The reproductive cycle of the sea urchin *Arbacia lixula* in Northwest Mediterranean: potential influence of temperature and photoperiod

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Abstract

We studied the reproductive cycle of the sea urchin *Arbacia lixula* in a subtidal population from Northeast Spain over four years using a gonadosomatic index and gonad histology. Our results show that the gonadosomatic index of *A. lixula* follows a seasonal cycle which peaks in May-July and attains its lowest values in October-November every year. The time course of the gonadosomatic index matched closely the photoperiod cycle. We also found a remarkable inter-annual variability in the maximum value of gonadosomatic index, which correlated with mean water temperature during the gonad growth period (winter and spring). Gonad histology was also in agreement with a single gametogenic cycle per year in this species. We explored the application of circular statistics to present and analyse gonadal development data, which allowed us to adequately handle the high intra-individual variability detected, with several developmental stages commonly found within the same gonad. The picture that emerged is one of a gametogenic timing driven by photoperiod, while the amount of reproductive output is determined by temperature. This is coherent with the tropical origin of the species and lends support to recent warnings about an increase in the abundance of this species in the Mediterranean as a result of global warming, with associated increased impact potential in sublittoral communities.

Introduction

The black sea urchin *Arbacia lixula* (Linnaeus, 1758) is one of the most abundant sea urchins in the Mediterranean (Benedetti-Cecchi et al. 1998; Palacín et al. 1998; Sala et al. 1998). Despite its increasingly recognized ecological importance (Bulleri et al. 1999; Guidetti et al. 2003; Guidetti and Dulcic 2007; Bonaviri et al. 2011; Gianguzza et al. 2011; Privitera et al. 2011; Gianguzza and Bonaviri 2013), it has been traditionally less studied than the Atlanto-Mediterranean edible sea urchin *Paracentrotus lividus*. The reproductive cycle of *P. lividus* is now well understood (e. g.: Fenaux 1968; Byrne 1990; Lozano et al. 1995; Fernandez and Boudouresque 1997; Sánchez-España et al. 2004; Barbaglio et al. 2007; Gianguzza et al. 2013), but little information exists on the reproductive cycle of *A. lixula* in the Mediterranean (Fenaux 1968; Régis 1979; Pedrotti 1993), though Tavares (2004) studied its reproductive biology in Brazil.

Despite being commonly considered as a typical Mediterranean species, *Arbacia lixula* is actually a species of tropical affinities (Stefanini 1911; Mortensen 1935; Tortonese 1965) which probably spread through the Mediterranean in the Upper Pleistocene (Wangensteen et al. 2012). It is presently distributed along shores of the tropical Atlantic, including Brazil, the African coast, Macaronesian archipelagos and the Mediterranean, where it may reach high population densities of more than 10 individuals m^{-2} (Guidetti et al. 2003). *A. lixula* is an omnivore tending to carnivory (Wangensteen et al. 2011) and has a high potential to impact shallow rocky areas. Its role in originating and maintaining barren zones is being increasingly recognized (Guidetti et al. 2003; Guidetti and Dulcic 2007; Bonaviri et al. 2011; Privitera et al. 2011). This species has experienced population increases in the past (Petit et al. 1950; Francour et al. 1994; Harmelin et al. 1995) and warnings have been issued about its potential future impact in the Mediterranean, considering the ongoing global warming trend (Gianguzza et al. 2011; Privitera et al. 2011).

Gametogenesis in Echinoidea (Pearse and Cameron 1991; Walker et al. 2007) involves the accumulation of nutritive phagocytes in the gonads, followed by nutrient transfer from these phagocytes to developing gametes and storage of mature gametes until broadcast spawning. This process is considered to be mainly regulated by photoperiod (Pearse et al. 1986; Bay-Schmidt and Pearse 1987; McClintock and Watts 1990) or temperature (Byrne 1990; Zamora and Stotz 1992; King et al. 1994). The gametogenic cycle may be followed up by histological methods or by measuring the gonad growth relative to body size (gonadosomatic index, GSI; Pearse and Cameron 1991). Fenaux (1968) found that the GSI for *Arbacia lixula* peaked during May-June-July in the French Riviera. Pedrotti (1993) reported a major peak of planktonic larval abundance of this species during October and November in the same area, while a lesser peak appeared in June. Both studies spanned only one reproductive cycle. Thus, long-term studies were lacking which would allow to assess inter-annual variability in the reproductive behaviour of *A. lixula* in the Mediterranean.

Most echinoderm species show remarkable natural fluctuations (Uthicke et al. 2009), which may be related with the regulation of their reproductive processes by external factors. Thus, the study of the natural inter-annual variability of their reproductive cycles and the assessment of the possible physical or biological factors that regulate these cycles are invaluable tools to predict future trends in the context of the ongoing climate change. With this goal, we monitored a population of *A. lixula* in natural conditions in Northwest Mediterranean during four years, in order to characterize the reproductive cycle of this species, to determine its inter-annual variability, and to assess the effects of photoperiod and inter-annual differences in temperature on the reproductive potential of this ecologically relevant echinoid.

Materials and methods

Sampling

Samples of *Arbacia lixula* were collected monthly by SCUBA diving at depths between 3 and 10 m at the littoral of Tossa de Mar (NE Spain, 41º 43.2' N, 2º 56.4' E) from November 2008 to September 2012. *A. lixula* densities were measured in the sampling area, using 6 transect lines of 50 square meters (Turon et al. 1995), at the beginning (November 2008) and at the end of the study (May 2013). Specimens were fixed in 4% formaldehyde. Only adult-sized sea urchins (test diameter size range 35.0 – 58.6 mm, mean 44.56 mm) were used for the study. Ten individuals per month were collected until August 2010, and twenty individuals per month were collected thereafter. December 2009 and October 2010 could not be sampled due to adverse meteorological conditions. One additional sample of twenty individuals was collected in June 2013 in order to check the robustness of the predictions for annual maximum gonadosomatic index based in mean temperature.

Gonadosomatic index analysis

Gonadosomatic index (GSI) was calculated as the ratio between the wet weight of the five gonads and the total wet body weight of the intact individual, (Meidel and Scheibling 1998; Sánchez-España et al. 2004; Martínez-Pita et al. 2008), using a precision scale (0.001 g). A total of 714 individuals from 46 monthly samples were measured. To check the absence of discrepancies related with the water content of the gonads, we calculated two other different gonadosomatic indices used in sea urchins literature: GSI-DW (ratio between the dry weight of the gonads and total dry weight; Byrne 1990; Lozano et al. 1995) and GSI-Nédélec (ratio between the dry weight of the gonads and the test diameter cubed; Nédélec 1983; Fernandez and Boudouresque 1997). These indices were plotted together with GSI, in order to assess their good correlation and the independence of our results from the chosen index (Fig S1).

Histological analysis

Histological sections (14 μm thick) of one paraffin-embedded gonad per individual collected between October 2009 and September 2012 were obtained in a Microm HM325 microtome and stained with hematoxylin-eosin. A total of 596 individuals (295 males and 301 females) from 34 monthly samples were analysed. Sex was determined and gonadal acini were classified into one of five developmental stages (spent – recovery – growing – premature – mature) adapted from the staging method used by Yoshida (1952). Due to high intra-individual heterogeneity, individual maturation states could not be adequately described by a single categorical stage. Instead, we used a circular coordinate system, in which the angular coordinate represents a continuous gonad maturation advance, and evenly separated angles were assigned to each of the five categoric developmental stages (Spirlet et al. 1998). A histological gonad maturation index (MI, angular variable) was calculated for each individual as the vectorial mean of 10 examined acini per individual. Monthly average maturation vectors were then calculated as vectorial means of individual MIs. The directions of these vectors represent the monthly mean values of the MI, whereas their modules are proportional to monthly inter-individual homogeneity.

The association between linear GSI and circular MI was assessed for each gonadal cycle by plotting GSI versus MI for each individual in a polar plot. In this plot, the angular coordinate is the MI and the radial coordinate is the GSI, and association between both variables is graphically assessed by whether the accumulation of individual points with high values of GSI is in the directions corresponding to high values of MI.

Temperature and photoperiod

Daily and monthly mean values for sea surface temperature (SST) were obtained from the nearby L'Estartit Meteorological Station (http://www.meteoestartit.cat). Measures of HOBO underwater temperature data loggers placed *in situ* at the sampling location during part of the study showed negligible differences with temperature recordings at L'Estartit (data shown in Fig. S2). Photoperiod data were obtained from the US Naval Observatory (http://aa.usno.navy.mil).

Statistical methods

Differences in individual GSI between sexes were assessed for every month using Mann-Whitney *U* tests and their significance was corrected for multiple tests using the Benjamini and Yekutieli (2001) FDR correction procedure. As no significant differences were found, both sexes were pooled for further analyses. Kruskal-Wallis non parametric ANOVAs, followed by Dunn's post hoc tests, were used to check for differences in GSI among months within each gonadal cycle and also to check for inter-annual differences among annual maximum values of GSI. Pearson correlation coefficients of monthly mean GSI with photoperiod were calculated separately for each gonadal cycle. The effect of sea surface temperature (SST) on annual maximum values of GSI was assessed by calculating Pearson correlation coefficient of this annual maximum GSI with mean SST during the gonad maturation period (averaging daily temperatures during the six months previous to the GSI peak).

Small sample sizes (n < 25) prevented us from using circular statistical tests such as Watson's U^2 (Fisher 1993; Zar 1996) for testing differences in MI. Instead, we used a balanced bootstrap procedure (Booth et al. 1993), where bias-corrected and accelerated confidence intervals (BCa; Efron 1987) for the monthly mean circular MI were calculated using a modified procedure from the R package BOOT (Canty and Ripley 2009), with 5,000 replicates. Differences of MI between sexes were then assessed by comparing these confidence intervals. As no differences between sexes were found, both sexes were pooled and bootstrap confidence intervals were recalculated for comparisons between months.

Circular-linear association between MI and GSI was assessed, for every cycle, calculating Mardia's (1976) circular-linear association coefficients, $R^2_{x\theta}$ and their significance was tested using a randomisation (permutation) procedure (Fisher 1993) with 10^6 replicates.

All statistical analyses were performed with RStudio (Rstudio Inc., Boston, MA, USA) or Sigmastat 3.1 (Systat Software Inc., Point Richmond, CA, USA). Graphical functions included in R packages CIRCULAR (Lund and Agostinelli 2010) and PLOTRIX (Lemon 2006) were used for graphical representations.

Results

Sea urchin densities

The densities of *Arbacia lixula* measured at the study site in the beginning (0.6 ± 0.8 individuals m^{-2} in November 2008) and in the end of the study (0.8 ± 0.3 individuals m^{-2} in May 2013) showed no significant differences ($p > 0.9$).

Gonadosomatic index

Fig. 1 shows the periodic behaviour of the gonadosomatic index (GSI) of *Arbacia lixula* throughout four complete annual cycles at the study site. Males and females were pooled together, since no significant differences were found in any sampled month (Mann-Whitney U test, all $P > 0.05$). An annual peak is apparent whereby the maximum value is attained every year during May-June-July. The minimal values occur every year in October. So, each reproductive cycle can be considered to span from October to September of the following year. Kruskal-Wallis tests found significant differences among months within every cycle (Table 1). An anomaly can be observed as a marked decrease of GSI during March of 2010, which could be explained by extreme low temperatures (see below). A remarkable correlation between monthly mean GSI and photoperiod was detected during three out of the four analysed cycles, with a somewhat less clear-cut relationship during the 2010 cycle (Table 2). Thus, the gonad build-up approximately starts with the winter solstice and the GSI peak occurs simultaneously with the summer solstice, suggesting that photoperiod may be the main factor regulating the timing of the gonadal cycle in *A. lixula*. Temperature, on the other hand, had cycles lagged by several months with respect to GSI cycles.

The magnitude of the annual maximum GSI showed a remarkable inter-annual variability, being significantly higher during the last two cycles than during the first two (Kruskal-Wallis followed by Dunn's test, $H_3 = 15.99$, $P = 0.001$). If we average the sea surface temperature (SST) during the gonad growth period (the six months previous to the peak, i.e. from December to May), the annual maximum value for GSI shows a strong correlation (r = 0.955, P = 0.04) with this mean SST. When we add the GSI data for June 2013 to this analysis, the correlation is enhanced (r = 0.962, P = 0.009; Fig. 2). An increment of 1ºC in mean SST originated a 2.5-fold increase in the maximum GSI. The first two cycles in our study were characterized by low mean SST values during the growth period and corresponded to GSI cycles showing a less defined peak (Fig.1), whereas the following two cycles were warmer years characterized by high mean SST values and a well-defined GSI peak. The SST values for 2013, on the other hand, belonged to the colder range observed in the study period (Fig. 2). The SST trend observed at Tossa de Mar and L'Estartit (Fig. S2) can be considered representative of the more general temperature recorded for these years in the Northwestern Mediterranean. Thus, the gonad growth of *A. lixula* can be considerably impaired during cold years with low winter and spring temperatures, whereas it can be enhanced by warmer temperatures during winter and spring.

Histology of the gonads

From the examination of histological sections of *Arbacia lixula* gonads, we could differentiate five gonadal maturation stages, namely: spent, recovery, growing, premature and mature, both in males and females (Fig. 3). In both sexes, the gametogenic process begins with a spent gonad (Fig. 3A and 3F), in which the acini are enclosed by a thin wall and are mostly void of cellular material. Relict spermatozoa or ova from the previous spawning event may be observed. Some growing nutritive tissue may be present, but never occupying a significant portion of the acinus. In the recovery stage (Fig. 3B and 3G), a dense meshwork of nutritive phagocytes occupies most of the acinal space. Primary spermatogonia and oogonia may occur near the acinal walls. Relict spermatozoa or ova may be present in different degrees of lysis. In the growing stage of the testes (Fig. 3C), nutritive tissue still occupies a considerable portion of the acini, but an empty space opens in the central area, where eosinophilic tails of developing spermatozoa can be observed. In the growing ovary (Fig. 3H), most developed oocytes are displaced towards the centre, while elongated, smaller ones are located near the acinal wall. In the premature testes (Fig. 3D), a mass of basophilic mature spermatozoa accumulates in the centre of the acinus, while a thick layer of nutritive tissue can still be observed in its periphery. In the premature ovary (Fig. 3I), oocytes at all stages of development occupy most of the space. Nucleoli and some remnant nutritive tissue are typically observed. The mature gonads (Fig. 3E and 3J) are densely packed with mature spermatozoa or ova and nutritive tissue is absent.

Arbacia lixula shows a striking intra-individual heterogeneity in gonad maturation, so that in most individuals, acini in different maturation stages can be found within one single gonad. This prevented us from assigning a categorical state of maturarion to any individual. Thus, we introduced a continuous circular maturation index (MI) to correctly describe the gonad maturation state. Also, a high degree of inter-individual variability can be found, so that in any given month, individuals belonging to different maturation stages can coexist (Fig. 4).

Fig. 4 shows the temporal variation of the MI throughout three complete reproductive cycles, corresponding to the last three cycles represented in the GSI graph (Fig. 1). The monthly mean vectors, as well as the individual MI for both males and females, are shown. Non-overlapping bootstrap confidence intervals for males and females were found only in one month (October 2009) out of 34 months compared, which is likely an artefact due to small sample size. Thus, both sexes were pooled for all the following analyses.

Although the inter-individual variability is consistently high, a remarkable match between mean MI vectors of corresponding months can be appreciated in Fig. 4 during the last two cycles, 2010–2011 (Fig. 4B) and 2011–2012. (Fig 4C). These correspond to the warmest years, when the GSI curve featured well-defined peaks. The majority of individuals were mature during May-June-July. The only noticeable difference is in the spawning event, which took place one month earlier in 2012

(August), as compared with 2011 (September).

In contrast, the first cycle in our histological data (2009–2010, Fig. 4A), which is also the coldest of our series, showed a very different behaviour from the two warmer cycles. The maturation vector is consistently retarded compared with the following cycles, not only during the coldest months, but also during the summer. Few mature individuals could be found, so that the mean MI vector did not attain the mature stage anytime during this cycle, halting instead at the premature stage.

Histologically mature *Arbacia lixula* individuals showed consistently high GSI values, and the individual GSI is significantly associated with the MI (Fig. 5 and Table 3). Nevertheless, Mardia's circular-linear association coefficient, $R^2_{x\theta}$, showed higher values and signification during the two warmest cycles, suggesting that, despite the significant association found, the GSI is less linked to the maturation stage during cold years, probably due to incomplete gonad maturation.

Discussion

The gonadosomatic index of *Arbacia lixula* at Tossa de Mar (Northwestern Mediterranean) follows a seasonal cycle which peaks in May – July, coinciding with the summer solstice, and attains its lowest values in October-November every year. A single annual spawning event can be inferred from the GSI trend, which would take place during the summer and early autumn, in agreement with the previous results of Fenaux (1968). The results from gonad histology also suggest a single gametogenic cycle per year in this species.

As temperature and photoperiod co-vary with a lag of a few months, it could be difficult to disentangle the effect of both variables. However, in *Arbacia lixula* photoperiod matches quite closely the time course of the GSI at our study site, especially in 2011 and 2012, while the temperature cycle is displaced by ca. 2-3 months (Fig. 1). This suggests that photoperiod rather than temperature drives the timing of gametogenesis. However, sea surface temperature (SST) seems to have also a critical effect in the reproductive cycle of *A. lixula*. The first two cycles of our series

(November 2008 to September 2010) were characterized by low maximum values of the GSI and corresponded to years when SST stayed considerably cold during winter and spring months. Conversely, the last two cycles (October 2010 to September 2012) showed high values for the annual maximum GSI and corresponded to years when SST during winter and spring months was exceptionally warm, compared with the climatic SST averaged over a 30-year period (1973-2002) (www.meteoestartit.cat). There was a high correlation of annual maximal GSI with mean SST during winter and spring months (Fig. 2). This suggests that the temperature prevailing during these months (December to May), which corresponded to the period of gonadal recovery and growth after spawning (Fig. 4), may be a main factor determining the magnitude of the annual maximum for GSI. The GSI value for June 2013 was also included in our analysis, in order to test the predictive capacity of our results. Temperatures in winter and spring of 2013 were relatively cold, and the result for the GSI closely matches the prediction of our linear regression (Fig. 2). Temperature, therefore, can be directly related with the annual variability in the reproductive output of this species.

Our data show that the reproductive behaviour of *Arbacia lixula* can be considerably affected by atypical cold episodes. During the winter of 2009-2010, the North Atlantic Oscillation recorded its lowest values since at least 1950 (Cohen et al. 2010) which caused extremely low temperatures across the Northern Hemisphere. Indeed, the mean SST for March of 2010 (11.8ºC) is the lowest of all temperatures recorded during our series, and lower than the historical mean over 30 years for this month (12.6ºC). This was reflected not only by a decline in the GSI of that month (Fig. 1) but also by a delay in the MI values during the following months of that reproductive cycle (Fig. 4A). Conversely, the last two cycles (Fig. 4B and 4C) correspond to warm years during which steady gonadal growth and maturation were observed, with predominance of mature individuals from May to July, and spawning events in August-September. The earlier spawning of August 2012 (compared with September 2011) could be related to the higher mean temperature during that month, compared with the same month of the previous year.

The inter-annual differences in the GSI-MI association plots (Fig. 5) also suggest a critical dependence of the reproductive cycle with temperature. The GSI was tightly correlated with maturation state during the two warmer cycles, attaining higher values for mature individuals, whereas this relationship was considerably weaker (albeit significant) during the coldest 2009-2010 cycle (Table 3), when all individuals had GSI values less than 10%.

An interesting question is whether GSI cycles with a sharp peak (such as those observed in the last two cycles of our study) correspond to the normal condition for *Arbacia lixula* reproduction in the Northwest Mediterranean or whether a gonadal cycle with a less-defined peak and low GSI values is the usual reproductive behaviour of the species in this area. It seems likely that a cycle with a sharp GSI peak is the normal condition of *A. lixula* in the tropical Atlantic, where it originated. But our study area in the Northwest Mediterranean usually attains the coldest SST values of the whole Mediterranean basin. The climatic value for the SST, averaged from 1973 to 2002, for the months of December to May is 13.43ºC. If we compare this historical value with the ones that occurred in the last five years (Fig. 2), we can conclude that the average reproductive behaviour of the species during that three-decade period must have been more similar to the two first cycles of our study, and that the two warmest years displaying sharp GSI peaks have to be considered as the abnormal situation. However, considering the current warming trend in the Mediterranean, these “abnormally high temperatures” could indeed become the rule in the near future, thus boosting the potential fecundity of this species of tropical affinities.

The reproduction of *Arbacia lixula* in the NW Mediterranean was first studied by Fenaux (1968). In this seminal work, she found that the GSI of *A. lixula* peaked regularly during May-June-July, with a spawning period extending from June to November, according to the presence of larvae in the plankton. She reported a delay between gonad maturation (which was achieved in March-April) and the beginning of spawning, concluding that the gametes would not be released until water temperature was over 20ºC. Thus, she argued that temperature was the main trigger of spawning in *A. lixula*. Our results for the GSI broadly agree with those of Fenaux, but they suggest that photoperiod could be the main factor determining the timing of the gonad maturation process, which is nevertheless considerably affected by temperature during the growing period. Our histological results showed that low temperatures during winter and spring may impair the gonad maturation process throughout the reproductive cycle, which could probably prevent Northwestern Mediterranean populations of *A. lixula* from successfully reproducing during cold years, even though the temperature during summer months reaches well over 20ºC.

High temperatures and heat waves have been shown to affect populations of many Mediterranean marine species (Lejeusne et al. 2010). Temperate invertebrate species may be negatively affected by high temperatures (e. g. Cerrano et al. 2000; Garrabou et al. 2001, 2009; Martin et al. 2002). Conversely, invertebrates of tropical affinities may be favoured by increasing temperatures (Francour et al. 1994). Our results suggest that the reproductive behaviour of *Arbacia lixula* may currently be subject to suboptimal temperature conditions in the Northwest Mediterranean and that its reproductive output could be improved by the ongoing warming trend.

Most works studying the gonad histology of echinoids assign a single, categorical maturation stage to any individual. This is useful only if the gonad maturation is a uniform process, producing individuals with homogenously matured gonads. However, the intra-individual variability in the maturation state of *A. lixula* gonads is strikingly high. Most individuals show gonadal acini in different maturation stages. For this reason, an integer scalar maturation index would not accurately describe most individuals. Alternatively, each individual is better characterized by a continuous maturation index, obtained from averaging the maturation state of several acini. To avoid problems arising from averaging mature and spent acini or individuals, this continuous index must not be a scalar number, but should be represented instead by a vector in a circular (polar) coordinate system (Spirlet et al. 1998). The use of a circular maturation index is a very powerful tool to analyse the gametogenic cycles of marine invertebrates, which can avoid the inherent problems of using scalar maturation indices to characterize a naturally cyclical process. The comparison among maturation states is straightforward in the circular monthly maturation vectors plots (Fig. 4). The polar coordinate system also allows to accurately compare between sexes or among months, and it is particularly useful for quickly comparing different years. The method also allows to perform *ad hoc* statistical analyses designed for this kind of data. We advocate the use of polar methods for the study of reproduction of iteroparous species, whenever cyclical stages can be defined.

Both males and females of *Arbacia lixula* shows remarkable inter-individual variability in its gonadal maturation state (Fig. 4). This maturation heterogeneity may also be found in other sea urchin species (e. g. Byrne 1990; Lessios 1991; Lozano et al 1995; Meidel and Scheibling 1998; Sánchez-España et al. 2004; Martínez-Pita et al. 2008). On the other hand, the presence of some mature individuals throughout most of the year could be a conserved trait from the tropical past of the genus *Arbacia* (Tavares 2004).

The main exogenous factors commonly reported to control the reproductive cycle of echinoids are temperature, photoperiod and food availability. Many works relate reproduction and development with temperature (Byrne 1990; Zamora and Stotz 1992; King et al. 1994; Lozano et al. 1995, López et al. 1998, Ling et al. 2008, Pecorino et al. 2013). It has been also demonstrated that GSI is correlated with food availability in many herbivorous sea urchin species (Fuji 1960; Ebert 1968; Pearse 1981; Fernandez and Boudouresque 1997; Guillou and Lumingas 1998, Hernández et al. 2006, Martínez-Pita et al. 2008). Thus, gonad index changes may depend on the amount of reserves accumulated rather than the maturation stage, and therefore the GSI alone would not be a good parameter to assess the gametogenic state of these species. However, *A. lixula* is an omnivore tending to carnivory (Wangensteen et al. 2011) whose densities are low to moderate in our sampling area. We measured the density values at the beginning and at the end of our study, and did not find any significant differences which could influence the gonad index. Food availability is unlikely to limit gonad growth in the study area at Tossa de Mar, which exhibits well developed sublittoral communities with high productivity throughout the year (Ballesteros 1988, 1993). Photoperiod has been suggested to control gonadal growth cycles in a variety of sea urchin species (Pearse et al. 1986; Bay-Schmidt and Pearse 1987; McClintock and Watts 1990; Walker and Lesser 1998; Brewin et al. 2000; Kelly 2001; Shpigel et al. 2004), including the congeneric *Arbacia dufresnii* (Brogger et al. 2010), though few of these studies have been carried out manipulating photoperiod during long periods of time (Walker and Lesser 1998; Dumont et al. 2006). Our results show that the GSI of *A. lixula* at our study site had a remarkable intra-cycle correlation with photoperiod, but the height of the maximum GSI peak was correlated with the mean temperature during the growing period. Thus, the photoperiod would predict “when” and the temperature is related to “how much” the gonads will grow. Our results with *A. lixula* agree with those of Spirlet et al. (2000) in *Paracentrotus lividus*, who suggested that temperature acted as an enhancer of the gametogenic process, but probably not as a trigger signal for the spawning in this species. In their study with *Strongylocentrotus droebachiensis*, Dumont et al. (2006) suggested that, once gametogenesis is initiated by photoperiod triggering, spawning cannot be halted by artificially altering the photoperiod. However, our results show that, in the case of *A. lixula*, the maturation process can be considerably disturbed by anomalous temperatures, which would then be a main modulator for the gametogenic process.

In accordance with its tropical origin, *A. lixula* showed a remarkable increase in its maximum gonadosomatic index with temperature at our study site. This could probably lead to a boost in its reproductive output in Northwest Mediterranean during warm years. This is in agreement with warning reports of increased abundance of *A lixula* in the Mediterranean attributable to increases in temperature (Francour et al. 1994; Harmelin et al. 1995). Given the ongoing global warming trend, *A. lixula* can potentially boost its negative impact (Privitera et al. 2011; Gianguzza et al. 2011) and become a serious threat for Mediterranean shallow rocky ecosystems in the near future. Other thermophilous sea urchins have been proven to be able to cause catastrophic shifts in newly colonized ecosystems as a consequence of climate change (e. g. Ling et al. 2009). A preventive monitoring of population densities of *A. lixula* would be desirable in potentially affected shallow water areas.

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Legends to figures

Fig. 1 Gonadosomatic index, GSI (% wet weight; means ± SE) of *Arbacia lixula* (pooled males and females) collected between November 2008 and September 2012 at Tossa de Mar (Spain, NW Mediterranean). Sea surface temperature data obtained from the Meteorological Station at L'Estartit. Photoperiod data obtained from the US Navy Observatory. Sample sizes: Nov 2008- Aug 2010: 10 individuals per month; Sep 2010 - Sep 2012: 20 individuals per month. Total individuals analyzed: 695.

Fig. 2 Relationship between sea surface temperature during the gonad growth period (averaged from December to May) and the maximum gonadosomatic index achieved by the sea urchin *Arbacia lixula* at Tossa de Mar (Spain) over a five-year period. Error bars indicate standard errors.

Fig. 3 Histological sections of *Arbacia lixula* male (A-E) and female (F-J) gonads illustrating the five maturation stages. A,F: Spent; B,G: Recovery; C,H: Growing; D,I: Premature and E,J: Mature. GNT: growing nutritive tissue; NT: nutritive tissue; GS: growing spermatozoa; S: spermatozoa; RO: relict ova; GO: growing ova; O: ova. Scale bar: 100 μm.

Fig. 4 Circular monthly maturation vector plots for *Arbacia lixula* at Tossa de Mar (Spain) throughout three consecutive reproductive cycles: 2009-2010 (a), 2010-2011 (b) and 2011-2012 (c). Solid and open bullets correspond to male and female individuals, respectively. Density estimates of the distributions are shown as dashed curves and 95% confidence interval for the mean direction (bootstrap) are shown as grey arcs. Average monthly sea surface temperatures are shown. Maturation stages are indicated: SPE: spent, REC: recovery, GRO: growing, PRE: premature and

MAT: mature. December 2009 and October 2010 could not be sampled due to adverse meteorological conditions.

Fig. 5 Polar plots showing the association between individual maturation indices (angular axis) and gonadosomatic indices (radial axis) during three consecutive annual cycles for *Arbacia lixula* at Tossa de Mar (Spain). Solid and open bullets correspond to male and female individuals, respectively. Maturation stages are indicated: SPE: spent, REC: recovery, GRO: growing, PRE: premature and MAT: mature.

Fig. S1 Comparison between monthly values (means ± SE) of the gonadosomic index chosen in this work (GSI, % wet weight) and two other gonadosomatic indices, GSI-DW (% dry weight) and Nédélec GSI (dry weight of the gonads divided by test diameter cubed).

Fig. S2 Monthly mean sea surface temperature measured at L'Estartit Meteorological Station (red dashed line) and daily mean sea surface temperature measured *in situ* at Tossa de Mar during part of the present study (blue continuous line) showing the good correlation between both measurements.

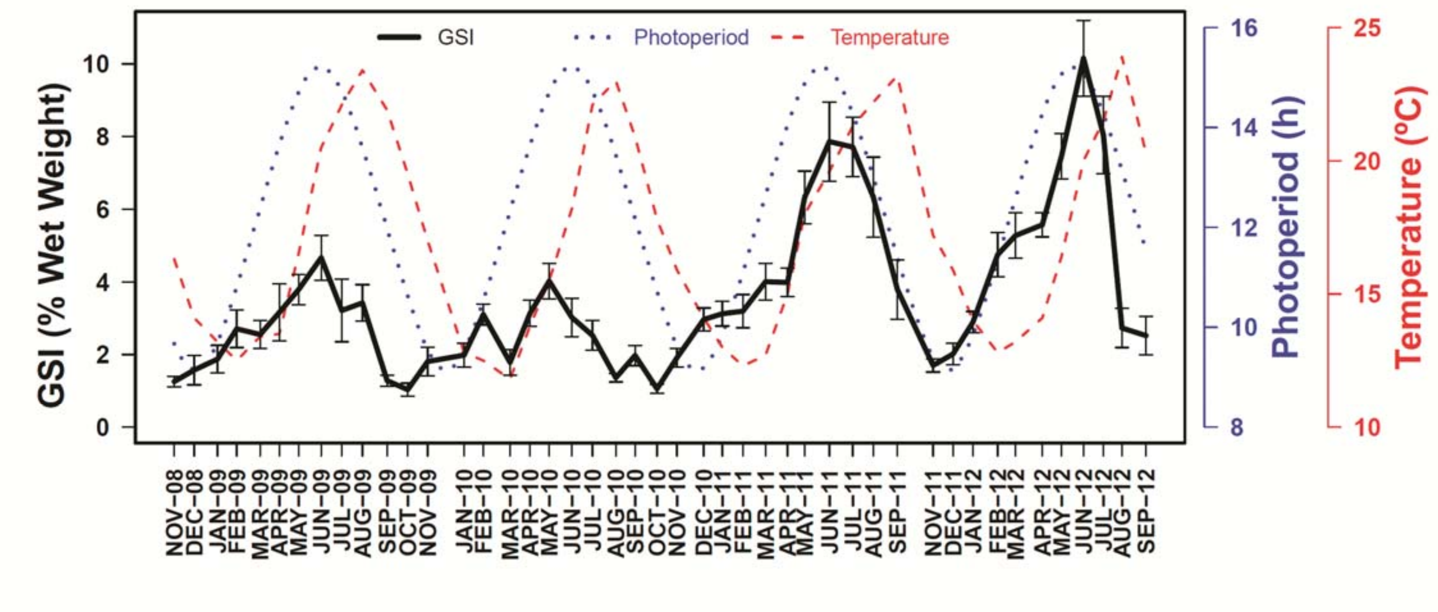
Legends to tables

Table 1 Kruskal-Wallis non parametric ANOVAs testing for differences among months in gonadosomatic indices of *Arbacia lixula* at Tossa de Mar (Spain) during four consecutive reproductive cycles. N: number of individuals used in each analysis.

Table 2 Pearson correlation coefficients of monthly mean gonadosomatic index of *Arbacia lixula* with photoperiod during each reproductive cycle. Significant correlations were found in all cases, except for the coldest cycle (2009-10), probably due to an anomalous gonadal cycle caused by cold temperatures.

Table 3 Mardia's circular-linear association coefficients, $R^2_{x\theta}$, for testing the association between individual maturation indices and gonadosomatic indices for *Arbacia lixula* during three consecutive reproductive cycles.

Figure 1


GSI
Photoperiod
Temperature
GSI (% Wet Weight)
0
2
4
6
8
10
Photoperiod (h)
8
10
12
14
16
Temperature (ºC)
10
15
20
25
NOV−08
DEC−08
JAN−09
FEB−09
MAR−09
APR−09
MAY−09
JUN−09
JUL−09
AUG−09
SEP−09
OCT−09
NOV−09
JAN−10
FEB−10
MAR−10
APR−10
MAY−10
JUN−10
JUL−10
AUG−10
SEP−10
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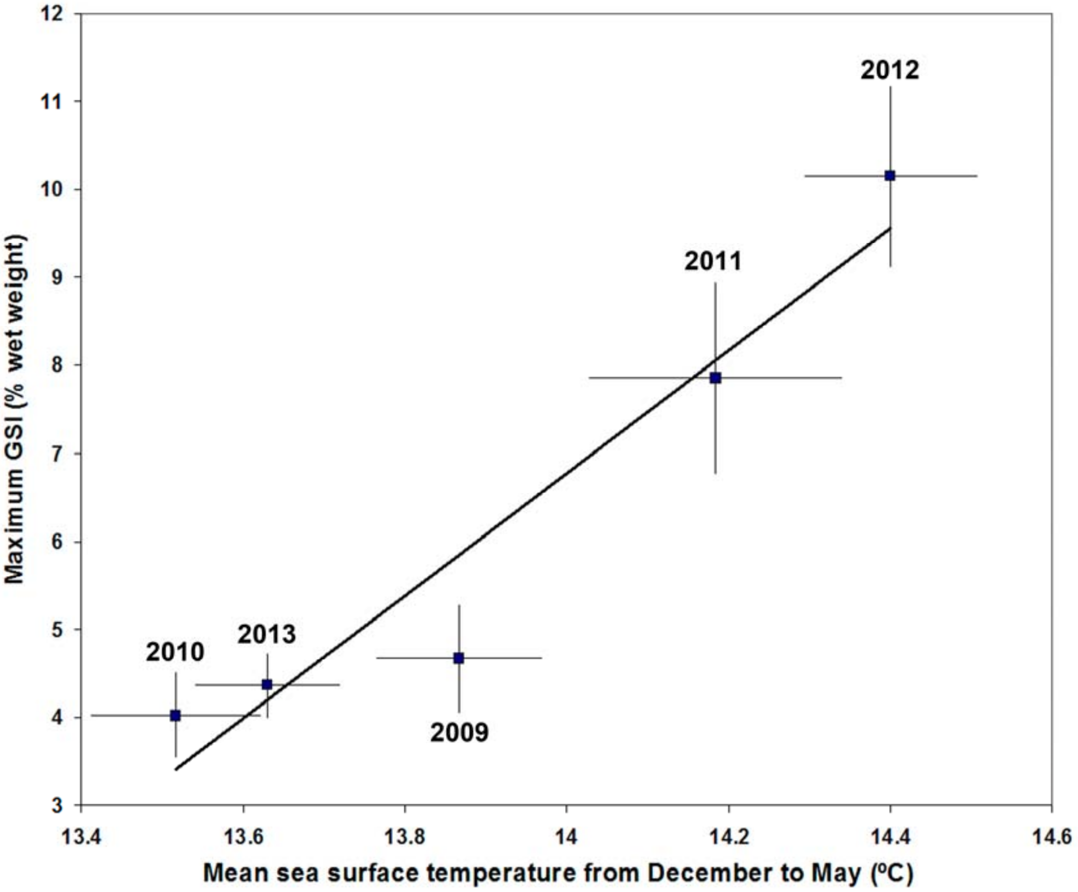
NOV−10
DEC−10
JAN−11
FEB−11
MAR−11
APR−11
MAY−11
JUN−11
JUL−11
AUG−11
SEP−11
NOV−11
DEC−11
JAN−12
FEB−12
MAR−12
APR−12
MAY−12
JUN−12
JUL−12
AUG−12
SEP−12

Figure 2



12
11
10
9
8
7
6
5
4
3
Maximum GSI (% wet weight)
2012
2011
2010
2013
2009
13.4
13.6
13.8
14
14.2
14.4
14.6
Mean sea surface temperature from December to May (ºC)

Figure 3

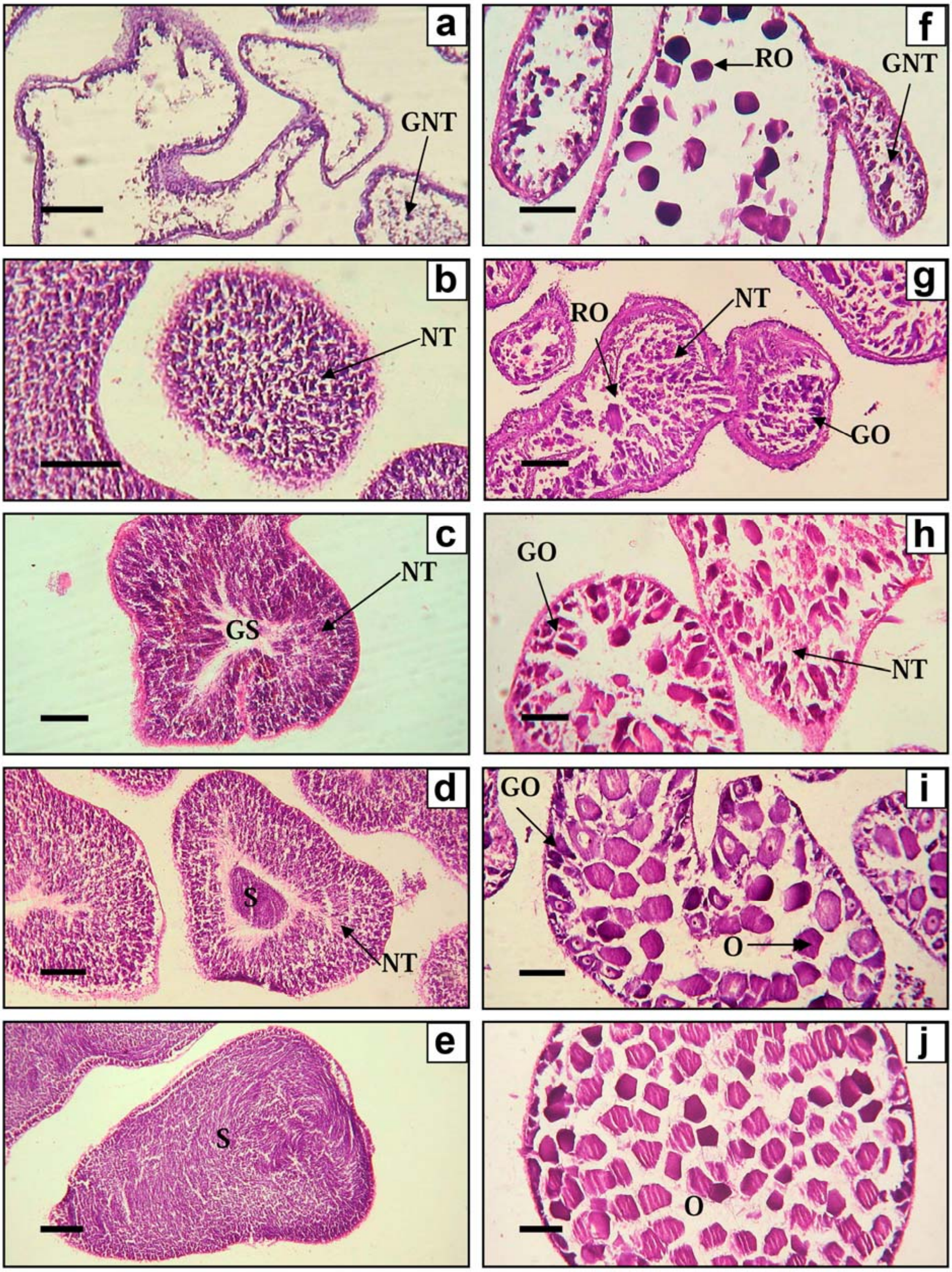


Figure 4

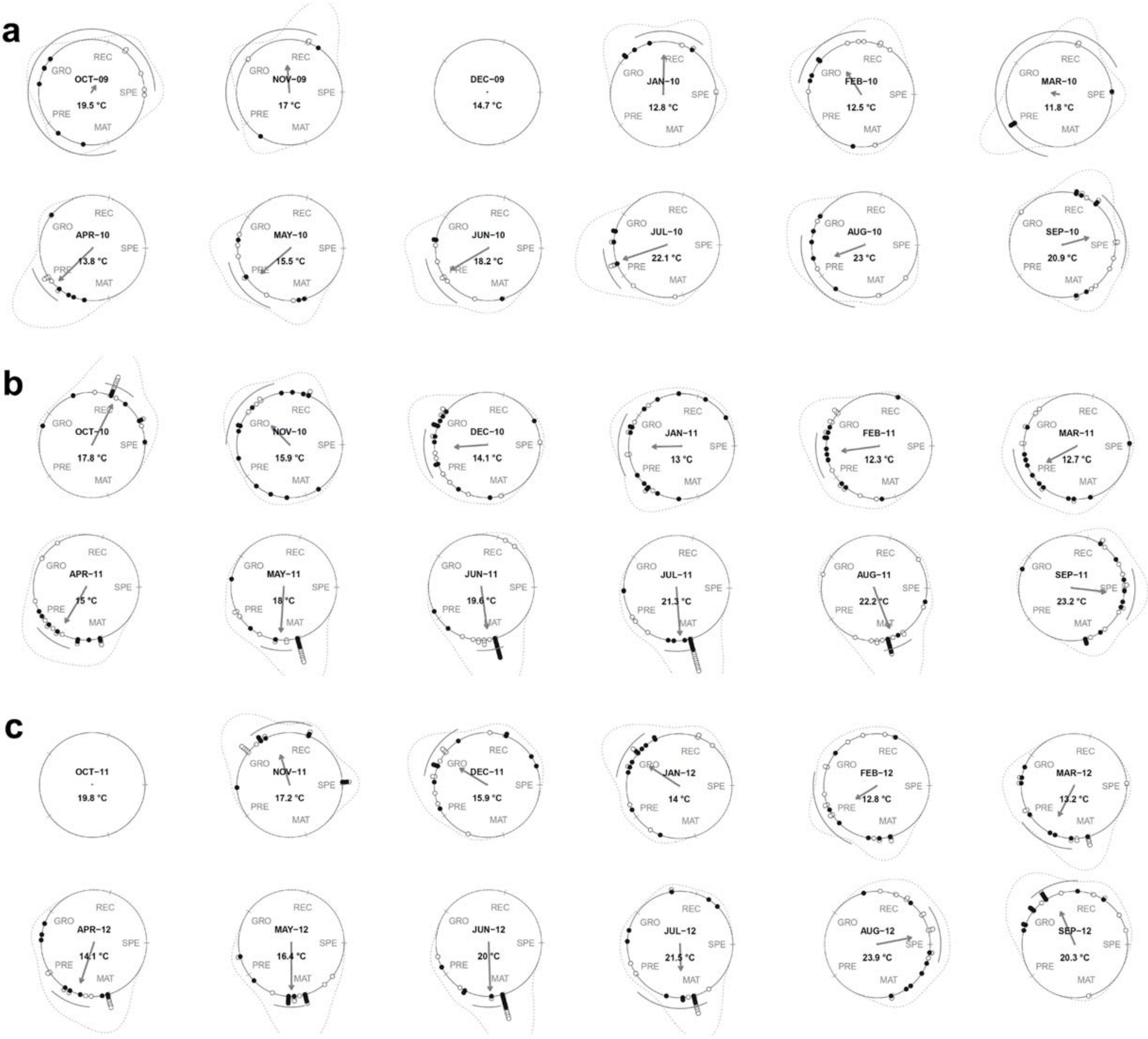

a
OCT−09 19.5 °C
NOV−09 17 °C
DEC−09 14.7 °C
JAN−10 12.8 °C
FEB−10 12.5 °C
MAR−10 11.8 °C
APR−10 13.8 °C
MAY−10 15.5 °C
JUN−10 18.2 °C
JUL−10 22.1 °C
AUG−10 23 °C
SEP−10 20.9 °C
b
OCT−10 17.8 °C
NOV−10 15.9 °C
DEC−10 14.1 °C
JAN−11 13 °C
FEB−11 12.3 °C
MAR−11 12.7 °C
APR−11 15 °C
MAY−11 18 °C
JUN−11 19.6 °C
JUL−11 21.3 °C
AUG−11 22.2 °C
SEP−11 23.2 °C
c
OCT−11 19.8 °C
NOV−11 17.2 °C
DEC−11 15.9 °C
JAN−12 14 °C
FEB−12 12.8 °C
MAR−12 13.2 °C
APR−12 14.1 °C
MAY−12 16.4 °C
JUN−12 20 °C
JUL−12 21.5 °C
AUG−12 23.9 °C
SEP−12 20.3 °C
REC
GRO
SPE
PRE
MAT

Figure 5

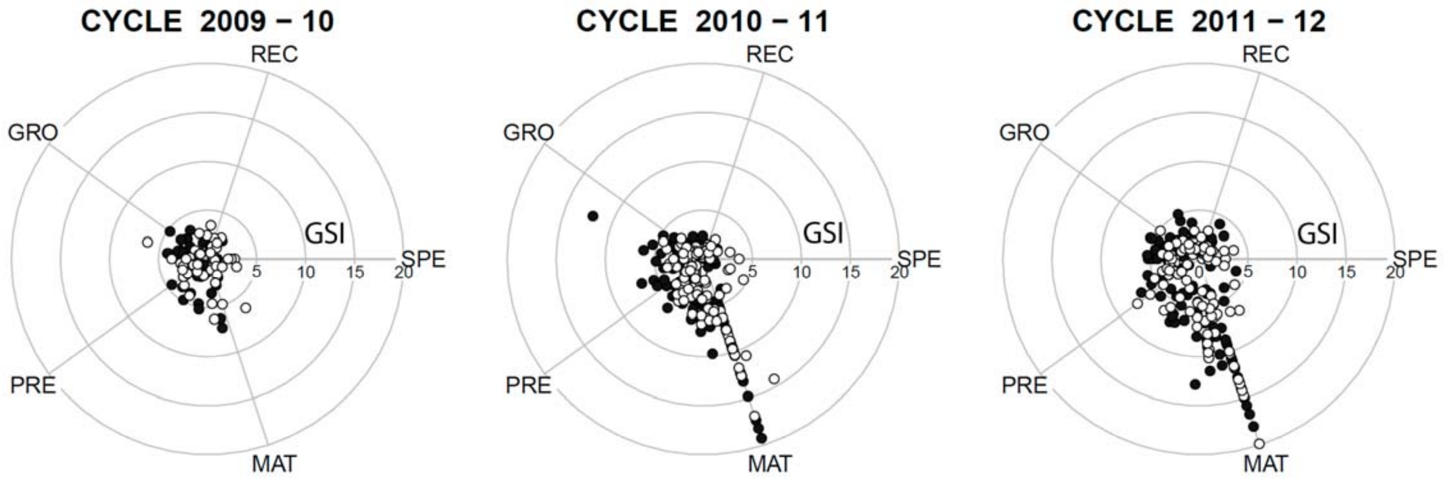

CYCLE 2009 – 10
CYCLE 2010 – 11
CYCLE 2011 – 12
REC
GRO
GSI
SPE
PRE
MAT
0
5
10
15
20

Table 1

Cycle	**N**	***H***	**d.f.**	***P*-value**
Nov 2008 – Sep 2009	110	41.08	10	1.1×10^{-5}
Oct 2009 – Sep 2010	121	51.19	10	1.6×10^{-7}
Oct 2010 – Sep 2011	246	99.13	11	2.7×10^{-16}
Nov 2011 – Sep 2012	218	122.27	10	2.2×10^{-16}

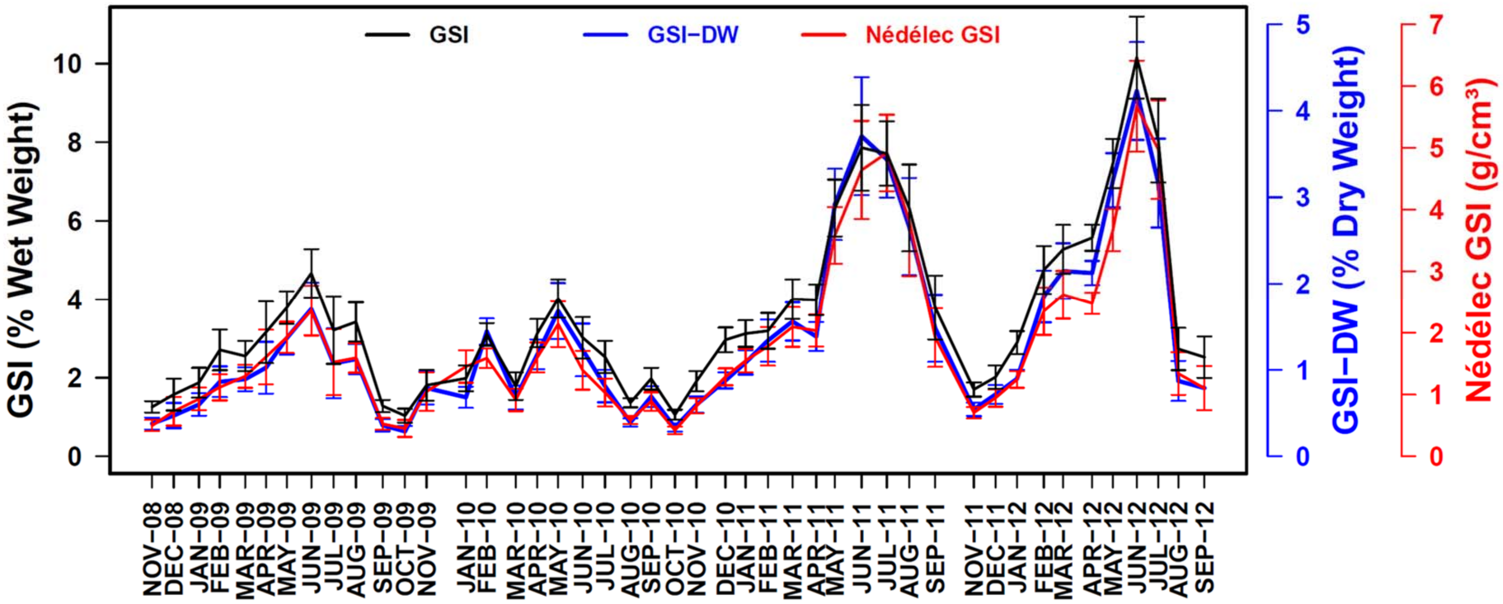
Table 2

Cycle	**Pearson *r***	***P*-value**
2008-2009	0.86	0.0007
2009-2010	0.51	0.11
2010-2011	0.90	0.00007
2011-2012	0.81	0.0023

Table 3

Cycle	**Males**	**Females**	**All**
2009-2010	$R^2_{x\theta} = 0.187$ $P = 0.0083$ n = 49	$R^2_{x\theta} = 0.162$ $P = 0.0061$ n = 59	$R^2_{x\theta} = 0.176$ $P = 3.5 \times 10^{-5}$ n = 108
2010-2011	$R^2_{x\theta} = 0.346$ $P < 10^{-6}$ n = 126	$R^2_{x\theta} = 0.456$ $P < 10^{-6}$ n = 118	$R^2_{x\theta} = 0.380$ $P < 10^{-6}$ n = 244
2011-2012	$R^2_{x\theta} = 0.445$ $P < 10^{-6}$ n = 104	$R^2_{x\theta} = 0.508$ $P < 10^{-6}$ n = 112	$R^2_{x\theta} = 0.473$ $P < 10^{-6}$ n = 216

Fig. S1


GSI
GSI−DW
Nédélec GSI
GSI (% Wet Weight)
GSI−DW (% Dry Weight)
Nédélec GSI (g/cm³)
0
2
4
6
8
10
0
1
2
3
4
5
0
1
2
3
4
5
6
7
NOV−08
DEC−08
JAN−09
FEB−09
MAR−09
APR−09
MAY−09
JUN−09
JUL−09
AUG−09
SEP−09
OCT−09
NOV−09
JAN−10
FEB−10
MAR−10
APR−10
MAY−10
JUN−10
JUL−10
AUG−10
SEP−10
OCT−10
NOV−10
DEC−10
JAN−11
FEB−11
MAR−11
APR−11
MAY−11
JUN−11
JUL−11
AUG−11
SEP−11
NOV−11
DEC−11
JAN−12
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MAR−12
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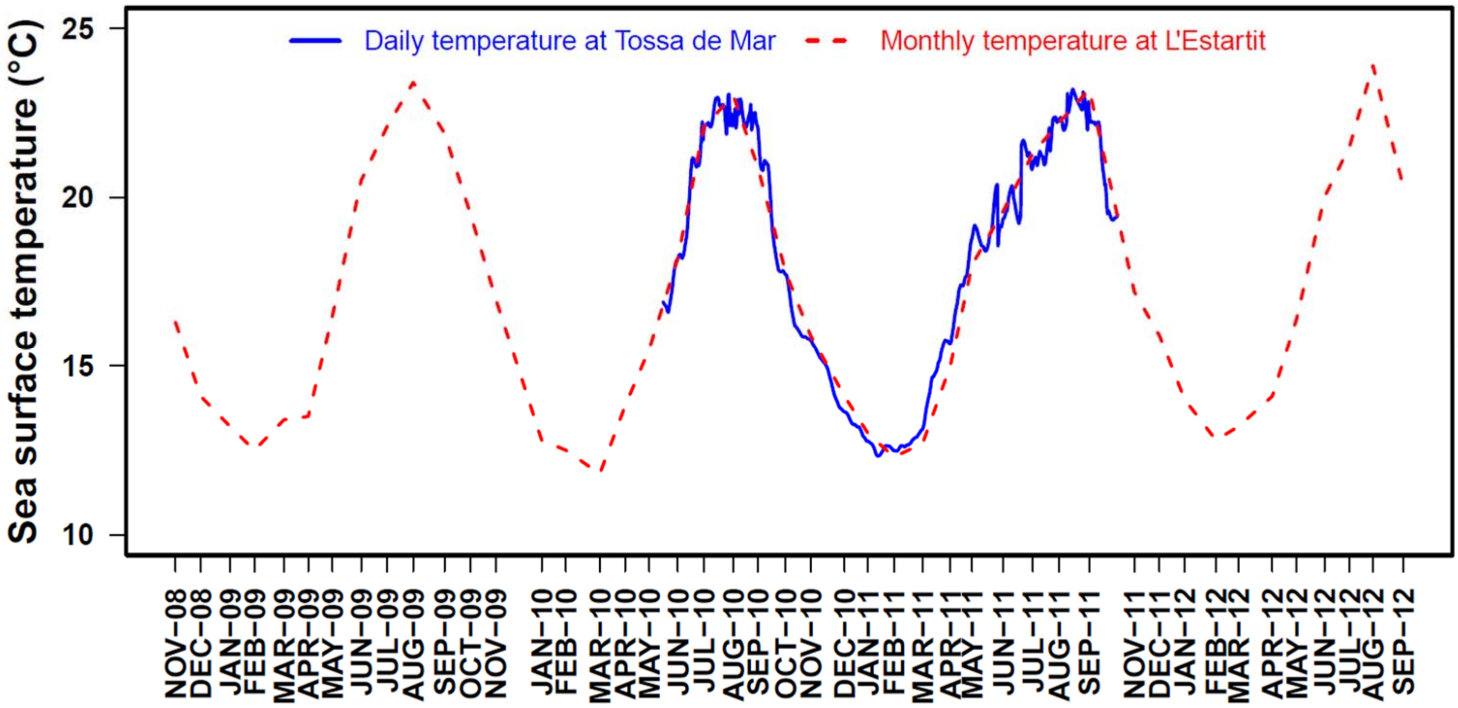
MAY−12
JUN−12
JUL−12
AUG−12
SEP−12

Fig. S2


Sea surface temperature (°C)
25
20
15
10
Daily temperature at Tossa de Mar
Monthly temperature at L'Estartit
NOV−08
DEC−08
JAN−09
FEB−09
MAR−09
APR−09
MAY−09
JUN−09
JUL−09
AUG−09
SEP−09
OCT−09
NOV−09
JAN−10
FEB−10
MAR−10
APR−10
MAY−10
JUN−10
JUL−10
AUG−10
SEP−10
OCT−10
NOV−10
DEC−10
JAN−11
FEB−11
MAR−11
APR−11
MAY−11
JUN−11
JUL−11
AUG−11
SEP−11
NOV−11
DEC−11
JAN−12
FEB−12
MAR−12
APR−12
MAY−12
JUN−12
JUL−12
AUG−12
SEP−12