REPORT

Photoperiod, temperature, and food availability as drivers of the annual reproductive cycle of the sea urchin *Echinometra* sp. from the Gulf of Aqaba (Red Sea)

Omri Bronstein · Yossi Loya

Abstract In spite of the efforts invested in the search for the environmental factors that regulate discrete breeding periods in marine invertebrates, they remain poorly understood. Here, we present the first account of the annual reproductive cycle of the pantropical sea urchin *Echinometra* sp. from the Gulf of Aqaba/Eilat (Red Sea) and explore some of the main environmental variables that drive echinoid reproduction. Monthly measurements of gonado-somatic indexes and histological observations of 20 specimens revealed a single seasonal reproductive cycle, with gametogenesis in males and females being highly synchronized. Gametogenesis commenced in June and peak spawning occurred between September and October. Gonado-somatic indexes were significantly correlated with seawater temperatures but not with photoperiod. The latter cycle lagged behind the gonado-somatic cycle by two months, suggesting that the onset of gametogenesis corresponds to shortening day length, while spawning may be driven by warming seawater temperatures. Gonads remained quiescent throughout the winter and spring (January through May) when temperatures were at their lowest. Chlorophyll-*a* concentrations increased significantly in the months following spawning (October through January). These high concentrations are indicative of high phytoplankton abundance and may reflect the increase in food availability for the developing larvae. Of the external test dimensions, length presented the highest correlation to body weight, indicating length as the best predictor for body size in *Echinometra*. Neither sexual dimorphism nor size differences between males and females were detected, and the sex ratios were approximately 1:1 in three distant *Echinometra* populations. Environmentally regulated reproduction, as occurs in sea urchins, might face severe outcomes due to anthropogenic disturbances to the marine environment. Consequently, there is a need to deepen our understanding of the mechanisms that drive and regulate this process in broadcast-spawning species.

Keywords Sea urchins · Reproductive cycle · Temperature · Photoperiod · Chlorophyll-*a* · Red Sea

Introduction

Sea urchins from the genus *Echinometra* (Camarodonta: Echinometridae) are highly common on most coral reefs and are often the most prevalent sea urchin species on the reefs they inhabit (McCartney et al. 2000; McClanahan and Muthiga 2001). They are commonly reported from shallow waters between the average low tide and depths of 10 m (McClanahan and Muthiga 2013), often showing patchy distributions with densities varying from 0.1 to 100 individuals m^{-2} over very short distances (Dumas et al. 2007). Of the widely distributed species of this genus, one species in particular, *Echinometra mathaei* (de Blainville), has received much attention in the scientific literature due to its significant ecological role in coral reef environments (McClanahan 1988a; McClanahan and Shafir 1990; McClanahan and Kurtis 1991) and has even been regarded by some as the world's most abundant sea urchin species (Palumbi and Metz 1991; Muthiga and Jaccarini 2005). *E. mathaei* play dual and contrasting roles in coral reef environments,

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mediated in large by their feeding behavior. Through feeding, they can reduce algal cover and break down reef substratum, which, at moderate densities, facilitates topographic complexity (Johnson et al. 2003) and enables coral settlement and growth (Bak 1990). However, as *E. mathaei* scrape the surface in the process of grazing (Lawrence and Sammarco 1982), they often remove large sections of the reef's hard substrata (Mokady et al. 1996; Bak 1994; Carreiro-Silva and McClanahan 2001). Consequently, at high densities, *E. mathaei*-caused bioerosion is considered a limiting factor for coral reef growth and survival (McClanahan and Kurtis 1991; Bronstein and Loya 2014). The reproductive cycle of *E. mathaei* has therefore received much attention in the scientific literature and has been examined over much of its range (Pearse 1968, 1969a; Pearse and Phillips 1968; Fujisawa and Shigei 1990; Drummond 1995; Alsaffar and Lone 2000; Muthiga and Jaccarini 2005). These studies portray mixed models of reproduction, with some populations reproducing continuously throughout the year (Pearse and Phillips 1968; Pearse 1969a; Kelso 1971), while others have restricted spawning periodicities (Fujisawa and Shigei 1990; Arakaki and Uehara 1991; Muthiga and Jaccarini 2005).

Gametogenesis in sea urchins typically follows a set of sequential maturation stages and is characterized by seasonal changes in gonad mass relative to total body mass (Fuji 1960; Byrne 1990; Walker et al. 2005). The processes underlying these stages involve the interaction of two cellular populations: somatic nutritive phagocytes (NPs) and germinal cells. At the onset of each reproductive cycle, gonads of both sexes increase in size due to extensive intragonadal nutrient storage by NPs (Walker et al. 2007). During vitellogenesis, these nutrients are transferred to the developing germ cells as the latter enlarge and increase in numbers. Mature gametes are then stored in the gonads until broadcast spawning.

The regulation of reproductive processes in marine invertebrates has long been discussed in the scientific literature (Mercier and Hamel 2009). Both timing and duration of breeding periods are crucial elements of the overall life history strategy of an organism (Boolootian 1966; Giese and Pearse 1974); therefore, the reproductive activity is thus usually timed to coincide with optimal environmental conditions for offspring survival (Mercier and Hamel 2009). In sea urchins, as in most broadcast-spawning species, high synchronization of both intra-population gamete synthesis and the environmental conditions at the time of spawning are required in order to maximize fertilization success and ensure survival of the offspring. This synchronization is believed to be mediated by external cues (Mercier and Hamel 2009). In that sense, the environmental variables that regulate reproduction in sea urchins have been a subject of interest ever since the notion was posited that invertebrates reproduce

during restricted annual periodicities (Orton 1920; Giese and Pearse 1974). The remarkable natural fluctuations exhibited by some echinoderm species (Uthicke et al. 2009) are often associated with regulation of their reproductive processes by environmental factors (Wangensteen et al. 2013). Thus, elucidating the potential environmental factors that regulate sea urchin reproduction greatly contributes to our ability to assess and predict the future of these prominent reef-structuring organisms, in particular in the context of climate change and the continuous increase in anthropogenic perturbations to the marine environment. Although not fully resolved in sea urchins, several environmental factors have been suggested to play an important role in regulating echinoid reproduction; among these factors are photoperiod (Kelso 1971; Pearse et al. 1986; Bay-Schmith and Pearse 1987; McClintock and Watts 1990; Walker and Lesser 1998; Shpigel et al. 2004), temperature (Byrne 1990; Pearse and Cameron 1991; Guillou and Michel 1993; King et al. 1994; Vaïtilingon et al. 2005; James et al. 2007), and food availability (Lawrence and Lane 1982; Muthiga and Jaccarini 2005; Harrington et al. 2007; Poorbagher et al. 2010).

The reproductive cycle of *Echinometra* had been thoroughly studied in the Gulf of Suez and northern Red Sea (Pearse 1969a). Pearse found marked differences in the reproductive behavior of *Echinometra* populations between the latter two regions, despite their geographic proximity. He reported a single spawning period (Jul–Sep) in the Gulf of Suez, in contrast to continuous spawning in the northern Red Sea. The reproductive cycle of *Echinometra* from the adjacent Gulf of Aqaba/Eilat (GOA/E), however, has never been studied in detail. Deciphering *Echinometra*'s reproductive cycle in the GOA/E will not only contribute to our understanding of these prominent reef-structuring sea urchins, but will also potentially help in clarifying some wider questions regarding the importance of geographical gradients, such as temperature and photoperiod, that regulate echinoid reproduction. Moreover, the need to further study the reproduction of these ecologically important species is further stressed in light of our recent study depicting *Echinometra* from the GOA/E and western Indian Ocean as a potentially new *Echinometra* species (Bronstein and Loya 2013).

Although echinoids are known to reproduce sexually (Pearse and Cameron 1991), their sex determination mechanism is still largely unknown (McClanahan and Muthiga 2013). In this respect, sex ratios may provide important clues to these mechanisms, as a sex ratio close to 1:1 may imply a chromosomal-dependent sex determination mechanism (Pearse and Cameron 1991). Biased sex ratios have been used as indicators of the mode of reproduction (gonochoric or hermaphroditic) of particular echinoid populations (Williamson and Steinberg 2002), as hermaphroditic populations typically have uneven adult
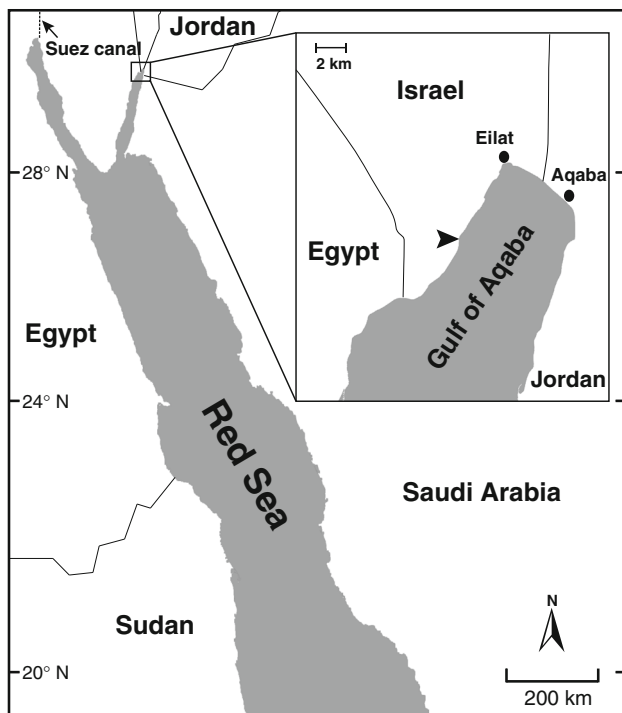
Fig. 1 Map of the northern Red Sea and Gulf of Aqaba. Collection sites of *Echinometra* specimens are marked by *arrowheads*

sex ratios (Gonor 1973; Sadovy and Shapiro 1987). In *Echinometra*, sex ratios are normally 1:1 (Pearse and Phillips 1968; Muthiga and Jaccarini 2005), although some exceptions of male (Pearse 1969a) or female (Pearse 1968) predominance have been recorded. Determining the sex ratios of naturally occurring *E. mathaei* populations may thus provide valuable data on the modes of reproduction and sex determination mechanisms of this widespread species.

Here, we investigated reproduction in *Echinometra* sp. with the aim of assessing the reproductive cycle of this most common echinoid in the GOA/E, and evaluating the relationship between the reproductive cycle of this species and photoperiod, seawater temperature, and phytoplankton concentrations. Histological observations and gonado-somatic indexes were used to depict the annual gametogenetic cycle and spawning periodicities. Allometric comparisons of males and females and differences in sex ratios were used to evaluate the modes of reproduction and resource allocation among naturally occurring *Echinometra* populations.

Materials and methods

Sample collection

Samples of *Echinometra* sp. were collected along the coast of Eilat in the GOA/E (Red Sea, 29°30′59.10″N, 34°55′34.63″E)

between January and December 2010, with one additional sampling conducted in February 2011 (Fig. 1). Twenty individuals were collected randomly on a monthly basis by snorkeling at depths of 1–3 m and brought to the laboratory at the Inter-University Institute (IUI) at Eilat for further analysis.

Allometry, sex ratios, and gonado-somatic indexes

The length, width, height, and weight of each specimen were recorded. Length and width of the tests were measured at the ambitus, while height was measured along the oral–aboral axis. Measurements to the nearest 0.5 mm were performed using thin-blade Vernier calipers to prevent interference by the spines. Weight was measured to the nearest 0.001 g after blotting each specimen dry for 5 min. After weighing and completion of external measurements, specimens were dissected and the gonads removed. Extracted gonads were weighed as described above, and the gonado-somatic index (GSI) was calculated as the ratio of the wet weight of the five gonads to the total wet body weight times 100 (Muthiga and Jaccarini 2005; Vaïtilingon et al. 2005; Walker et al. 2005). Sex was determined by observing a small piece of gonad under a light microscope to allow comparisons of the allometric measurements between males and females and to test for possible biases in sex ratios. Samples from Zanzibar (06°01′20.99″S, 39°25′30.21″E) in the western Indian Ocean, and Okinawa (26°38′03.44″N, 127°51′51.24″E) in the Indo-West Pacific, as well as 37 additional samples from Eilat, collected between June 2007 and the current study, were added to the analyses for comparison with other *Echinometra* populations. In total 297, 481, and 83 specimens were analyzed from Eilat, Zanzibar, and Okinawa, respectively.

Histological analysis

On completion of the GSI measurements, gonads were fixed in Bouin's solution for 24 h and then stored in 70 % ethanol. Preserved gonads were dehydrated, embedded in paraffin, and sectioned at 7 μm. Three replicates of each gonad, at approximately 50 μm intervals, were sectioned and stained with hematoxylin and eosin, and the clearest of the three sections was analyzed. As gametogenesis was in synchrony in all five gonads, and homogeneous throughout their lengths (Pearse 1969a; Byrne 1990; Williamson and Steinberg 2002), only the middle portion of a single gonad was used in the analysis. Male and female gonads were classified into four gametogenetic stages corresponding to fluctuations in both the NP and germ cell populations, following the staging methods used by Walker et al. (2005). These stages are (I) inter-gametogenesis and NP phagocytosis, (II) pre-gametogenesis and NP renewal (III)

gametogenesis and NP utilization, and (IV) end of gametogenesis, NP exhaustion, and spawning. Observations and measurements were made under a Nikon eclipse 90i light microscope mounted with a Nikon DS-Ri1 high-resolution documentation system (Nikon Instruments Inc.).

The size frequency distributions of oocytes and ova were assessed by measuring the first 50 oocytes and ova encountered in each ovary. Data from five females per month were pooled, giving a sample size of 250 oocytes and ova, respectively. Only primary oocytes sectioned through the nucleolus and ova sectioned through the nucleus were considered for this analysis.

Environmental measurements

Sea surface temperatures (SST) and chlorophyll-*a* measurements were obtained from the Israel National Monitoring Program at the Gulf of Eilat (http://www.meteo-tech.co.il/eilat-yam/eilat_periodical_en.asp). Monthly mean values of SST and chlorophyll-*a* concentrations were calculated from daily measurements. Photoperiod was calculated from data obtained from the Wise Observatory, Israel (http://wise-obs.tau.ac.il/).

Statistics

Data analyses were performed using R software for statistical computing (R Core Team 2013). All data were tested for normality and homogeneity of variance prior to deciding upon the appropriate statistical test. When data violated the test assumptions of normal distribution and homoscedasticity, nonparametric tests or permutation analysis were performed. Permutations were performed using the packages lmPerm (Wheeler 2010) and coin (Hothorn et al. 2008) for data analysis, allowing all permutations of Y (i.e., Perm = "Exact"). The allometric relations of weight to length, width, and height were fitted as permutation regression models (data log-transformed) with sex (two levels) and sites (three levels). As length presented the best fit to the model compared to width and height ($r^2 = 0.94$, $r^2 = 0.71$, and $r^2 = 0.39$, respectively), we present only length data here. Chi-squared tests were used to test possible differences in sex ratios. Differences in GSI were evaluated following Wangensteen et al. (2013); Mann–Whitney U tests were applied to test for between-sexes differences for every month (p values corrected for multiple testing using the Benjamini and Yekutieli FDR correction; Benjamini and Yekutieli 2001). As no significant differences were found, both sexes were pooled for further analyses. Kruskal–Wallis nonparametric ANOVAs, followed by Dunn's post hoc tests, were used to check for differences in GSI among months. To reassert the statistical validity of monthly GSI differences, permutation

ANCOVAs were fitted to the data (with gonad weight as the response variable and total body weight as the covariate). Monthly mean GSI values were tested against monthly mean SST, chlorophyll-*a*, and photoperiod using Spearman's rank correlation. Pair-wise Kolmogorov–Smirnov tests were applied to test for monthly differences in size frequency distributions between oocytes and ova. p values were adjusted for multiple testing to minimize false discovery rate, using the Bonferroni correction. Monthly mean diameters of oocytes and ova were compared using permutation ANOVAs followed by Tukey's HSD post hoc.

Results

Sex ratios and allometry

The sex ratios did not differ significantly from a ratio of 1:1 for *Echinometra* from the GOA/E (140 males, 157 females; Chi-square test, $x^2 = 1.73$, $df = 1$, $p = 0.188$) and Okinawa (46 males, 37 females; Chi-square test, $x^2 = 0.96$, $df = 1$, $p = 0.323$). However, in Zanzibar the male-to-female ratio deviated significantly from a ratio of 1:1 (267 males, 214 females; Chi-square test, $x^2 = 5.84$, $df = 1$, $p = 0.016$), with males appearing more abundant than females. No hermaphrodites were observed in any of the samples.

The weights and lengths of sea urchins from Eilat, Zanzibar, and Okinawa all had positively and highly significant correlations ($r^2 = 0.94$, $p < 2.2e-16$ for Eilat; $r^2 = 0.93$, $p < 2.2e-16$ for Okinawa; $r^2 = 0.95$, $p < 2.2e-16$ for Zanzibar), demonstrating weight increase as the sea urchins grew and elongated; the linear regression lines of the log-transformed data are shown in Fig. 2a–c. No significant differences were observed between the regressions of males and females at all sites.

Gonad histology

Four different gonad maturation stages have been recognized for male and female *Echinometra* sp. from Eilat (GOA/E). Although these stages appeared successively throughout the duration of the study, some variation among individuals from the same monthly sample was occasionally observed. The cellular events of oogenesis and spermatogenesis are listed below.

Testes

Stage I (Inter-gametogenesis and NP phagocytosis; Fig. 3a): The testicular lumen is largely devoid of contents.
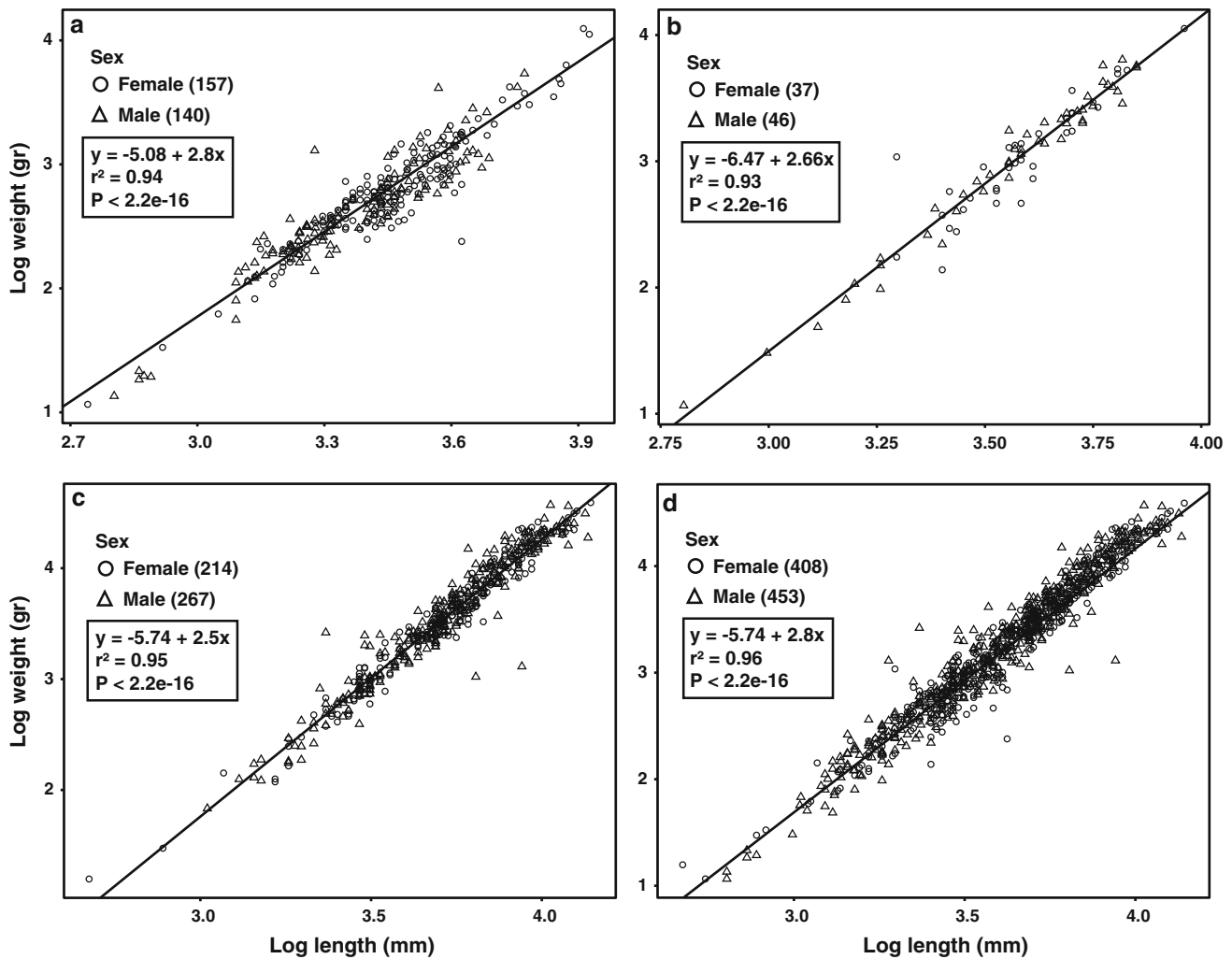
Fig. 2 Linear regression models of length and weight (log-transformed) in three populations of *Echinometra*: **a** Eilat, **b** Okinawa, **c** Zanzibar, and **d** the pooled data from all locations. Females are denoted by *circles*; males by *triangles*. *Numbers* in parentheses represent sample sizes. The regression equations are given in the *boxes* with respective r^2 and *p* values

A thin basophilic layer of amitotic spermatogonia and primary spermatocytes line the ascinal wall and relict spermatozoa may sometimes be present. NPs begin to accumulate nutrients near the ascinal wall, although the general appearance of the NP layer is that of a thin meshwork, largely depleted. In early-spent testes, relict spermatozoa may sometimes be present in the center of the acini, but these will later be resorbed by NP phagocytosis.

Stage II (Pre-gametogenesis and NP renewal; Fig. 3b): Amitotic and mitotic spermatogonia emerge near the ascinal wall and occasionally project centrally as primary spermatocytes. The NP layer proliferates as the ascinus is filled with growing NP cells.

Stage III (Gametogenesis and NP utilization; Fig. 3c): All stages of germ cells are evident in the germinal epithelium, gradually maturing from amitotic spermatogonia near the ascinal wall, to new spermatozoa accumulating in the testicular lumen. NPs deplete and are displaced from the center as this stage progresses until finally remaining as a thin luminal layer near the ascinal wall.

Stage IV (End of gametogenesis, NP exhaustion, and spawning; Fig. 3d): The testicular lumen is densely packed with the highest numbers of new spermatozoa for the year. The NP layer is at its annual low and is restricted to the ascinus periphery.

Ovaries

Stage I (Inter-gametogenesis and NP phagocytosis; Fig. 3e): A thin layer of NPs line the ascinal wall while the lumen appears largely empty. In early-spent ovaries, relict oocytes and large unspawned ova may sometimes occupy the lumen. As this stage progresses, the relict gametes will eventually be phagocytosed and resorbed by the NPs.
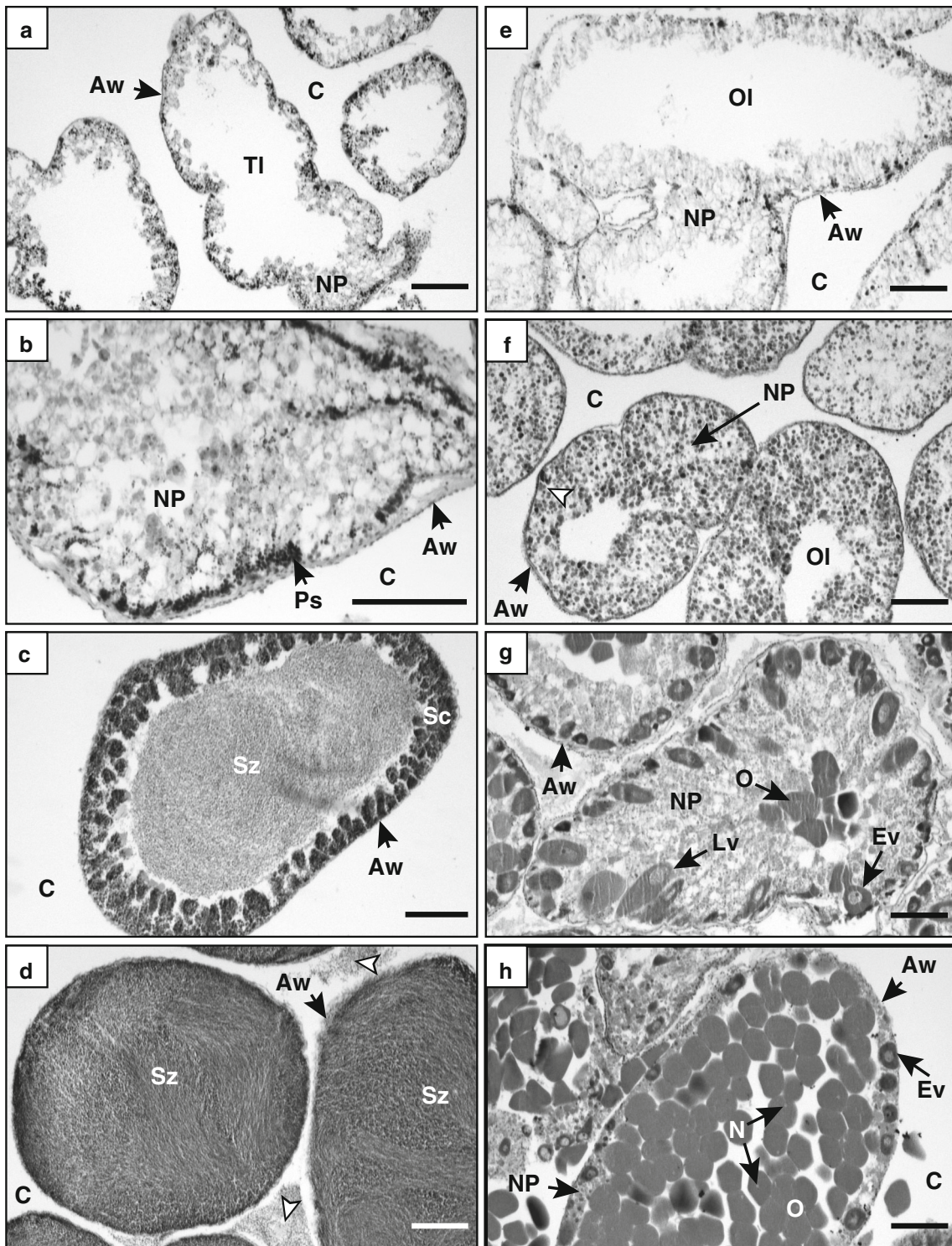
◄ **Fig. 3** Histology of testes (**a**–**d**) and ovaries (**e**–**h**) of *Echinometra* sp. from Eilat (Gulf of Aqaba). Cross-sections through acini representing reproductive stages I–VI. *Stage I* inter-gametogenesis and NPs phagocytosis. Gonads are largely devoid of contents, showing spermatozoan-free lumen in males (**a**), and ova-free lumen in females (**e**). A thin layer of NPs is present along the ascinal walls in both sexes. *Stage II* pre-gametogenesis and NP renewal. NPs proliferate throughout the gonads from the ascinal wall to the center, gradually filling the lumen of testis (**b**) and ovaries (**f**). Limited groups of spermatogonia and primary spermatocytes and clusters of early oocytes (*open arrowhead*) start to be visible near the testicular and ovarian walls, respectively. *Stage III* gametogenesis and NP utilization. Spermatogenic cells increase in number as new spermatogonia develop basally while spermatocytes migrate to the testicular lumen, where they accumulate as newly matured spermatozoa (**c**). Extensive growth of primary oocytes as they mature and migrate to the ovarian lumen, where they accumulate as fully mature ova (**g**). *Stage VI* end of gametogenesis, NP exhaustion, and spawning. The NP layer in both testes and ovaries is largely depleted and reduced to a thin layer along the ascinal wall. The testicular lumen is densely packed with spermatozoa. Occasionally, spermatozoa are evident in the coelom (*open arrowheads*) (**d**). Ovarian lumen densely packed with mature ova, while oocytes at different maturation stages are evident near the ascinal wall (**h**). *Scale bars* represent 100 μm. *Aw* ascinal wall, *C* coelom, *Ev* early vitellogenetic oocyte, *Lv* late vitellogenetic oocyte, *NP* nutritive phagocytes, *O* ova, *Ol* ovarian lumen, *Tl* testicular lumen, *N* nucleus, *Sc* spermatocytes, *Sz* spermatozoa, *Ps* primary spermatocytes

Stage II (Pre-gametogenesis and NP renewal; Fig. 3f): The NPs grow and extend into the lumen and by the end of this stage occupy most of the ovary. Clusters of early oocytes are apparent along the ascinal wall. Ova are largely absent from the lumen although relict degenerating ova may occasionally be present at the center.

Stage III (Gametogenesis and NP utilization; Fig. 3g): All stages of germ cells are visible at this stage including amitotic and mitotic oogonia as well as early and late oocytes. With the onset of vitellogenesis, primary oocytes increase, with most remaining in contact with the ascinal wall. At the end of this stage, the first mature ova may start to appear in the ovarian lumen. NPs are still filling the lumen, although as oocytes grow they progressively occupy less space.

Stage IV (End of gametogenesis, NP exhaustion, and spawning; Fig. 3h): As vitellogenesis continues, the growing primary oocytes detach from the ascinal wall and project toward the center of the ascinus where they accumulate as ova. Oocytes at other maturation stages are still present in the germinal layer near the ovarian wall. By the end of this stage, ovaries are densely packed with mature ova and the NPs are completely exhausted.

Gametogenetic cycle

The relative monthly frequencies of the four maturation stages are given for both males (Fig. 4a) and females (Fig. 4b). Males and females appeared remarkably synchronized throughout their gametogenetic cycle. Spent males and females were mainly present from January to June, with some spent females evident through July–August. During these same months, stage II specimens were also present although to a slightly lesser extent. Gametogenesis initiated in June and progressed rapidly, reaching a peak in July (although some stage III specimens were also observed in other months). Maturation and spawning occurred from midsummer to early winter (August–December) when the frequency of stage IV specimens was highest. This stage peaked in September–October, later showing signs of decline as winter progressed. Gametogenesis appeared to be continuous throughout the spawning period, as evident from the basal frequencies of stage III specimens during this period. The relative frequencies of February 2011 were similar to those of February 2010, supporting the stability of the observed annual cycle.

Oocytes size frequency distribution

The monthly size frequency distributions of oocytes and ova are given in Fig. 5. A significant difference in oocyte size distribution was observed between May and the following months (pair-wise Kolmogorov–Smirnov, $D = 0.568$, $p < 0.05$) with no significant change in the latter (pair-wise Kolmogorov–Smirnov, $p > 0.05$ for all comparisons). Oocyte mean diameters varied significantly between months (permutation ANOVA, $df = 4$, $p < 2.2e{-}16$). The most significant increase in oocyte mean diameter occurred between May and June (from 22.15 to 37.5 μm, respectively), followed by no significant changes in oocyte mean diameters. Mature ova were first recorded in June, 1 month following oocyte appearance. Ova mean diameters varied significantly between months (permutation ANOVA, $df = 4$, $p < 2.2e{-}16$). Ova continued to be present throughout the duration of reproductive stages III and IV, ending in December. Ova mean diameter increased from an annual low in June of 55.93 ± 0.56 μm (mean $\pm$ SE), to a maximum in October of 61.13 ± 0.41 μm (mean $\pm$ SE), after which ova mean diameter decreased. This pattern coincided with the observed gametogenetic cycle and subsequent spawning.

Gonado-somatic indexes (GSI)

The GSI of *Echinometra* sp. in the GOA/E undergoes an annual cycle (Fig. 6). As no significant differences in individual GSI between males and females were found in any of the sampled months (Mann–Whitney U test, all FDR corrected $p > 0.05$), their data were pooled monthly. Kruskal–Wallis tests showed significant differences in GSI values between months (Kruskal–Wallis test, $H = 157.19$, $df = 12$, $p < 2.2e{-}16$). Due to the possibility of drawing erroneous conclusions when applying analyses of variance to ratios (Packard and Boardman 1988), additional analyses of
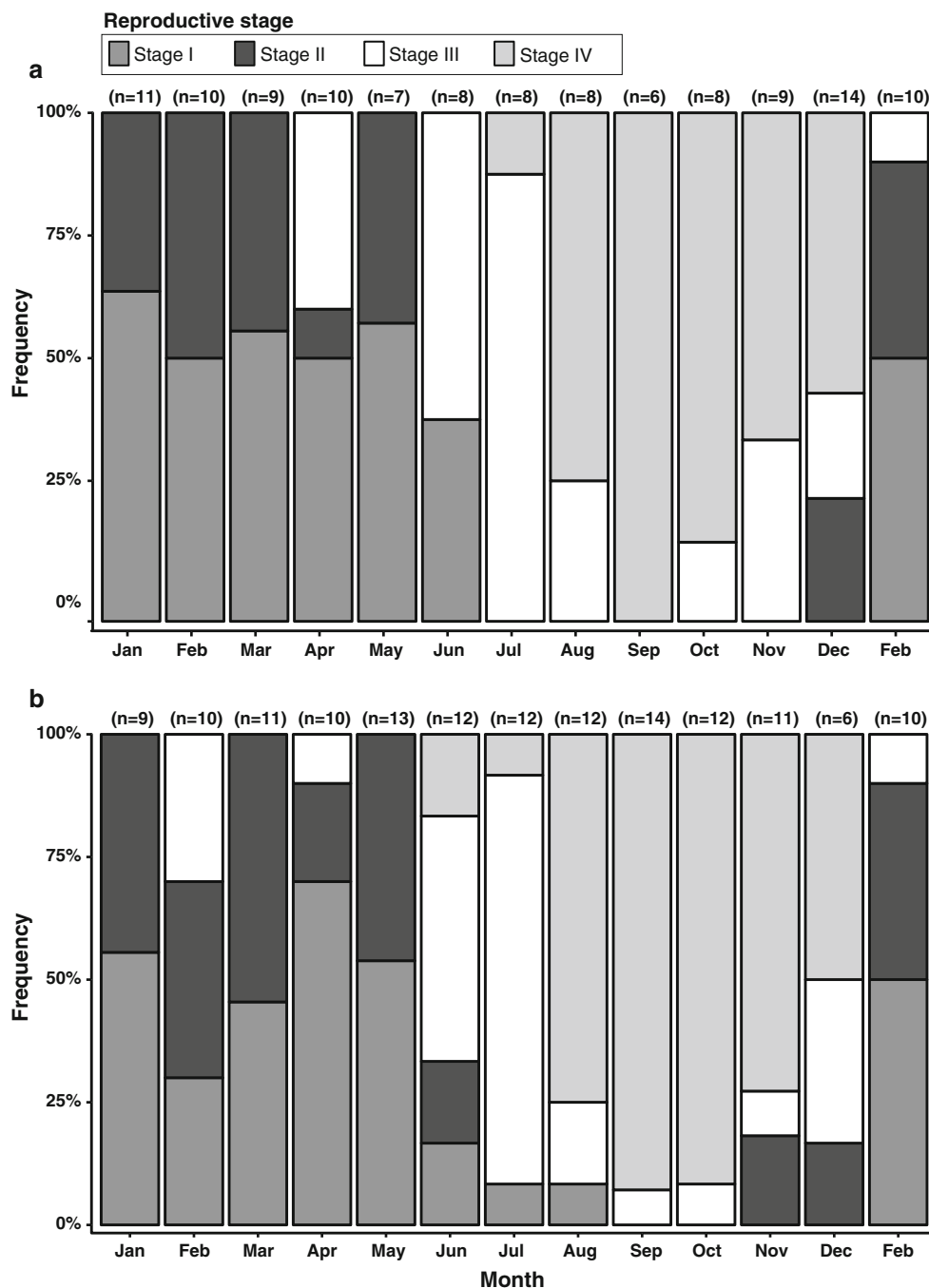
Fig. 4 The gametogenetic cycle of *Echinometra* sp. from Eilat (Gulf of Aqaba). The relative frequencies (%) of gonad developmental stages in monthly samples of **a** males and **b** females. Frequencies are based on monthly histological analysis of 20 specimen studied from January 2010 to February 2011. Colors indicate reproductive stages I, II, III, and VI. Reproductive stages correspond to: *I* inter-gametogenesis and NPs phagocytosis; *II* pre-gametogenesis and NP renewal; *III* gametogenesis and NP utilization; *VI* end of gametogenesis, NP exhaustion, and spawning

covariance (ANCOVA) were applied to test for monthly differences in gonad weight. This analysis confirmed the observed monthly differences (permutation ANCOVA, $p < 2e-16$). GSI reached an annual peak during August–September and was followed by a drop in the index value in October, indicating a single annual spawning event (Fig. 6a).

GSI values reached their annual minimum from March through May, after which GSI buildup was observed. The GSI values in February 2011 were similar to those of February 2010. Monthly mean GSI levels had a significant positive correlation with SST (Spearman rank correlation, $r_s = 0.6$, $p < 2.2e-16$; Fig. 6b). The gametogenetic cycle followed the
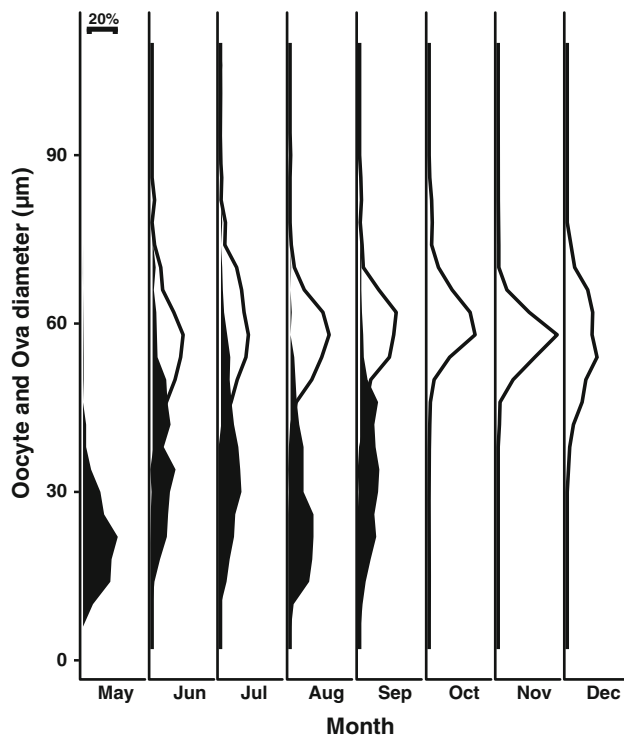
Fig. 5 The size distribution of ova (*open polygons*) and oocytes (*filled polygons*) of female *Echinometra* sp. collected at the coral reefs of Eilat during May through December 2010. Mean oocyte and ova diameters (μm) were obtained from measurements of 50 cells each, in five female specimens per month

increase in mean water temperatures and reached peak GSI values during the warmest months of the year. Chlorophyll-*a* concentrations and photoperiod had little to no correlation with GSI (Spearman rank correlations, $r_s = -0.13$, $p = 0.04$ and $r_s = -0.09$, $p = 0.14$, respectively). Nonetheless, the annual fluctuations in chlorophyll-*a* concentrations lagged by 4 months with respect to the annual GSI cycle, as reflected in the strong positive correlation achieved when chlorophyll-*a* concentrations were shifted by 4 months backwards (Spearman rank correlation, $r_s = 0.81$, $p = 0.0014$). Similarly, the annual photoperiod cycle preceded that of GSI by 2 months (Spearman rank correlation, $r_s = 0.69$, $p = 0.0111$; for the forward shifted photoperiod cycle).

Discussion

Geographical patterns of *Echinometra* reproduction

The paradigm of continuous reproduction in the tropics, with increased restriction of the breeding season with increased latitude, has been widely discussed in the scientific literature for many marine invertebrates (Orton 1920; Giese 1959; Bauer 1992). The wide distributional range of *E. mathaei* has consequently made it a prime

candidate for testing this paradigm. Pearse (1968) noted a latitudinal threshold of 27°N, above which *E. mathaei* spawning is expected to be restricted to the summer months, whereas closer to the equator these sea urchins are expected to shift toward continuous spawning. Nonetheless, exceptions to this paradigm exist, even for *E. mathaei*, such as in the case of the Kenyan populations, where a seasonal reproductive cycle was noted, despite their proximity to the equator (latitude 4°S; Muthiga and Jaccarini 2005). In the current study specimens were collected from Eilat, at the northern tip of the GOA/E, above Pearse's latitudinal threshold for summer-restricted spawning. Despite a slight deviation in the spawning season between Pearse (1969a) and the current study (Jul–Sep and Sep–Oct, respectively), our findings conform to Pearse's latitudinal threshold. Thus, in the case of the reproductive cycle of *Echinometra*, the Gulfs of Suez and Aqaba bear high similarity, while also being distinct from other parts of the Red Sea where spawning is continuous.

That the latter two gulfs are distinct from the rest of the Red Sea may be attributed to their northerly position and the consequent environmental conditions that gradually change according to latitude. However, these two gulfs also bear marked differences from one another in many of their environmental and biological characteristics, and as such raise intriguing questions regarding the role of possible environmental drivers of echinoid reproduction. While the Gulf of Suez is long, narrow, and shallow (250 km long, 32 km average width, and only about 35 m in average depth), the GOA/E, though shorter and narrower (160 km long with an average width of 16 km), is considerably deeper, averaging 650 m and reaching more than 1,800 m at its deepest point (Loya 2004; Monismith and Genin 2004; El-Shenawy et al. 2006). James and Pearse (1969) demonstrated the marked faunistic differences between the two gulfs and attributed these differences to the depth differences between them. They note, based on species distribution and abundance, that the environmental conditions within the GOA/E are more similar to those in the Red Sea than to those in the Gulf of Suez. Nonetheless, detailed faunistic and hydrologic data on these three basins (i.e., Gulf of Suez, GOE/A and the Red Sea) are still needed in order to fully understand the mechanism that facilitates the different reproductive strategies between the two gulfs and the rest of the Red Sea. Even at this point, however, it is safe to assume that timing of the reproductive activity of *Echinometra* is dependent on multiple co-acting environmental factors rather than on a single determining variable (Pearse 1969a).

Gametogenesis

The reproduction of *Echinometra* from the GOA/E follows an annual cycle, with gametogenesis initiating in June and
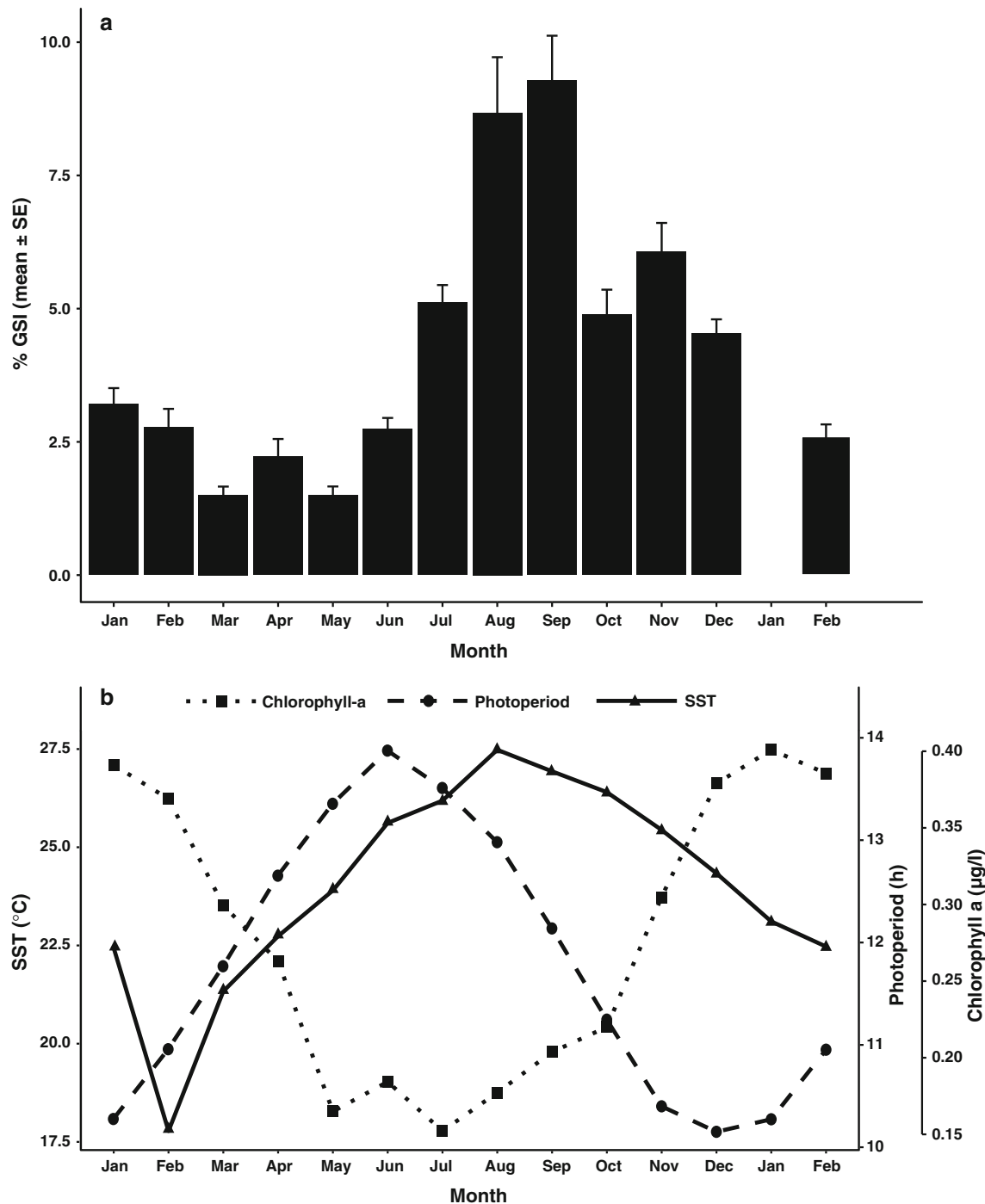
Fig. 6 Monthly mean values of the GSI of *Echinometra* sp. from the Gulf of Aqaba and the mean monthly values of selected environmental variables. **a** Monthly GSI values (mean ± SE) for *Echinometra* sp. at Eilat (Gulf of Aqaba) during 2010–2011 ($n = 20$ specimens per month). No data are available for January 2011. **b** Monthly mean SST (°C), photoperiod (h), and chlorophyll-

a (µg l^{-1}). Mean photoperiod was calculated from data obtained from the Wise Observatory, Israel (http://wise-obs.tau.ac.il/). Mean SST and chlorophyll-a were obtained from the Israel National Monitoring Program at the Gulf of Eilat (http://www.meteo-tech.co.il/eilat-yam/eilat_periodical_en.asp)

spawning occurring between September and October. As in most studies of echinoid gonad histology (Wangensteen et al. 2013), the low intra-individual variability and uniform gonad maturation of *Echinometra* from the GOA/E

facilitated assigning a single categorical maturation stage to any given individual. Histological observations of the gonads of *Echinometra* from the GOA/E revealed that gametogenesis in males and females was highly

synchronized. Mature males and females predominated from August to November with no apparent shift in the duration of this phase between the two sexes, in contrast to a prolonged spawning period observed in males of *E. mathaei* from Kenya (Muthiga and Jaccarini 2005) and the Gulf of Suez (Pearse 1969a). In the latter two regions the longer spawning period of males was explained as an adaptive strategy to enhance fertilization success by ensuring fertilization of females spawning late or out of season (Muthiga and Jaccarini 2005). However, as no females appear to spawn out of season in the GOA/E, male spawning may have consequently evolved to converge to female spawning periods, thus maximizing fertilization success.

Oogenesis took approximately 2–3 months, similar to *E. mathaei* from Kenya (Muthiga and Jaccarini 2005) and the Gulf of Suez (Pearse 1969a). The presence of a large population of oocytes in the ovaries is indicative of the continuous process of vitellogenesis and oocyte maturation from May to September. Ovaries began accumulating ova as early as June and had attained a high ova content by September, prior to spawning, when oogenesis was completed. However, in many echinoid species (e.g., *Diadema setosum*, *Prionocidaris baculosa*, and *Lovenya elongate*) including *E. mathaei*, not all oocytes mature to ova, as some excess oocytes disintegrate and undergo phagocytosis by the NPs, thus facilitating the reallocation of nutrients to the remaining growing oocytes (Pearse 1969b, 1970). When a large number of excess oocyte are produced and later phagocytosed, the oocytes size frequency distribution appears bimodal, as was demonstrated for *E. mathaei* from the Gulf of Suez (Pearse 1969a). In contrast, when a low number of excess oocytes are being produced, a continuous progression from small to large oocytes is apparent and manifested as a unimodal distribution (Muthiga and Jaccarini 2005). In this respect, *Echinometra* from the GOA/E are similar to *Echinometra* from Kenya, producing few excess oocytes per gametogenetic cycle.

Environmental regulation of the reproductive cycle

Temperature

Seasonal temperature variations have been shown by many to play an important role in regulating echinoid reproduction (e.g., Pearse 1969a; Sakairi et al. 1989; Byrne 1990; King et al. 1994; Pecorino et al. 2014; Wangensteen et al. 2013). Temperature was significantly correlated with *Echinometra*'s reproductive cycle in the GOA/E. Similar to the Gulf of Suez, its reproductive cycle is positively correlated with rising SST in summer. However, the temperatures in the latter two gulfs are not identical; those in the Gulf of Suez range from 18 °C in winter to 28 °C in

summer (Pearse 1969b), while in the GOA/E, they range from 20.5 °C (winter, northern Gulf) to 27.3 °C (summer, southern Gulf; Loya 2004). Nonetheless, although the GOA/E experiences a narrower range of temperatures, never reaching the maxima or minima of those of the Gulf of Suez, there seems to be no difference in *Echinometra*'s reproductive cycle between the two gulfs. In another example, *E. mathaei* populations from Rottnest Island, Western Australia, spawn continuously (Pearse and Phillips 1968) in seawater temperatures cooler than those experienced by Kenyan *E. mathaei* populations that spawn seasonally (Muthiga and Jaccarini 2005; 18–22 and 24–31 °C, respectively). Thus, a seawater temperature threshold is not likely in *Echinometra*, as had been previously suggested (Pearse and Phillips 1968). Alternatively, variation in temperature, rather than an absolute temperature threshold, may be the key exogenous signal for spawning in *Echinometra*.

Photoperiod

Photoperiod has also been noted as one of the main exogenous factors that control the reproductive cycle in echinoids (Pearse et al. 1986; Walker and Lesser 1998; Kelly 2001; Shpigel et al. 2004). However, no correlation between GSI and photoperiod was found for *Echinometra* from the GOA/E. These results appear to be in agreement with Pearse (1969a), who similarly found no such correlation in *Echinometra* populations from the adjacent Gulf of Suez (which experiences similar photoperiod conditions). Nonetheless, the longest mean day length was reached in June, 2 months prior to the peak in GSI and at the time of gametogenesis initiation, as the photoperiod cycle lagged 2 months behind the GSI cycle (Fig. 6). Thus, although photoperiod does not seem to be the trigger for spawning, unlike several other echinoid species, long days may inhibit gametogenesis (Pearse et al. 1986; Walker and Lesser 1998), and shortening days may serve as the cue for gametogenesis initiation. A similar tendency of gametogenesis initiation triggered by the onset of shortening day length was also reported in other echinoid species, such as *Strongylocentrotus droebachiensis* from the Gulf of Maine (Walker and Lesser 1998).

Food availability

Echinoid spawning was noted to coincide with phytoplankton blooms in temperate environments (Himmelman 1975). However, as food levels can be quite variable among years and locations, food abundance is unlikely to directly entrain reproductive cycles (Mercier and Hamel 2009). Food availability is nonetheless an important factor for the developing larvae and newly recruited young

(Lessios 1981). In Kenya, *E. mathaei* was reported to spawn during the annual peak in chlorophyll-*a* concentrations (Muthiga and Jaccarini 2005); and in the Gulf of Suez, fluctuations in food availability were suggested to play an important role in regulating the reproductive periodicities of the echinoids *Prionocidaric baculosa* and *Lovenia elongate* (Pearse 1969b). In the GOA/E, *Echinometra* spawn prior to the annual peak concentration in chlorophyll-*a*. The timing of spawning nevertheless coincides with the sharp, constant rise in chlorophyll-*a* concentrations. The latter concentrations constantly increase in the months following spawning, and the annual peak is reached in January, approximately 4–5 months post spawning. Thus, the developing larvae are ensured a constant supply of food throughout the early stages of their lives.

Sex ratios and allometry

In contrast to the relatively large body of the literature on the environmental drivers of their reproductive cycle, far less progress has been made in understanding the underlying mechanisms of sexual reproduction in echinoids. Currently, no external morphological characters are recognized as distinguishing between male and female *Echinometra* (McClanahan and Muthiga 2013). Similarly, in the current study, despite meticulous examination of more than 860 specimens, no external sexual dimorphism was evident. We further explored the sexual variance in *Echinometra* by means of allometric comparisons of different populations in the Indo-West Pacific. Biotic factors such as food availability, as well as abiotic factors such as wave action, have been shown to influence urchin morphology (Lewis and Storey 1984; Dafni 1986). Our data nonetheless show a highly conserved size allometry in *Echinometra*, as reflected in the similar regressions obtained from the three study sites (i.e., the GOA/E, Zanzibar, and Okinawa), despite the likely biotic and abiotic differences between these sites (e.g., temperature, photoperiod, salinity, wave action, chlorophyll-*a* concentration, coral and macroalgae abundance, etc.; Sakai and Yamazato 1984; McClanahan 1988b; Loya 2004; Bronstein and Loya 2014). Of the three measurable axes of the oval-shaped *Echinometra* (length, width, and height), length had the highest correlation to weight, establishing it as the most reliable character for size evaluation among the three body axes. No difference between males and females was detected with respect to size, that is, males did not appear to be bigger than females or vice versa, at any of the sites (Fig. 2a–c). Figure 2d summarizes the length–weight regression of the pooled *Echinometra* data and reflects the exceptionally high coefficient of determination ($r^2 = 0.96$) for the length–weight regression. To date, little is known regarding the

mechanism that determines sex in echinoids (McClanahan and Muthiga 2001, 2013). Sex ratios have, however, been used as a broadscale diagnostic indicator of the mode of reproduction (Sadovy and Shapiro 1987; Williamson and Steinberg 2002) and our data conform to those for gonochorism in *Echinometra* from the GOA/E and Okinawa (sex ratio of 1:1), and although male predominance was recorded in *Echinometra* populations from Zanzibar, no hermaphrodites were observed in any of the samples. Species with a chromosomal (i.e., genetic) sex determination mechanism, such as *Paracentrotus lividus* (Lipani et al. 1996), are expected to allocate equal effort to producing male and female offspring because random meiosis would lead to a mean sex ratio of 0.5 (Gianguzza et al. 2009). Thus, as largely no size differences or sex ratio disparities were observed between the two sexes across highly diverse environments, sex allocation in *Echinometra* appears to be an inherent trait with little sexual plasticity in respect to environmental conditions.

Environmental threats to echinoid reproduction

Changes to key environmental factors that regulate breeding in sea urchins, such as photoperiod and temperature, may have fundamental consequences on their reproduction, potentially leading to the reduction or even functional collapse of entire populations. Such changes may act on global (Hoegh-Guldberg et al. 2007) or local (Aubrecht and Elvidge 2008; Aubrecht et al. 2008) scales, but are often the consequence of human action. As gametogenesis in echinoids appears to be regulated by day length, exposure to artificial light due to coastline development may disrupt their natural reproductive cycle. Although exposure to artificial light was shown to directly disrupt reproduction in several marine invertebrates, such as corals (Jokiel et al. 1985) and polychaete worms (Franke 1999), the full extent of this disturbance is still poorly understood (Longcore and Rich 2004; Aubrecht et al. 2008). Nonetheless, such exposure poses a potential threat, mainly to species in which most of the population occupies shallow waters, as in the case of *Echinometra* (McClanahan and Muthiga 2001). Warming due to climate change may shift the cue for spawning to earlier months in the year, thus disrupting reproduction by (a) preventing the completion of the reproductive cycle (if the temperature-dependent spawning is shifted to earlier months while the photoperiod-dependent gametogenesis remains the same), or (b) by offsetting the synchronization between food supply, larval development, and juvenile growth if a time gap arises between spawning and the timing of the annual algal blooms. Though these hypotheses need further testing for validation, the accelerated development of coastal areas in the vicinity of coral reefs, such as in the GOA/E, calls for immediate scientific evaluation of these potential threats.

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