a diameter ≥200 μm (Fig. 3C); however, during April, May and September, smaller oocytes (≤200 μm) with a mauve-purple stained acidophilic ooplasm, were also observed (Fig. 3B, D).

The number of eggs mm^{-2} differed significantly among months (Table 1), with the highest value in April (32.3 ± 4.1 eggs mm^{-2}) (Fig. 4). Pair-wise tests grouped together gonads with the highest numbers of eggs mm^{-2}, which were collected in April, May and September. This group was significantly different from the other months tested ($p \leq 0.05$).

Oocyte development stages were distinguished in 4 different maturation classes: early differentiated oogonia, previtellogenic, vitellogenic and mature oocytes (Fig. 4). Statistical analysis was significant (Table 1), suggesting that, although mature oocytes were found throughout the year, gonads had qualitatively and quantitatively different composition of oocytes over time.

3.4. Fecundity index

The FI analysis displayed statistically significant fluctuations throughout the year (Table 1, Fig. 5). Similar to the GSI, values of the FI were low from late autumn (December: 1.08 ± 0.18) to March (0.63 ± 0.05), increasing abruptly later between April and May (3.18 ± 0.23). FI values declined more gradually than GSI values, with low summer values (August: 0.71 ± 0.11). FI values displayed a second sharp increase in September (4.94 ± 0.35), before declining again at the beginning of autumn. The lowest values of both GSI and FI were in February (0.63 ± 0.05). FI and GSI values were significantly positively linearly correlated ($r = 0.84$, $p < 0.01$).

3.5. Biochemical analysis

The biochemical composition of *P. noctiluca* female gonads differed significantly among months (Table 1). The CAP analysis, made on centroids, indicated that changes in the biochemical composition of gonads through the year were mainly due to differences in the relative proportions of lipid and protein contents among months (Fig. 2, Supplementary material); specifically, the gonadal lipid content showed the greatest change depending on the season (Pearson correlation coefficient, CAP2 axis = 0.98). Protein contents also were strongly correlated with CAP1 axis (0.89).

A cluster analysis based on lipid content variation identified three groups of samples. The first group was formed by gonads with the lowest lipid concentration (February, 91.72 ± 2.12 μg mg^{-1}). Lipid concentrations in gonads were much higher in April (136.81 ± 16.52 μg mg^{-1}) and clustered with gonads sampled in May, November and December.

The protein concentrations were highest in December (187.83 ± 6.38 μg mg^{-1} OM) (Fig. 6), declined progressively until May (147.45 ± 19.94 μg mg^{-1} OM), reached a second high concentration in August (179.91 ± 9.65 μg mg^{-1} OM) and declined again during autumn months. Carbohydrate concentrations did not change significantly during the year ($F_{8, 37} = 1.78$; $P = 0.13$) (Fig. 6).

The annual trend of organic matter in the gonads (Fig. 6) revealed the highest OM concentration in late autumn (December: 59.26 ± 1.67% of DW). The gonadal OM concentration progressively declined from winter to late summer (from December: 59.27 ± 1.68% DW to August: 41.29 ± 1.81% DW). Homogeneity in the variances was significantly different ($C = 0.25$, $p > 0.01$).

4. Discussion

In the present study, the gametogenic cycle and reproductive potential of *P. noctiluca* female gonads were investigated during one year, integrating morphometric (gonad-somatic and fecundity indices), histological (gonadal maturation, oocyte differentiation patterns) and biochemical analyses (protein, lipid, and carbohydrate concentrations). These methods are recognized as suitable tools to identify spawning periods in different animal taxa (Arafa et al., 2012; Kharat and Khillare, 2013; Sandrini and Avian, 1991), but they were applied here together for the first time to study the oogenesis of a jellyfish species. This work will provide a reference basis for future assessments on reproductive potentials of outbreak-forming jellyfish.

P. noctiluca has a holoplanktonic epipelagic life cycle and,

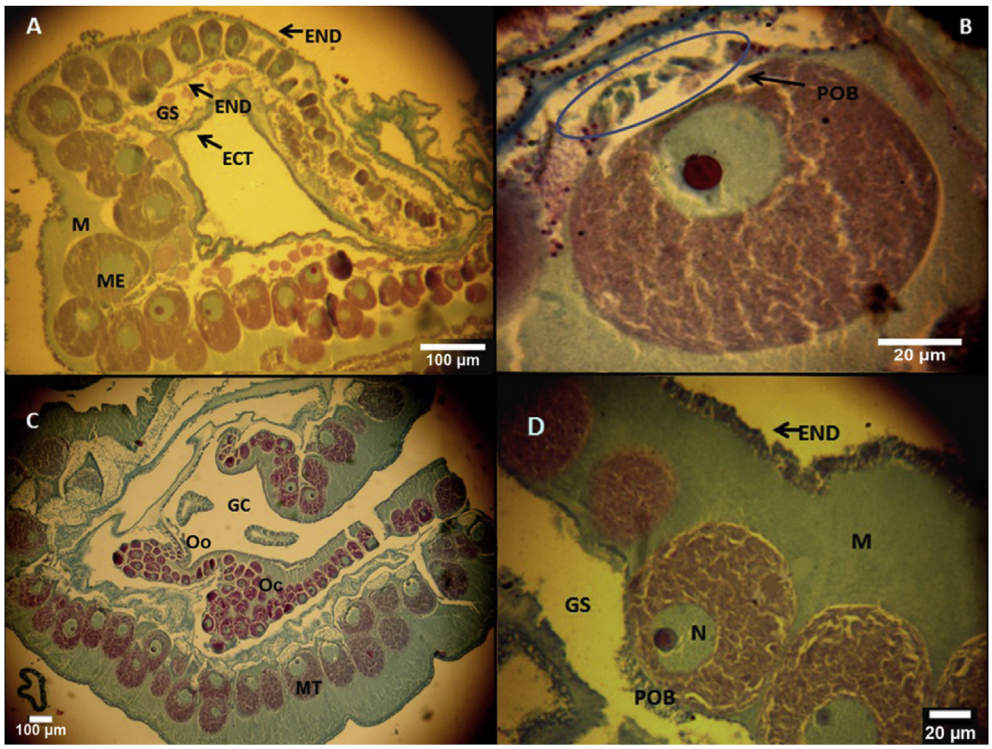

Fig. 3. A and C-Traverse section through *Pelagia noctiluca* female gonad, B-Detail of mature egg with associated paraovular body, D-Detail of mature eggs ready for spawning. ECT, ectoderm; END, endoderm; GC, gastric cavity; GS, genital sinus; M, mesoglea; ME, mature egg; POB, paraovular body; N, nucleus; Oo, pre-vitellogenic oocutes; Oc, vitellogenic Oocytes.

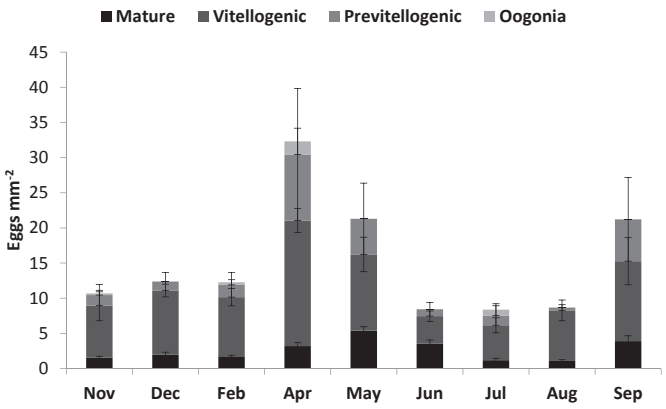

Fig. 4. Monthly variations (mean ± standard error) in total number of eggs and egg size classes (mean ± standard error) of *Pelagia noctiluca* in the Strait of Messina (November 2011–September 2012).

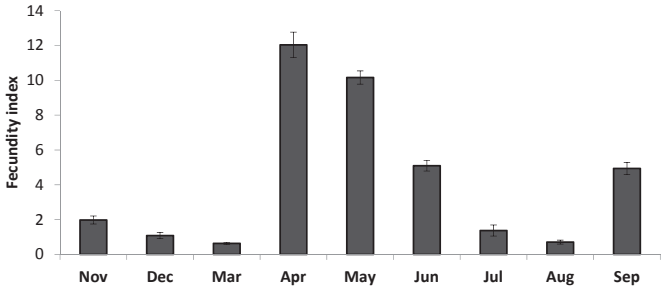

Fig. 5. Monthly variations (mean ± standard error) of the fecundity-index for *Pelagia noctiluca* in the Strait of Messina (November 2011–September 2012).

therefore, its occurrence is much less predictable because of the lack of a sessile polyp stage. So far, no investigations have combined different approaches to address the importance of sexual reproduction in the emergence of jellyfish blooms. Our results confirmed the findings of previous studies, showing seasonal changes of *P. noctiluca* gonadal activity (Goy et al., 1989; Rosa et al., 2013; Sandrini and Avian, 1991). Furthermore, female jellyfish had oocytes at all different maturation stages throughout the year. Because of this, *P. noctiluca* may support an iteroparous reproduction strategy (Canepa et al., 2014a), as in many cnidarians and other marine taxa (Baillon et al., 2014; Benayahu and Loya, 1986; Coma et al., 1995; Hamel et al., 1993; Kennedy et al., 2011).

Oocyte size distribution was shown to be a good proxy to assess female gonad maturity for Semaestomeae and for Rhizostomeae jellyfish (Toyokawa et al., 2009). *P. noctiluca* mature oocytes (>200 µm in diameter) from North Adriatic Sea are found imbedded in the gonadal tissue most of the year (Avian and Sandrini, 1991). The population of *P. noctiluca* from the Strait of Messina showed a similar long temporal pattern but, interestingly, the gonads of medusae sampled in April, May and September contained the largest numbers of mature eggs per area unit, mostly with diameters <200 µm. Therefore, *P. noctiluca* sexual reproduction seems to occur throughout the year, with two seasonal peaks (autumn, spring) of spawning and embryonic development.

Egg size is a key factor in animal reproductive strategies, whose variability is determined by a trade-off between quantity and quality of offspring (Olive, 1985). Most life-history models predict that females will produce many small eggs in environmentally favourable conditions and fewer larger eggs in unfavourable conditions (Lucas and Lawes, 1998; Olive, 1985; Roff, 1992). Both abiotic and biotic factors have been proposed to control egg size variability, as an adaptation to local environmental conditions. For instance, anchovy oocyte size and egg batch size differed between two

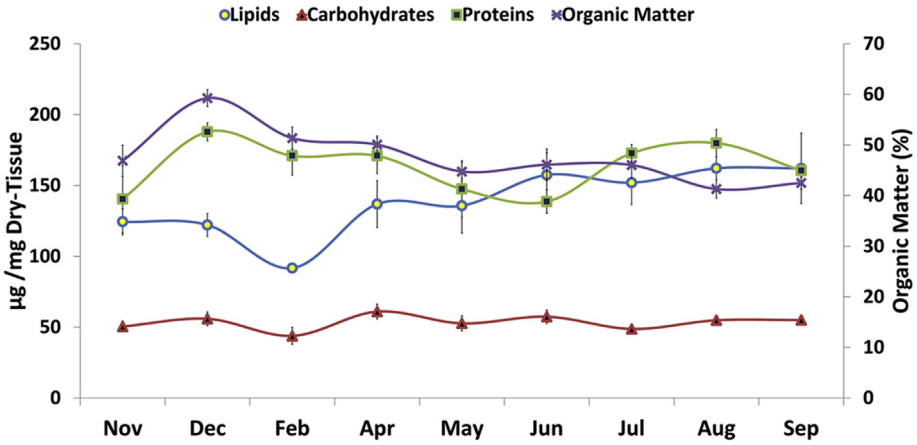

Fig. 6. Temporal changes in the organic matter content (as percentage of gonadal weight), carbohydrate, protein and lipid contents in gonads of female *Pelagia noctiluca* (mean ± SD) in the Strait of Messina (November 2011–September 2012).

spawning grounds separated by 15 degrees of latitude and marked sea water temperature differences (Leal et al., 2008). A direct relationship of egg numbers with temperature, but an inverse relationship with egg size also have been documented in other fish species (Kokita, 2003). In addition to potential sea water temperature effects, food availability may also play a role in determining differences in oocyte number and size and maximizing offspring survival in the spawning habitat (Castro et al., 2002; Llanos-Rivera and Castro, 2004). Indeed, the investigated habitat of *P. noctiluca* (the Strait of Messina) has strong environmental forcing, with a peak of the annual upwelling cycle in late spring until late summer (Azzaro et al., 2007). The characteristic "biological richness" of upwelling systems appears at all levels of the trophic chain. Nutrient-rich waters associated with upwelling promote phyto- and zooplankton pulses that sustain marine food chains (Boero, 1994; Boero et al., 2008). Therefore, seasonal differences in food quality might trigger different reproduction strategies. Accordingly, a previous study on *Aurelia aurita* suggested that the restricted food availability in Horsea Lake (UK) limited the energy investment in sexual reproduction by the production of few, large planula larvae, while the well-fed medusae from Southampton Water adopted an opportunistic strategy by producing many small planulae (Lucas and Lawes, 1998). Comparably, in the Strait of Messina, abundant zooplankton availability following the spring phytoplankton bloom (Azzaro et al., 2007; Bandelj et al., in prep.) can be correlated with the occurrence of *P. noctiluca* females bearing a high number of small (<200 µm) mature oocytes. In autumn months with lower prey availability, the reproductive strategy of *P. noctiluca* specimens of comparable sizes (Tab.) apparently showed a seasonal switch to produce fewer larger eggs (>200 µm). Our analyses showed these differences were not related to jellyfish size but uniquely to a temporal effect (Supplementary material).

The analysis of egg size composition pointed out that *P. noctiluca* in the Strait of Messina across the year exhibits two rounds of sexual reproduction, from germ cell differentiation to spawning. In both cycles, three phases can be recognized: [I] gamete differentiation, [II] differentiation coupled with spawning, and [III] mainly spawning events. Gonads from specimens collected in April and September were in the first phase, characterized by previtellogenic oocytes. In May, gonads were already in the second phase when spawning and new oocyte differentiation occurred, with intermediate features (occurrence of mature and previtellogenic-vitellogenic eggs). The third and last phase with much reduced egg differentiation and major spawning events occurred in June or in November and December. Oocyte differentiation and spawning of mature eggs were kept at intermediate levels during the remaining months (February, July and August).

These overall differences in gonad development and oocyte differentiation can be related to seasonal variations of the environmental conditions. Changes in temperature are known to act directly on the metabolic rate of *A. aurita*, or indirectly on the food supply through primary and secondary production increments (Lucas and Lawes, 1998). Somatic growth and sexual reproduction are competitive processes in terms of the energetic investment of assimilated food (Lilley et al., 2014a,b; Lucas, 2001). Yet, different food availability may favour either growth or reproductive development. *A. aurita* medusae invested 4% of assimilated food energy in sexual reproduction under conditions of elevated food supply; while during low food periods, those values decreased to 2% (Schneider, 1989). Overall, the amount of energy allocated to sexual reproduction increases with food availability and well-fed jellyfish populations have higher GSI than food-limited populations (Lucas, 2001; Lucas and Lawes, 1998). Accordingly, we hypothesize that seasonal phytoplankton and zooplankton blooms may accelerate the oogenesis rate in *P. noctiluca*, i.e. increasing the proliferation of endodermal germ cells, their differentiation into new oogonia, and governing the timing and onset of spawning periods.

The umbrella diameters (50–90 mm) of *P. noctiluca* medusae sampled in the Strait of Messina (Fig. 1) had a uni-modal size class distribution during most of the year. These results can be related with the egg size composition of female gonads examined in the present study (Fig. 4). Mature eggs were found all year, which may indicate a constant supply of new jellyfish throughout the year; however, new cohorts were identified after the two main spawning peaks, suggesting a differential survival rates in different months. During the spring, jellyfish diameters ranged from 35 to 145 mm (Fig. 1). The small jellyfish represented a new cohort of individuals produced during the previous autumn. The largest medusae, up to 145 mm, belonged to another cohort, possibly from the preceding spring. *P. noctiluca* is known to live not only in surface waters, but it has been recorded down to 1400 m depth (Franqueville, 1971). Recently, new analysis demonstrated that *P. noctiluca* outbreaks along the Catalan coasts occurred in the proximity of two main marine canyons (Benedetti-Cecchi et al., 2015). In the Strait of Messina, the peak of upwelling cycle occurs from late spring to early summer (Azzaro et al., 2007). Genetic data on *P. noctiluca* from the Strait of Messina revealed high level of inbreeding and individual cohesiveness of population subunits (Aglieri et al., 2014),

suggesting small-scale and oceanographic patterns may contribute to temporary genetic structure and limited dispersal. Overall, this information may support the finding of additional jellyfish cohorts in late spring and early summer months and the hypothesis of seasonal vertical migrations of *P. noctiluca* population subunits by upwelling and down-welling water circulation cells, such as those along coastal canyon corridors (Boero, 2012; Canepa et al., 2014a). Following the two cycles of sexual reproduction, *P. noctiluca* jellyfish might spend part of the year at lower epipelagic or even mesopelagic depths along the continental slope, with an increasing investment for germ cell differentiation and gonad maturation. By late winter – early spring, when sea temperature are low, massive outbreaks of large, adult *P. noctiluca* individuals occur (Rosa et al., 2013). These episodes usually are recorded at coastal locations in the proximity to the upper margins of marine canyons and upwelling areas, such as Aeolian Islands archipelago waters and the Strait of Messina (NE Sicily), the Island of Elba (Tuscany) and the continental platform of the Ligurian Sea (Boero, personal communication). From January to March, large *P. noctiluca* can be found on surface waters even in daylight, often exhibiting an unusual swimming behaviour with individuals swimming in pairs (Canepa et al., 2014a).

We used here two positively correlated indexes, the gonad-somatic and fecundity indexes (GSI and FI), to identify the spawning periods of *P. noctiluca*. Yet, these morphometric indexes, together with the overall histological analyses of gonads, indicate two different spawning periods, a pulse of reproduction in spring, from April to June–July, and a second period from September to November, reaching the minimal values in the coldest months from December (GSI) to March (FI). The size of jellyfish specimens used for the histological and morphometric analyses was generally consistent all year round (except April). Therefore, the observed changes in the GSI (Fig. 2) and FI (Fig. 5) should be attributed to changes in the amount of energy invested in gonadal development at the different months, which is related to concomitant changes of environmental conditions.

Sea water temperature, together with quantity and quality of available food resources, are known as major drivers of gonadal output (Ben-David-Zaslow and Benayahu, 1999; Harland et al., 1992; Stimson, 1987). Temperature is usually considered to be a key factor for initiating gonad development, whereas the extent and success of the reproductive processes directly depend on ingested food or on previously stored reserves (Lubet, 1959).

The estimated Pearson's coefficient (PPMCC) between sampled *P. noctiluca* jellyfish size and the GSI over the entire year was not significant. The largest variability of GSI among *P. noctiluca* medusae within a sampling group was in April and September, probably due to changes in their reproductive effort by increasing the relative energy investment for gonadal growth, leading to heterogeneous GSI among individuals of the same size with gonads at different development stages. The gonadal and somatic tissues over the jellyfish life may undergo different growth rates, according to the proportional investment towards gonadal or somatic tissues in different periods, leading to variable results as either allometric or isometric growth.

The GSI may be not useful to identify spawning periods in any jellyfish population composed of a wide size range of individuals over different months, because the sexual reproduction effort might be masked by the parallel growth of somatic tissues. For the investigated *P. noctiluca* population in the Strait of Messina, the relationship between gonadic dry weight (GDW) and jellyfish diameter (JD) is linear (GLM model with Poisson distribution: $p_{value=}$ 0.005).

Conversely, the FI does not include the somatic tissues; therefore, we consider this index to be the most appropriate to identify potential spawning periods of jellyfish like *P. noctiluca*, without any potential interference due to possible isometric growth of soma and gonads. In addition, the FI showed longer potential spawning periods than the GSI. Therefore, the FI values provided a reliable explanation for the occurrence of *P. noctiluca* all year round in the Strait of Messina, and for the homogeneity of jellyfish size through different months in our study. Further investigations on different jellyfish species or on *P. noctiluca* populations other than in the Strait of Messina will clarify whether the calculation of FI may be used as a standard method to investigate the spatial and temporal dynamics of holoplanktonic jellyfish species.

The female gonadal organic matter content may represent another useful proxy to understand reproductive dynamics of jellyfish, as a reflection of differential energy investments between somatic and gonadic tissues, which may fluctuate according to endogenous and environmental control mechanisms (e.g. food abundance and temperature) (Olive, 1985). The content of OM in the female gonads of *P. noctiluca* (as percentage of total OM) was different in the two potential spawning periods. The highest gonadal OM value was recorded in late autumn, with poor quality and quantity of available zooplankton food; conversely, a low gonadal OM content was observed in spring (Fig. 6), at the time of the highest food availability (Bandelj et al., in prep.). Indeed, an increased amount of organic matter invested in the production of offspring is a strategy to ensure reproductive success under food shortage conditions (Olive, 1985).

The gross biochemical composition of *P. noctiluca* gonads showed consistent reserves of proteins, abundant quantities of lipids, and lower levels of carbohydrates (Fig. 6). However, the relative proportions of these molecules depend on the season, probably related to the quality and quantity of ingested food, the sea water temperature, and the reproductive cycle period (Lucas and Lawes, 1998; Roff, 1992). Our results showed that proteins represent the main component of *P. noctiluca* gonads and that they are significantly related to the gonadal reproductive cycle, with the lowest concentrations recorded during the spawning months by the emission of eggs and the embedding mucus strands (November, May, June; Fig. 6). This finding agreed with the cycle of gonad development and oocyte differentiation, because yolk protein contents increase during oocyte growth and maturation to rapidly disappear when eggs are released from the gonadal tissues (Martinez-Pita Marsh and Watts, 2007).

In general, lipids stored in marine invertebrate eggs are used by the larvae as an energy supply (Holland, 1978). In molluscs, lipids are selectively accumulated in the ovary during the reproductive period (Giese, 1969). Similarly, seasonal variations of lipid and fatty acid contents have been documented during the reproductive cycle of the pennatulacean *Renilla koellikeri* (Pernet et al., 2002). This benthic anthozoan colony accumulates large amounts of fatty acids and lipids just prior to spawning, followed by the subsequent decrease after egg release. By contrast, lipids in *P. noctiluca* gonads appear to be the substrates for energy storage, but uncoupled to the spawning cycles. Lipid concentrations increased in the spring simultaneously with the phytoplankton bloom (Azzaro et al., 2007) and remained constant, or even higher, after the probable spawning event in late spring. Gonads in *P. noctiluca* change over time; the vitellogenesis phase occurs consistently throughout the year, and therefore in these organs are required high amounts of lipids continually. Apparently, the large amounts of total lipids stored throughout the summer could provide a metabolic reserve for periods of food shortage, during which jellyfish might maintain optimal physiological performances and keep the gametogenic cycle throughout the year.

In conclusion, in the Strait of Messina, *P. noctiluca* has the potential to reproduce all year long, although the number of eggs

produced and their quality can grow exponentially when the environmental conditions are favourable. This plasticity make *P. noctiluca* an excellent bloomers, as already proposed (Dawson and Hamner, 2008). Histological and morphometric analyses (oocyte differentiation, GSI and FI) revealed that late spring and autumn were the periods of greatest energy investment in sexual reproduction by *P. noctiluca*. The FI was the best indicator of the temporal windows for reproduction, which probably is related to environmental conditions. *P. noctiluca* spawning events and egg fertilization occurred mainly in May and October, when the sea surface temperature was around 19 °C. At this temperature, the time needed for planula metamorphosis into ephyrae is only 92 h, while at 13 °C, metamorphosis may require up to 168 h (Avian and Sandrini, 1991), thereby increasing the risk of mortality by predation (Avian, 1986).

This study showed that investigations on jellyfish sexual reproduction may provide biological information relevant for management of coastal zones affected by outbreaks of gelatinous taxa. When abundant, *P. noctiluca* as well as other predator jellyfish can exert significant impact on zooplankton communities and food webs, eventually affecting the success of fish reproduction, either by direct predation on fish larvae or by competition for available food resources (Purcell and Arai, 2001). Tourism and aquaculture are also two main human activities in coastal areas that may be severely affected by unexpected jellyfish blooms, with large economic losses (De Donno et al., 2014; Graham et al., 2014; Purcell et al., 2014a; Richardson et al., 2009). The ecological importance of sexual reproduction and inter-population connectivity was rarely considered in this context (Aglieri et al., 2014), whereas so more attention was paid to asexual mechanisms of bloom formation (Canepa et al., 2014b; Pascual et al., 2014; Purcell, 2007; Purcell et al., 2012). Overall, our results suggest that observations on life cycles and sexual reproduction may support forecasting analyses aiming to [I] identify locations and periods of greatest bloom potential, and [II] apply adaptive countermeasures (e.g. mapping best locations for aquaculture, informative campaigns at tourist hot spots) to secure safe implementation of human activities in coastal areas.

Acknowledgements

Our sincere thanks to Dr. Jennifer E. Purcell for scientific advice and critical reading of the original manuscript. The research leading to these results received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under Grant Agreement No. 266445 for the project Vectors of Change in Oceans and Seas Marine Life, Impact on Economic Sectors (VECTORS – http://www.marine-vectors.eu). Logistic/technical support was also provided by the FP7 EU projects COCONET, PERSEUS, the ENPI CBCMED programme MED-JELLYRISK, and by the Italian Flagship project RITMARE.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ecss.2016.01.002.

References

Aglieri, G., Papetti, C., Zane, L., Milisenda, G., Boero, F., Piraino, S., 2014. First evidence of inbreeding, relatedness and chaotic genetic patchiness in the holoplanktonic jellyfish *Pelagia noctiluca* (Scyphozoa, Cnidaria). PLoS One 9, 1–15. http://dx.doi.org/10.1371/journal.pone.0099647.

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58, 626–639. http://dx.doi.org/10.1139/cjfas-58-3-626.

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525. http://dx.doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2.

Ansell, A., 1972. Distribution, growth and seasonal changes in biochemical composition for the bivalve *Donax vittatu* (da Costa) from Kames Bay, Millport. J. Exp. Mar. Biol. Ecol. 10, 137–150. http://dx.doi.org/10.1016/0022-0981(72)90099-8.

Arafa, S., Chouaibi, M., Sadok, S., El Abed, A., 2012. The influence of season on the gonad index and biochemical composition of the sea urchin *Paracentrotus lividus* from the Golf of Tunis. Sci. World J. 1–8. http://dx.doi.org/10.1100/2012/815935.

Arai, M.N., 1997. A Functional Biology of Scyphozoa. London.

Arai, M.N., 2001. Pelagic coelenterates and eutrophication : a review. Hydrobiologia 69, 69–87.

Avian, M., 1986. Temperature influence on in vitro reproduction and development of *Pelagia noctiluca* (Forskål). Boll. Zool. 53, 385–391. http://dx.doi.org/10.1080/11250008609355528.

Avian, M., Sandrini, L., Stravisi, F., 1991. The effect of seawater temperature on the swimming activity of *Pelagia noctiluca* (Forsskål). Boll. Zool. 58, 135–141.

Avian, M., Sandrini, L.R., 1991. Oocyte development in four species of scyphomedusa in the northern Adriatic Sea. Hydrobiologia 216/217, 189–195.

Azzaro, F., Decembrini, F., Raffa, F., Crisafi, E., 2007. Seasonal variability of phytoplankton fluorescence in relation to the Straits of Messina (Sicily) tidal upwelling. Ocean Sci. Discuss. 4, 415–440.

Baillon, S., Hamel, J.-F., Mercier, A., 2014. Protracted oogenesis and annual reproductive periodicity in a deep-sea octocoral. Mar. Ecol 1–15. http://dx.doi.org/10.1111/maec.12236.

Bandelj V., Milisenda G., Fuentes V.L., Piraino S., Solidoro C., A Physiology-based Growth Model for the Jellyfish Pelagia noctiluca and Its Application to the Strait of Messina (Mediterranean Sea). In prep

Barnes, H., Blackstock, J., 1973. Estimation of lipids in marine animals tissues: detailed investigation of the sulphophosphovanill in method for "'total'" lipids. J. Exp. Mar. Biol. Ecol. 12, 103–118.

Benayahu, A., Loya, Y., 1986. Sexual reproduction of a soft coral: synchronous and brief annual spawning of *Sarcophyton glaucum* (Quoy & Gaimard, 1833). Biol. Bull. 170, 32–42.

Ben-David-Zaslow, R., Benayahu, Y., 1999. Temporal variation in lipid, protein and carbohydrate content in the Red Sea soft coral *Heteroxenia fucsescens*. J. Mar. Biol. Assoc. U. K. 79, 1001–1006.

Benedetti-Cecchi, L., Canepa, A., Fuentes, V., Tamburello, L., Purcell, J.E., et al., 2015. Deterministic factors overwhelm stochastic environmental fluctuations as drivers of jellyfish outbreaks. PLoS One 10 (10), e0141060. http://dx.doi.org/10.1371/journal.pone.0141060.

Bhattacharya, C.G., 1967. A simple method of resolution of a distribution into gaussian components. Biometrics 23, 115–135.

Boero, F., 1994. Fluctuations and variations in coastal marine environments. Mar. Ecol. 15, 3–25. http://dx.doi.org/10.1111/j.1439-0485.1994.tb00038.x.

Boero, F., 2012. Il ritorno di MeteoMedusa. Focus (Madison) 237, 92–94.

Boero, F., 2013. Review of Jellyfish Blooms in the Mediterranean and Black Sea. Rome.

Boero, F., Bouillon, J., Gravili, C., Miglietta, M., Parsons, T., Piraino, S., 2008. Gelatinous plankton: irregularities rule the world (sometimes). Mar. Ecol. Prog. Ser. 356, 299–310. http://dx.doi.org/10.3354/meps07368.

Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Mar. Biol. 104, 275–289.

Canepa, A., Fuentes, V., Sabatés, A., Piraino, S., Boero, F., Gili, J.-M., 2014a. *Pelagia noctiluca* in the Mediterranean Sea. In: Pitt, K.A., Lucas, C.H. (Eds.), Jellyfish Blooms SE – 11. Springer, Netherlands, pp. 237–266. http://dx.doi.org/10.1007/978-94-007-7015-7_11.

Canepa, A., Purcell, J.E., Belmar, M.B., Acevedo, M., Gentile, M., Fuentes, V., 2014b. Salinity effects on asexual reproduction of *Carybdea* sp. (Cnidaria: Cubozoa). J. Plankton Res. 36, 585–590. http://dx.doi.org/10.1093/plankt/fbt124.

Carrasco, C., Navarro, J., Leiva, G., 2006. Biochemical composition and tissue weight of *Chorus giganteus* (Gastropoda: Muricidae) exposed to different diets and temperatures during reproductive conditioning. Interciencia 31, 376–381.

Castro, L.L.R., Llanos, A., Blanco, J., Tarifeño, E., Escribano, R., Landaeta, M., 2002. Latitudinal variations in spawning habitat characteristics: influence on the early life history traits of the anchoveta, *Engraulis ringens*, off northern and central Chile. GLOBEC Rep. 16, 42–45.

Coma, R., Ribes, M., Zabala, M., Gili, J., 1995. Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. Mar. Ecol. Prog. Ser. 117, 173–183.

Dawson, M.N., Hamner, W.M., 2008. A character-based analysis of the evolution of jellyfish blooms: adaptation and exaptation. Hydrobiologia 616, 193–215. http://dx.doi.org/10.1007/s10750-008-9591-x.

De Donno, A., Idolo, A., Bagordo, F., Grassi, T., Leomanni, A., Serio, F., Guido, M., Canitano, M., Zampardi, S., Boero, F., Piraino, S., 2014. Impact of stinging jellyfish proliferations along south Italian coasts: human health hazards, treatment and social costs. Int. J. Environ. Res. Public Health 11, 2488–2503. http://dx.doi.org/10.3390/ijerph110302488.

Delap, M., 1906. Notes on the rearing, in aquarium, of *Aurelia aurita* L. and *Pelagia perla* (Slabber). Rep. Sea Inld Fish. Ire. 7, 22–26.

Dubois, M., Gilles, K., Hamilton, J., Reber, P., Smith, F., 1956. Colorimetric method for determination of sugar and related substances. Anal. Chem. 28, 350–356.

Fernández, M.D., Camacho, A.P., 2005. Histological study of the gonadal development of *Ruditapes decussatus* (L.)(Mollusca: Bivalvia) and its relationship with available food. Sci. Mar. 69, 87–97.
Franqueville, C., 1971. Macroplancton profond de Méditerranée. Téthys 3, 15–16.
Gabe, M., 1968. Technique histologique. Massouet e. ed. Paris.
Gayanilo, F., Sparre, P., Pauly, D., 2002. FAO–ICLARM Fish Stock Assessment Tools (FiSAT II): User's Manual. Int. Cent. Living Aquat. Resour. Manag. Food Agric. Organ, United Nations, Rome.
Giese, A., 1959. Comparative physiology: annual reproductive cycles of marine invertebrates. Annu. Rev. Physiol. 21, 457–576.
Giese, A., 1969. A new approach to the biochemical composition of the mollusc body. Ocean. Mar. Biol. Ann. Rev. 7.
Gori, A., Linares, C., Viladrich, N., Clavero, A., Orejas, C., Fiorillo, I., Ambroso, S., Gili, J.-M., Rossi, S., 2013. Effects of food availability on the sexual reproduction and biochemical composition of the Mediterranean gorgonian *Paramuricea clavata*. J. Exp. Mar. Biol. Ecol. 444, 38–45. http://dx.doi.org/10.1016/j.jembe.2013.03.009.
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. 36, 269–279.
Graham, W.M., Gelcich, S., Robinson, K.L., Duarte, C.M., Brotz, L., Purcell, J.E., Madin, L.P., Mianzan, H., Sutherland, K.R., Uye, S., Pitt, K.A., Lucas, C.H., Bøgeberg, M., Brodeur, R.D., Condon, R.H., 2014. Linking human well-being and jellyfish: ecosystem services, impacts, and societal responses. Front. Ecol. Environ. 12, 515–523. http://dx.doi.org/10.1890/130298.
Hamel, J.-F., Himmelman, J.H., Dufresne, L., 1993. Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Duben and Koren). Biol. Bull. 184, 125–143. http://dx.doi.org/10.2307/1542223.
Hamner, W.M., Dawson, M.N., 2009. A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. Hydrobiologia 616, 161–191. http://dx.doi.org/10.1007/s10750-008-9620-9.
Harland, A., Davies, P., Fixter, L., 1992. Lipid content of some Caribean corals in relation to depth and light. Mar. Biol. 113, 357–361.
Hasselblad, V., 1966. Estimation of parameters for a mixture of normal distributions. Technometrics 8, 431–444.
Hertwig, O., Hertwig, R., 1979. Die Actinien, Anatomisch und Histologisch mit besonderer beriicksichtigung des Nervenmuskelsystems. Jena.
Holland, D., 1978. Lipid reserves and energy metabolism in the larvae of benthic marine invertebrates. In: Malins, D., Sargent, J. (Eds.), Biochemical and Biophysical Perspectives in Marine Biology, vol. IV, pp. 85–123. London.
Huang, X., Yin, Y., Shi, Z., Li, W., Zhou, H., Lv, W., 2010. Lipid content and fatty acid composition in wild-caught silver pomfret (*Pampus argenteus*) broodstocks: effects on gonad development. Aquaculture 310, 192–199. http://dx.doi.org/10.1016/j.aquaculture.2010.10.015.
Johansen, D., 1940. Plant Microtechique. McGraw-Hil, New York, NY.
Kennedy, J., Gundersen, A.C., Høines, Å.S., Kjesbu, O.S., 2011. Greenland halibut (*Reinhardtius hippoglossoides*) spawn annually but successive cohorts of oocytes develop over 2 years, complicating correct assessment of maturity. Can. J. Fish. Aquat. Sci. 68, 201–209. http://dx.doi.org/10.1139/F10-149.
Kharat, S., Khillare, Y., 2013. Gonadosomatic index, ova diameter and fecundity of fresh water hill stream teleost *Nemacheilus moreh* (Sykes). Int. J. Bioassays 2, 992–995.
Kogovšek, T., Bogunović, B., Malej, A., 2010. Recurrence of bloom-forming scyphomedusae: wavelet analysis of a 200-year time series. Hydrobiologia 645, 81–96. http://dx.doi.org/10.1007/s10750-010-0217-8.
Kokita, T., 2003. Potential latitudinal variation in egg size and number of a geographically widespread reef fish, revealed by common-environment experiments. Mar. Biol. 143, 593–601. http://dx.doi.org/10.1007/s00227-003-1104-x.
Kramp, P., 1924. Medusae. Rep. Danish Ocean. Expedit. Mediterr. Adjac. Seas, 1908-1910 2, H 1:1–67.
Laegdsgaard, P., Byrne, M., Anderson, D.T., 1991. Reproduction of sympatric populations of *Heliocidaris erythrogramma* and *H. tuberculata* (Echinoidea) in New South Wales. Mar. Biol. 110, 359–374.
Leal, E.M., Castro, L.R., Claramunt, G., 2008. Variabilidad en el tamaño de ovocitos y fecundidad parcial de anchoveta (*Engraulis ringens*, Jenyns 1842) from two spawning areas off the Chilean coast. Sci. Mar. 73, 59–66. http://dx.doi.org/10.3989/scimar.2009.73n1059.
Lilley, M., Elineau, A., Ferraris, M., Thiery, A., Stemmann, L., Gorsky, G., Lombard, F., 2014a. Individual shrinking to enhance population survival: quantifying the reproductive and metabolic expenditures of a starving jellyfish, *Pelagia noctiluca*. J. Plankton Res. 36, 1585–1597. http://dx.doi.org/10.1093/plankt/fbu079.
Lilley, M., Ferraris, M., Elineau, A., Berline, L., Cuvilliers, P., Gilletta, L., Thiéry, A., Gorsky, G., Lombard, F., 2014b. Culture and growth of the jellyfish *Pelagia noctiluca* in the laboratory. Mar. Ecol. Prog. Ser. 510, 265–273. http://dx.doi.org/10.3354/meps10854.
Llanos-Rivera, A., Castro, L.R., 2004. Latitudinal and seasonal egg-size variation of the anchoveta (*Engraulis ringens*) off the Chilean coast. Fish. Bull. 102, 207–212.
Lo Bianco, S., 1909. Notizie biologiche riguardanti specialmente il periodo di maturità sessuale degli animali del Golfo di Napoli. Mitt. Zool. Stn. Neapel 8, 513–761.
Lowry, O., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265.
Lubet, P., 1959. Recherches sur le cycle sexuel et l'emission des gametes chez les Mytilides et les Pectinidees (Mollusques bivalves). Rev. Trav. Inst. Pech. Marit. 23, 389–548.
Lucas, C., 2001. Reproduction and life history strategies of the common jellyfish, *Aurelia aurita*, in relation to its ambient environment. Hydrobiologia 451, 229–246.
Lucas, C.H., Lawes, S., 1998. Sexual reproduction of the scyphomedusa *Aurelia aurita* in relation to temperature and variable food supply. Mar. Biol. 131, 629–638. http://dx.doi.org/10.1007/s002270050355.
Malej, A., 1989. Behaviour and trophic ecology of the jellyfish *Pelagia noctiluca* (Forsskal, 1775). J. Exp. Mar. Ecol. 126, 259–270.
Malej, A., Vukovič, A., Vukovic, A., 1986. Some data on the metabolism of *Pelagia noctiluca* in the Gulf of trieste. Nov. Thalass. 8, 107–111.
Mariottini, G.L., Giacco, E., Pane, L., 2008. The mauve stinger *Pelagia noctiluca* (Forsskål, 1775). Distribution, ecology, toxicity and epidemiology of stings. A review. Mar. Drugs 6, 496–513. http://dx.doi.org/10.3390/md20080025.
Martinez-Pita Marsh, A., Watts, S., 2007. Biochemical and energy requirements of gonad development. In: Lawrence, J.M. (Ed.), De-velopments in Aquaculture and Fisheries Science, Edible Sea Urchins: Biology and Ecology, vol. 32, pp. 35–53. Amsterdam.
Milisenda, G., Rosa, S., Fuentes, V.L., Boero, F., Guglielmo, L., Purcell, J.E., 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, e94600.
Mills, C.E., 2001. Jellyfish blooms : are populations increasing globally in response to changing ocean conditions . Hydrobiologia 451, 55–68.
Molinero, J., Ibanez, F., Nival, P., 2005. North Atlantic climate and northwestern Mediterranean plankton variability. Limnol. Ocean. 50, 1213–1220.
Morand, P., Carré, C., Biggs, D.C., 1987. Feeding and metabolism of the jellyfish *Pelagia noctiluca* (scyphomedusae, semaeostomae). J. Plankton Res. 9, 651–665.
Morand, P., Goy, J., Dallot, S., 1992. Recrutement et fluctuation à long-terme de *Pelagia noctiluca* (Cnidaria, Scyphozoa). Ann. l'Institut Océanogr. 68, 151–158.
Olive, P., 1985. Physiological adaptations and the concepts of optimal reproductive strategy and physiological constraints in marine invertebrates. In: Physiologi. The Company of Biologists Ltd, Cambridge.
Pascual, M., Fuentes, V., Canepa, A., Atienza, D., Gili, J.-M., Purcell, J.E., 2014. Temperature effects on asexual reproduction of the scyphozoan *Aurelia aurita* s.l.: differences between exotic (Baltic and Red seas) and native (Mediterranean Sea) populations. Mar. Ecol. 1–9. http://dx.doi.org/10.1111/maec.12196.
Pernet, V., Gavino, V., Gavino, G., Anctil, M., 2002. Variations of lipid and fatty acid contents during the reproductive cycle of the anthozoan *Renilla koellikeri*. J. Comp. Physiol. B 172, 455–465. http://dx.doi.org/10.1007/s00360-002-0268-x.
Pillay, K.K., Nair, N.B., 1973. Observations on the biochemical changes in gonads and other organs of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) during the reproductive cycle. Mar. Biol. 18, 167–198. http://dx.doi.org/10.1007/BF00367985.
Purcell, J., 2007. Environmental effects on asexual reproduction rates of the scyphozoan *Aurelia labiata*. Mar. Ecol. Prog. Ser. 348, 183–196. http://dx.doi.org/10.3354/meps07056.
Purcell, J.E., 2005. Climate effects on formation of jellyfish and ctenophore blooms: a review. J. Mar. Biol. Assoc. U. K. 85, 461–476. http://dx.doi.org/10.1017/S0025315405011409.
Purcell, J.E., Arai, M.N., 2001. Interactions of pelagic cnidarians and ctenophores with fish : a review. Hydrobiologia 451, 27–44.
Purcell, J.E., Atienza, D., Fuentes, V., Olariaga, A., Tilves, U., Colahan, C., Gili, J.-M., 2012. Temperature effects on asexual reproduction rates of scyphozoan species from the northwest Mediterranean Sea. Hydrobiologia 690, 169–180. http://dx.doi.org/10.1007/s10750-012-1047-7.
Purcell, J.E., Baxter, E.J., Fuentes, V., 2014a. Jellyfish as Products and Problems for Aquaculture. Woodhead Publishing.
Purcell, J.E., Tilves, U., Fuentes, V.V.L., Milisenda, G., Olariaga, A., Sabatés, A., 2014b. Digestion times and predation potentials of *Pelagia noctiluca* eating fish larvae and copepods in the NW Mediterranean Sea. Mar. Ecol. Prog. Ser. 510, 201–213. http://dx.doi.org/10.3354/meps10790.
Richardson, A.J., Bakun, A., Hays, G.C., Gibbons, M.J., 2009. The jellyfish joyride : causes, consequences and management responses to a more gelatinous future. Trends Ecol. Evol. 24, 312–322. http://dx.doi.org/10.1016/j.tree.2009.01.010.
Roff, D., 1992. The Evolution of Life Histories. Theory and Analysis. Chapman & Hall, New York, NY.
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. J. Mar. Syst. 111–112, 97–107. http://dx.doi.org/10.1016/j.jmarsys.2012.10.001.
Russell, F., 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., Pagès, F., Atienza, D., Fuentes, V., Purcell, J.E., Gili, J.-M., 2010. Planktonic cnidarian distribution and feeding of Pelagia noctiluca in the NW Mediterranean Sea. Hydrobiologia 645, 153–165. http://dx.doi.org/10.1007/s10750-010-0221-z.
Sandrini, L., Avian, M., 1983. Biological cycle of *Pelagia noctiluca*: morphological aspects of the development from planula to ephyra. Mar. Biol. 74, 169–174.
Sandrini, L., Avian, M., 1989. Feeding mechanism of *Pelagia noctiluca* (Scyphozoa: Semaeostomeae); laboratory and open sea observations. Mar. Biol. 102, 49–55.

Sandrini, L., Avian, M., 1991. Reproduction of *Pelagia noctiluca* in the central and northern Adriatic Sea. Hydrobiologia 216/217, 197–202.
Schneider, G., 1989. The common jellyfish *Aurelia aurita*: standing stock, excretion and nutrient regeneration in the Kiel Bight, Western Baltic. Mar. Biol. 100, 507–514.
Slattery, M., McClintock, J.B., 1995. Population structure and feeding deterrence in three shallow-water antarctic soft corals. Mar. Biol. 122, 461–470. http://dx.doi.org/10.1007/BF00350880.
Stimson, J., 1987. Location, quantity and rate of change in quantity of lipids in tissue of hawaiian hermatypic corals. Bull. Mar. Sci. 41, 889–904.
Torreiro, M., Garcia-Martinez, P., Catoira, J., Mosquera, G., 1998. Seasonal variation in biochemical composition in gonads of the sea urchin, *Paracentrotus livid*. In: Mooi, R., Telford, M. (Eds.), Proceedings of the 9th International Echinoderm Meeting. Rotterdam, pp. 753–758.
Toyokawa, M., Shimizu, A., Sugimoto, K., Nishiuchi, K., Yasuda, T., 2009. Seasonal changes in oocyte size and maturity of the giant jellyfish, *Nemopilema nomurai*. Fish. Sci. 76, 55–62. http://dx.doi.org/10.1007/s12562-009-0187-9.
Underwood, A.J., 1997. Experiments in Ecology. Cambridge University Press, Cambridge. http://dx.doi.org/10.1017/CBO9780511806407.
Watanabe, T., Itoh, A., Murakami, A., Tsukashima, Y., Kitajima, C., Fujita, S., 1984. Effect of nutritional quality of diets given to broodstocks on the verge of spawning on reproduction of ted sea bream. Bull. Jpn. Soc. Sci. Fish. 50, 1023–1028.
Zagami, G., Badalamenti, F., Guglielmo, L., Manganaro, a., 1996. Short-term variations of the zooplankton community near the Straits of Messina (North-eastern Sicily): relationships with the hydrodynamic regime. Estuar. Coast. Shelf Sci. 42, 667–681. http://dx.doi.org/10.1006/ecss.1996.0043.
Zavodnik, D., 1987. Spatial aggregation of the swarming jellyfish *Pelagia noctiluca* (Scyphozoa). Mar. Biol. 94, 265–269.