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Comparison of quantitative gonad maturation scales in a temperate oyster (*Crassostrea gigas*) and a sub-tropical oyster (*Crassostrea corteziensis*)

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Abstract :

Quantitative scales to evaluate maturity of female gonads were compared for a temperate oyster, Crassostrea gigas and a tropical oyster, Crassostrea corteziensis grown in the same locality. C. gigas had well-defined maturation and spawning periods and a resting phase in winter; in C. corteziensis mature individuals occurred most of the year and there were several spawning peaks. Of the quantitative scales used here, average oocyte diameter and gonad coverage area, much used for C. gigas, and ovary maturity index, less used, were inadequate to distinguish the reproductive pattern of C. corteziensis, since they both skewed the degree of maturation in vitellogenic stages in favor of C. gigas. Maximum oocyte diameter and maximum cytoplasm area were different among species at vitellogenic stages and also in previtellogenic stages. Nucleus: cytoplasm ratio was significantly different in previtellogenic and spawned stages between C. gigas and C. corteziensis. The Index of gonadal development was skewed in favor of C. gigas in postvitellogenic stage. The only scale that was comparable between species was reproductive potential, but it also was one of the most laborious. Other ordinal scales commonly used, such as a visual external evaluation of the gonad, only classified correctly a quarter of the stages.

Keywords : Female, histology, mollusk, oyster, reproduction, vitellogenesis

Introduction

A continuous scale of maturation is more accurate to describe reproductive potential in mollusks, since it can be correlated to biochemical or physiological variables or used as a co-variable for analyses. Several quantitative scales that are based on continuous data have been proposed; for example, a comparison of gonadal to visceral mass area or gonad coverage area in *C. gigas* (Mori 1979; Enriquez-Diaz 2004; Fabioux et al. 2005) and *C. viriginica* (Heferman & Walker 1989; Barber et al. 1991), and in *C. gigas* males (Lannan 1980). A variation of this is the follicle cross-sectional area relative to total cross-sectional area (Allen & Downing 1986) and the ratio of the weight of the photographic print area occupied by ova to the photographic print weight of the gonad in *C. gigas* (Muranaka & Lannan 1984). The number of mature oocytes in relation to total oocytes was used to determine stage of gonadal development in *C. gigas* (Lannan 1980). Oocyte diameter has been proposed to evaluate the degree of maturation in oysters (Lango-Reynoso et al. 2000; Ren et al. 2003; Chavez-Villalba et al. 2002; Li et al. 2006) proposed using average oocyte diameter as a tool for assigning maturation stages in *C. gigas*.

However, for tropical oysters there are scarce studies that report quantitative data on maturation. Among the existing scales, the gonadosomatic index (GSI), calculated as gonad weight in relation to body weight has been reported for *C. corteziensis* (Frias-Espericueta et al. 1997, 1999) and *C. iridescens* (Páez-Osuna et al. 1993; Frias-Espericueta et al. 1997). However, Frias-Espericueta et al. (1997) concluded that measuring GSI in oysters was inaccurate because the gonad cells develop spread within the mantle. The area occupied by gonad relative to somatic tissue, termed gonad coverage area (GCA) has been reported for *C. corteziensis* (Rodríguez-Jaramillo et al. 2008). The oocyte diameter, widely used for *C. gigas*, has also been tested in *C. rhizophorae* (Ferreira et al. 2006) and *C. corteziensis* (Rodríguez-Jaramillo et al. 2008).

The shortage of quantitative maturation data in tropical oysters is further complicated when the objective is to compare reproductive potential between species. For example, the oocyte diameter in same stage oocytes is bigger in *C. gigas* compared to *C. corteziensis*, which can skew data to a false advance stage of maturation in the former. In accordance, Ferreira et al. (2006) also concluded that differences in oocytes do not allow for direct use of scales developed for *C. gigas*, to *C. rhizophorae*, a tropical oyster. Relative scales, as GSI, have been applied to compare maturation in different species of fish (David-Gómez & Gerson-Araujo 2004; Brewer et al. 2007). A maturity index (MI), calculated by the number of individuals at each maturity stage, has been applied to compare different species of echinoderms (Egea et al. 2011). The reproductive output index (ROI=% area of propagules/total area of tissue scanned) has been applied to compare different species of sponges (Whalan et al. 2007; Leong & Pawlik 2011). The objective of this study was to compare quantitative methods used for assigning continuous scales of maturation in *C. gigas*, and analyzing if the same scale can be use to reflect variations in the reproductive pattern of *C. corteziensis*, in an effort to make comparable maturation grade in both species.

Materials and methods

Site, origin and sampling

Oysters were sampled from Bahía Ceuta, on the coast of the state of Sinaloa in the Northwest of Mexico. *C. corteziensis* were sampled in 2005-2006, as described in Rodríguez-Jaramillo et al. (2008), while *C. gigas* were sampled in 2007, as described in Galindo-Sanchez et al. (2008). Upon arrival, the specimens were inspected and measured (total weight, length, width, and wet tissue weight). The oyster meat was removed from the shell and a 2-mm cross-section was processed and the maturation stage frequency was analyzed as described in Rodríguez-Jaramillo et al. (2008).

Visual test for maturity

The visual maturity was done based on the method described by Costil et al. (2005) and modified here by assigning a number to the perceived stage of maturation of the gonad after opening the oyster:

1. Immature: Thin oysters
2. Early maturation or post-spawning: partially thin oysters
3. Late maturation: fleshy individuals
4. Mature or ready to spawn. Individuals that seem "veined"

Quantitative histology

Oocyte frequency: Frequency was calculated by counting each type of oocyte in three regions of the ovary of each female that appeared within an area of 1.44 mm^2at 20×. A total of 8457 oocytes were analyzed for *C. gigas*, and 9870 for *C. corteziensis.*

Oocyte area and diameter: The diameter and area of oocytes were determined with digitalized images at 40×and 100× for ovogonias (Image Pro Plus software v. 7.0 Media Cybernetics, Silver Spring, MD, USA). Approximately 40–50 oocytes in three randomly selected regions of a slide were observed if the nucleus was visible. Since oocytes deviate markedly from a sphere, the theoretical diameter (TD) was calculated from the total area (A) of each oocyte using the following formula: $TD= \sqrt{4A/\pi}$ (Saout et al. 1999).

Reproductive scale: The average diameter of oocytes was calculated for each female, and females were classified according to the scale proposed by Lango-Reynoso et al. (2000) for *C. gigas* based on oocyte diameter:

Early gametogenesis =3.0-12.0 µm

Growing = 12.1-30.0 µm

Mature =30.1-41.0 µm

Degenerating =41.1-60.0 µm

Gonad coverage area: The area occupied by the gonad was determined using an image system analyzer (Image Pro Plus v.7.0) at 4× (7.9mm²) from a mean of three different slices from each specimen. The image analysis was based on the intensity of the tissue-specific color and the female gonad and visceral area were automatically calculated in pixels and expressed in µm². The area reported as the gonadal coverage area (GCA) was calculated as: GCA= (gonad occupation area/total area) ×100.

Ovary Maturity Index: The ovary maturity index was calculated as the total area occupied by each oocyte in each gonad, using the following formula: GCA x Oocyte area x total number of oocytes, modified from Arcos et al. (2005) to analyze maturation in shrimp.

Index of Gonadal Development: The index of gonadal development was calculated as the ratio of postvitellogenic oocytes in relation to the total number of oocytes present in the gonad, as described by Lannan (1980).

Nucleus: cytoplasm ratio: The area of the nucleus was determined for each oocyte similarly to the area for oocytes using digitalized images at 40× and 100× for ovogonia. The ratio was calculated as nucleus: cytoplasm ratio= nucleus total area/cytoplasm total area without nucleus.

Reproductive potential: Determined based on fecundity as described by Heffernan and Walker (1989), modified here to include oocytes in all stages of maturation, was calculated using the following equation: $(4/3\pi)\ r^3\ N$, where r = radius of the oocyte and

N = total number of oocytes, but only the oocytes present in 1.44 mm^2at 20× were counted.

Statistical analysis

The stages of visual maturation were compared to the stages of reproduction by a X^2.

Oocyte measurements of each female were analyzed with a nested ANOVA (40-50 oocytes per female when possible, 10 oysters per month). Morphometric analyses, GCA, and oocyte frequencies per oyster were analyzed by one-way ANOVA, followed by Newman-Keuls post hoc analysis to assess the significant differences (P<0.05) between months (14 levels) or maturation stages (4 levels).

All statistical analyses were made using Statistica™ v. 6.0. Percentage results were transformed to arcsine before ANOVA but only untransformed means are presented. All data are reported as mean ± standard error, except where indicated.

Results

Maturation stage frequency

Females of *C. corteziensis* were found in several stages of maturation during most of the year, postvitellogenic females were found from March to November, and spawned females were observed from May to December. From December to February, a large proportion of immature females were present (Fig. 1A).

In contrast, spawning occurred in late summer and during autumn in females of *C. gigas*, and no matured females were found before May (Fig. 1B).

Average oocyte diameter

The average oocyte diameter of both species is shown in Fig. 2. The oocytes of *C. gigas* were approx. 25µm in January and grew till reaching their biggest size in April

(more than 40µm), then decreased in size in May and further still in June, and increased again in July and August, after which they reached their smaller size in October.

No significant differences were found for oocyte diameter of *C. corteziensis*.

Gonad coverage area

The GCA of *C. gigas* was lowest in January and from there, increased till reaching highest values in April and May. It decreased from June till reaching values similar to January in August, where it remained stable till October.

In *C. corteziensis* the GCA increased from January and February when values were lowest, till reaching highest values in April. In May and June it decreased to intermediate values and then increased in August to values similar to April. In September it decreased again to values similar to May, and then decreased to values similar to January from October to December (Fig. 3).

Reproductive potential

The reproductive potential for *C. gigas* increased from January to February and still more to its highest values in March, and then decreased in April and even more in May. It increased again in June and decreased to similar levels of January in July, and then decreased to lowest values in August and October (Fig. 4).

The same index calculated for *C. corteziensis* showed lowest levels in January, then increased from February till reaching the highest peak in April, decreasing in May and June, and reaching a second peak in August till October, and then decreased in November to reach values similar to January in December.

Ovary maturity index

The ovary maturity index calculated for *C. gigas* increased from January and February to intermediate values in March, and then to highest levels in April, then gradually decreasing from May till reaching in August and October similar values to January (Fig. 5).

The same index calculated for *C. corteziensis* showed lowest levels in January, then increased from February till reaching a peak in April, decreasing in May and June, and reaching a second peak in August; decreasing in September till reaching, from October on, similar values to February and January.

Index of gonadal development

The Index of gonadal development of *C. gigas* showed significant differences during the year, increasing to maximum values in April, and lowest values in January.

The same index calculated for *C. corteziensis* showed significant differences, with higher values in April. The rest of the year presented similar high values form a homogeneous group, falling down to the lowest values in November, and recovering in December (Fig. 6).

Oocyte and cytoplasm size

Quantitative measurements by stage of maturation are presented in Table 1. There were significant difference in average oocyte diameter between *C. gigas* and *C. corteziensis*. In *C. gigas*, largest oocytes were found in vitellogenic females, with significantly smaller oocytes in the other three stages of maturation. In *C. corteziensis*, spawned and vitellogenic females had the largest oocytes, followed by postvitellogenic females, with the smaller oocytes in previtellogenic stage. The average oocyte diameter of vitellogenic *C. gigas* was significantly larger than the average oocyte diameter in *C. corteziensis*.

Maximum oocyte diameter followed a similar trend, but significant differences were found between previtellogenic and spawned stages in *C. gigas*, and between vitellogenic and previtellogenic stages in *C. corteziensis*. There were significant differences between species in the previtellogenic and vitellogenic stage.

Maximum cytoplasm area was biggest in vitellogenic stage of *C. gigas*, which was significantly different from postvitellogenic stage and spawned stage. In *C. corteziensis*, the vitellogenic stage was significantly different from previtellogenic stage. There were significant differences in the cytoplasm area between species in the previtellogenic and vitellogenic stages.

Nucleus:*Cytoplasm ratio*

The Nucleus:cytoplasm ratio was highest in spawned stage of *C. gigas*, and in previtellogenic stage of *C. corteziensis*, compared to other stages for each species. There were significant differences for the Nucleus:cvtoplasm ratio between species in the previtellogenic and spawned stage.

Gonad coverage area

The GCA was analyzed in relation to maturation stages, the highest values were found in vitellogenic females, with no differences among the other three stages in either species. However, the values of GCA were significantly larger in the vitellogenic stage of *C. Gigas* compared to *C. corteziensis*.

Reproductive potential

Reproductive potential classified by stage, is presented in Table 1. Vitellogenic females had the highest values, while the other stages presented similar values in *C. corteziensis* females. In *C. gigas*, spawned females had the lowest values.

Ovary maturity index

This index had the highest values in the vitellogenic stage compared to other stages in both species. The ovary maturity index was significantly higher in *C. gigas* in females in vitellogenic stage, compared to *C. corteziensis* females in the same stage (Table 1).

Index of gonadal development.

This index had the highest values in the postvitellogenic stage and the lowest in the previtellogenic. The index of gonadal development was significantly higher in *C. gigas* in females in postvitellogenic stage, compared to *C. corteziensis* females in the same stage (Table 1).

Visual test for maturity and stages of gonad development

The four stages of the visual test for maturity were determined only for *C. corteziensis* and are presented in Table 2. The histological analysis found that 16.7% of oysters classified as in late maturation of spawning using the visual scale were, in fact, immature. Only 22.7% of the oysters classified as being in early maturation were in previtellogenic stage. The best results were obtained with the vitellogenic stage, where 50% were classified as late maturation by the visual scale. Oysters in postvitellogenic or spawned stage were mostly classified as immature.

Reproductive scale and stages of gonad development

The four stages of the reproductive scale assigned by oocyte size, similar to what has been described for *C. gigas* by Lango-Reynoso et al. (2000) but modified for *C. corteziensis* that has smaller oocytes, is presented in Table 2. The histological analysis found that most (71%) of oysters classified as in early maturation were in previtellogenic stage, and 50% of oysters classified as growing were in vitellogenic

stage. Oysters in postvitellogenic stage were classified as mature (32%) or degenerating (43%); the least asserts were found for spawning stage in which females were mostly classified as growing (50%), mature (43%) or early maturation and degenerating, both with 29%.

Discussion

Maturation stages are usually subjective and largely depend on the experience of the classifier and on previous work for the same or similar species (Barber & Blake 1991). There are different numerical scales that can be used to sort individual oysters into maturation stages in a more objective way, and one that is widely used for scallops and other aquatic organisms because of its simplicity, is the visual maturity, that has the advantage of being easily applied and not expensive (Barber& Blake 1991; Palacios et al. 2003). However, since oocytes in oysters are not profusely pigmented, as is the case for scallops, the assignment of a visual maturation is difficult, and it has the additional disadvantage of generating ordinal data. We used the visual scale to predict maturation stage in oysters, but have found that some oysters were classified as immature even when by the ordinal histological scale they were rated as in vitellogenesis or completely mature (Table 2): 25% of immature oysters were classified as immature when using a visual scale, even if they were spawned, while 52% were classified as spawned in the visual scale, when they were in vitellogenic stage, according to histology. In total, only about a quarter of the *C. corteziensis* oysters were correctly classified.

Lango-Reynoso et al. (2000) proposed a system for classifying *C. gigas* females into maturation stages based on the size of the oocytes present in the gonad; thus females with oocytes smaller than 6.5µm were considered in early gametogenesis, when half the gonad of a female had oocytes of approx. 21µm and the other half oocytes of 34µm, the female was in growing stage, when most (90%) of oocytes were larger than 30-41µm the female was considered mature, and when most of the gonad had oocytes

smaller than 5.5µm while one part of oocytes were larger than 46µm, the gonad was degenerating. This scale has since been applied in several works to the same species (Chávez-Villalba et al. 2002; Ren et al. 2003). We classified maturation stages for *C. corteziensis* on the basis of oocyte diameter, a scale termed reproductive scale, as proposed for *C. gigas* by Lango-Reynoso et al. (2000), but modified here because oocytes in *C. corteziensis* are smaller than that of *C. gigas*. We did found some oocytes that seemed overmature, possible a result of distention of endoplasmic reticulum, and were larger (45 µm) than postvitellogenic oocytes, in contrast to 60 µm reported by Lango-Reynoso et al. (2000) for *C. gigas*. As can be seen in Table 2, early maturation was the most correctly classified stage, with 71% of females in previtellogenic stage, followed by 50% of vitellogenic females classified in growing stage. In contrast, the mature and degenerated stages were mostly classified as spawned or postvitellogenic, respectively. Nevertheless, the scale proposed by Lango-Reynoso et al. (2000) was far better in predicting maturation stage than the visual scale. Thus, the ranges used for assigning a maturation stage should be adapted according to species and possibly for different populations from different ecosystems.

One of the most used continuous scales to indicate the state of maturity is the relation between gonad weight and body weight, termed gonadosomatic index (GSI). It has the advantage of being inexpensive, quick, objective, and that it generates continuous data. GSI has been extensively used in *C. gigas* (Ren et al. 2003; Ngo et al. 2006) and *C. corteziensis* (Páez-Osuna et al. 1993; Frias-Espericueta et al. 1997, 1999). However and in contrast to scallops and other marine organisms, the gonad in oysters can be difficult to separate from adjacent tissue (Mori 1979; Frías-Espericueta et al. 1997). In addition, the weight of the gonad "corrected" by body weight can still be dependent of body weight, particularly when animals are growing (de Vlaming et al. 1982; Grant & Tyler 1983b; Packard & Boardman 1988; West 1990; Palacios et al. 2003). Furthermore, GSI is not a precise reflection of gonad activity because there can

be temporal differences in the recruitment of oocytes or fluid content in the ovary tissues external to oocytes during maturation (Grant & Tyler 1983a; Palacios et al. 2004) or in the digestive gland (Grant & Tyler 1983b), that affect gonad or total weight. Costil et al. (2005) observed that even if no gametes remained in the follicles, the presence of abundant connective tissue might induce visual of fatness. The same applies to gonad biomass estimated by stereology, since it requires the weight of the gonad for the calculations (Enriquez-Diaz 2004). A further disadvantage of this methodology is that it requires three sections of the gonad, anterior, middle and rear, and this reduces the amount of gonad that can be used for other additional test such as biochemistry and genetics. To overcome the difficulty of dissecting and weighing a gonad dispersed in other tissues, some studies have measured the gonad thickness using calipers (Lossanoff 1965), the weight of the area of the photographic print occupied by ova to the weight of the gonad (Muranaka & Lannan 1984), or the follicle or gonad tissue area in relation to the other tissue, usually determined by image analyses (Mori 1979; Lannan, 1980; Allen & Downing 1986; Hefferman & Walker 1989; Barber et al. 1991; Enriquez-Diaz 2004; Fabioux et al. 2005; Quintana 2005). This ratio has received several names and has been evaluated by slightly different protocols by each group; for commodity is termed here gonad coverage area (GCA). The GCA has the advantage of being quantitative and relatively quick and simple to analyze, although image analysis requires previous histological process, which can be expensive and time consuming. GCA can provide continuous data in completely immature oysters, but also in males, as demonstrated in *C. gigas* by Lannan (1980). The GCA can indicate when the female is fully mature, as can be seen in figure 3, but the differences in a gonad that is maturing or spawning is not so evident. This is further observed in Table 1, where no differences were found among stages other than vitellogenic stage. A similar result was obtained by Brewer et al. (2007) in different fish species, who proposed using GSI till fishes appear to be in

mature condition and then to incorporate histology to pinpoint specific periods when spawning occurs.

Oocyte size is independent of the body size (Grant & Tyler 1983a; Palacios et al. 2003) and can be used as a covariable to correct for gonad maturation in females (Palacios et al. 2003). However, when average oocyte diameter was used to reveal the annual reproductive pattern of *C. corteziensis*, no significant differences were found among the sampled periods (Fig. 2). In a similar analysis, oocyte maximum area or diameter has been proven efficient in organisms that have partial spawns and thus present a wide distribution of oocytes of different sizes or cohorts (Palacios et al. 2003). Maximum oocyte area was slightly better revealing the annual reproductive pattern than average oocyte diameter, since there were significant differences as a result of the sampling period, but a Tukey posthoc analysis revealed that there were only differences between maximum oocyte diameter in December and May for *C. corteziensis* (results not shown). The spawning periods were not evidenced using maximum oocyte diameter. The analysis per stage presented in Table 1 revealed that the vitellogenic stage was well separated in both species using oocyte size, but the spawned stage was only significantly separated in *C. gigas*.

There are several scales that are based on ratios or indexes. These have the advantage of using relative scales of reproduction and enables comparison of reproductive output to be made among species. One of these has been extensively used in medicine to determine cell growth; the Nucleus:cytoplasm ratio. The Nucleus:cytoplasm ratio has been applied to analyze oocyte growth with good results in marine organisms (Pastorinho et al. 2003; Van der Molen 2003; Arjarasirikoon et al. 2004) and in mollusks (Rodríguez-Jaramillo et al. 2001). However, when using this ratio to predict maturation stages here, we could not distinguish between vitellogenic stage and other stages, although spawned stage of *C. gigas* and previtellogenic stage of *C. corteziensis* were different from other stages (Table 1). This ratio allowed thus to

reveal the resting period of December and January, but not gonad maturation. It also has the disadvantage of being quite laborious.

The Ovary Maturity Index, modified from that proposed by Arcos et al. (2005) could distinguish the vitellogenic stage, but not the other stages (Table 1). It also has the disadvantage of using the oocyte area, which increases significantly the values of the vitellogenic stage in *C. gigas*, and it is laborious to measure.

The Reproductive Potential, calculated in relation to number of oocytes and their size, based on the formula for fecundity proposed by Heffernan & Walker (1989) that was based on the analysis of postvitellogenic oocytes, and that was modified here to include oocytes in all stages of maturation, is a good predictor of maturation stage, particularly for *C. gigas*. Of all the analysis, this was the only one where the values were not significantly different between species, indicating this to be the best way to compare these two species. However, it was by far the most laborious of all the analysis.

The Index of Gonadal Development proposed by Lannan (1980) could distinguish the postvitellogenic stage en *C. gigas*, but not the other stages (Table 1). It also has the disadvantage of using the ratio of postvitellogenic oocytes in relation to the total number of oocytes present in the gonad, which increases significantly the values of the vitellogenic and postvitellogenic stages in *C. corteziensis*, and it is laborious to measure.

The comparison of the annual reproductive pattern using some of these different methods of analysis on the same females was of particular interest, because there are differences that may lead to an erroneous conclusion in relation to gametogenesis and spawning, particularly when comparing reproductive effort between species. The quantitative methods that are significantly different per stage between *C. gigas* and *C. corteziensis*, indicate that comparison is skewed, since a more advanced maturation can be assigned to *C. gigas* for the same stage. This is the case of oocyte size, GCA, and

ovary maturity index; even the last two are a relative measure of maturity. The only scale where the values of *C. corteziensis* were similar to and even surpassed the ones in *C. gigas*, was again, Reproductive potential. This scale is by far the best to use for comparing this two species, or using as a covariable when comparing biochemical reserves during maturation. In any case, differences among methods should be considered when establishing a reproductive period for each species, since there can be differences among them that makes comparison complex.

In conclusion, there are strong variations among the different methods tested to generate quantitative data on gonad maturation, particularly when comparing different species. GSI, abundantly used in scallops and other marine organisms, cannot be straightforward applied to oysters in general. Oocyte size can be a relatively adequate predictor of maturation stage, but it does not represent the reproductive pattern in tropical oysters with an extended reproductive period and several partial spawns. Nucleus:cytoplasm ratio might only evidence the resting period, while the ovary maturity index is skewed in favor of *C. gigas*. The Ovary Maturity Index, the Reproductive Potential, and the GCA, all continuous and objective scales, seem to be the more adequate measure of maturity to compare oysters of different species. However, the Ovary Maturity Index and the Reproductive Potential are much more laborious, since they requires additional measures. So for general maturation analysis the GCA could be enough, but to pinpoint the specifics of maturation between species, the Reproductive Potential is better.

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References

Allen Jr SK, Downing SL. 1986. Performance of triploid Pacific oysters, *Crassostrea gigas* (Thunberg). I. Survival, growth, glycogen content and sexual maturation in yearlings. J. Exp. Mar. Biol. Ecol. 102:197-208.

Arjarasirikoon U, Kruatrachue M, Sretarugsa P, Chitramvong Y, Jantataeme S, Upatham ES. 2004. Gametogenic processes in the pearl oyster, *Pteria penguin* (Röding, 1789) (Bivalvia, Mollusca). J. Shellfish Res. 23:403-409.

Arcos F, Racotta IS, Palacios E, Ibarra AM. 2005. Ovary development at the onset of gametogenesis is genetically determined and correlated with reproductive traits at maturity in shrimp *Litopenaeus (Penaeus) vannamei*. Mar. Biol. 148:339-346.

Barber BJ, Blake NJ. 1991. Reproductive physiology. In: Shumway SE (ed.) Scallops: Biology, Ecology and Aquaculture. Elsevier, West Boothbay Harbor, p 377-428.

Barber BJ, Ford SE, Wargo RN. 1991. Genetic variation in the timing of gonadal maturation and spawning of the eastern oyster *Crassostrea virginica* (Gmelin). Biol. Bull. 181:216-221.

Brewer SK, Rabeni CF, Papoulias DM. 2007. Comparing histology and gonadosomatic index for determining spawning condition of small-bodied riverine fishes. Ecol. Fresh. Fish 17: 54-58.

Chávez-Villalba J, Barret J, Mingant C, Cochard J-C, Le Pennec M. 2002. Autumn conditioning of the oyster *Crassostrea gigas*: A new approach. Aquaculture 210:171-186.

Costil K, Royer J, Ropert M, Soletchnik P, Mathieu M. 2005. Spatio-temporal variations in biological performances and summer mortality of the Pacific oyster *Crassostrea gigas* in Normandy (France). Helgol Mar. Res. 59:286-300.

David-Gómez I, Gerson-Araujo F. 2004. Reproductive biology of two marine catfishes (Siluriformes, Ariidae) in the Sepetiba Bay, Brazil. Rev. Biol. Trop. 52(1): 143-156.

Delaporte M, Soudant P, Moal J, Marty Y, Samain JF. 2005. Incorporation and modification of dietary fatty acids in gill polar lipids by two bivalve species *Crassostrea gigas* and *Ruditapes philippinarum*. Comp. Biochem. Physiol. 140B: 460–470.

De Vlaming V, Grossman G, Chapman F. 1982. On the use of the gonadosomatic index. J. Fish Physiol. 1:31-39.

Egea E, Mérigot B, Mahé-Bézac C, Féral JP, Chenuil A. 2011. Differential reproductive timing in *Echinocardium* spp.: The first Mediterranean survey allows interoceanic and interspecific comparisons. C. R. Biologies. 334:13-23.

Enriquez-Díaz M. 2004. Variabilité et bioénergétique de la reproduction chez l'huître creuse *Crassostrea gigas*. 2004. PhD Thesis, Université de Bretagne Occidentale. 1-216 pp.

Fabioux C, Huvet A, Le Souchu P, Le Pennec M, Pouvreau S. 2005. Temperature and photoperiod drive *Crassostrea gigas* reproductive internal clock. Aquaculture 250:458-470.

Ferreira MPA, Paixao LF, Alcantara-Nieto CP, Santos SSD, Rocha RM. 2006. The analysis of fecundity, based on the radius and total number of oocytes, was a good reflection of maturation as described by traditional histology. Int. J. Morphol. 24:437-442.

Frias-Espericueta MG, Osuna-Lopez J I, Paez-Osuna F. 1999. Gonadal maturation and trace metals in the mangrove oyster *Crassostrea corteziensis*: seasonal variation. Sci. Total Environ. 231:115-123.

Frias-Espericueta M, Páez-Osuna F, Osuna-López JI. 1997. Seasonal changes in the gonadal state of the oysters *Crassostrea iridescens* and *Crassostrea corteziensis* (Filibranchia: Ostreidae) in the Northwest coast of Mexico. Rev. Biol. Trop. 45:1061-1065.

Grant A, Tyler PA. 1983a. The analysis of data in studies of invertebrate reproduction. II. The analysis of oocyte size/frequency data, and comparison of different types of data. Int. J. Invert. Reprod. 6:271-283.

Grant A, Tyler PA. 1983b. The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturation indices. Int. J. Invert. Reprod. 6:259-269.

Hefferman PB, Walker RL. 1989. Quantitative image analysis methods for use in histological studies of bivalve reproduction. J. Moll. Stud. 55:135-137.

Hurtado MA, Racotta IS, Arcos F, Morales-Bojórquez E, Moal J, Soudant P, Palacios E. 2012. Seasonal biochemical composition, fatty acids, and sterols in female *Crassostrea corteziensis* oysters in relation to the reproductive cycle. Comp. Biochem. Physiol, Part B. 163:172-183.

Galindo-Sanchez CE, Ibarra AM, Cruz P. 2008. Genetic diversity among four different world-wide lines of Pacific oyster *Crassostrea gigas* by microsatellites. Poster. PHYSIOMAR 08, Brest Francia.1-4 Septiembre.

Lango-Reynoso F., Chávez-Villalba J, Cochard J-C, Le Pennec M. 2000. Oocyte size, a means to evaluate the gametogenic development of the Pacific oyster (*Crassostrea gigas)* Thunberg. Aquaculture 190:183-199.

Lannan JE. 1980. Broodstock management of *Crassostrea gigas*I. Genetic and environmental variation in survival in the larval rearing system. Aquaculture 21:323-336.

Leong W, Pawlik JR. 2011. Comparison of reproductive patterns among 7 Caribbean sponge species does not reveal a resource trade-off with chemical defenses. J. Exp. Mar. Biol. Ecol. 401:80-84.

Li Q, Liu W, Shirasu K, Chen W, Jiang S. 2006. Reproductive cycle and biochemical composition of the oyster *Crassostrea plicatula* Gmelin in an eastern coastal bay of China. Aquaculture 261:752-759.

Loosanoff VL. 1965. Gonad development and discharge of spawn in oysters of Long Island sound. Biol. Bull. 129:546-561.

Mori K. 1979. Effects of artificial eutrophication on the metabolism of the Japanese oyster. Mar. Biol. 53:361-369.

Muranaka M, Lannan JE. 1984. Broodstock management of *Crassostrea gigas:* environmental influences on broodstock conditioning. Aquaculture 39:217-228

Ngo TTT, Kang SG, Kang DH, Sorgeloos P, Choi KS. 2006. Effect of culture depth on the proximate composition and reproduction of the Pacific oyster, *Crassostrea gigas* from Gosung Bay, Korea. Aquaculture 253:712-720.

Packard GC, Boardman TJ. 1988. The misuse of ratios, indices and percentages in ecophysiological research. Physiol. Zool. 6:1-9.

Páez-Osuna F, Zazueta-Padilla HM, Osuna-López JI. 1993. Biochemical composition of the oysters *Crassostrea iridescens* Hanley and *Crassostrea corteziensis* Hertlein in the Northwest coast of Mexico: seasonal changes. J. Exp. Mar. Biol. 170:1-9.

Palacios E, Racotta IS, Villalejo-Fuerte M. 2003. Assessment of ovarian development and its relation to mating in wild and pond-reared *Litopenaeus vannamei* shrimp in a commercial hatchery. J. World Aquacult. Soc. 34:446-477.

Palacios E, Racotta IS, Ibarra AM, Ramírez JL, Millan A, Avila S. 2004. Comparison of biochemical composition and muscle hypertrophy associated with the reproductive cycle of diploid and triploid *Argopecten ventricosus* scallops. J. Shellfish Res. 23:483-489.

Pastorinho MR, Antunes CP, Marques JC, Pereira ML, Azeiteiro UMM, Morgado FM. 2003. Histochemistry and histology in planktonic ecophysiological processes determination in a temperate estuary (Mondego River estuary, Portugal). Acta Oecologica 24: S235-S243.

Quintana R. 2005. Effect of estradiol-17β on the gonadal development of diploid and triploid female eastern oysters PhD Thesis. Universidad Autónoma de Baja California. 1-138 pp.

Ren JS, Marsden ID, Ross AH, Schiel DR. 2003. Seasonal variation in the reproductive activity and biochemical composition of the Pacific oyster (*Crassostrea gigas*) from the Marlborough Sounds, New Zealand. New Zealand J. Mar. Fresh. Res. 37:171-182

Rodríguez-Jaramillo C, Maeda-Martínez AN, Valdez ME, Reynoso-Granados T, Monsalvo-Spencer P, Prado-Ancona D, Cardoza-Velasco F, Robles-Mungaray M, Sicard MT. 2001. The effect of temperature on the reproductive maturity of the penshell *Atrina maura* (Sowerby 1835). J. Shellfish Res. 20:39-47.

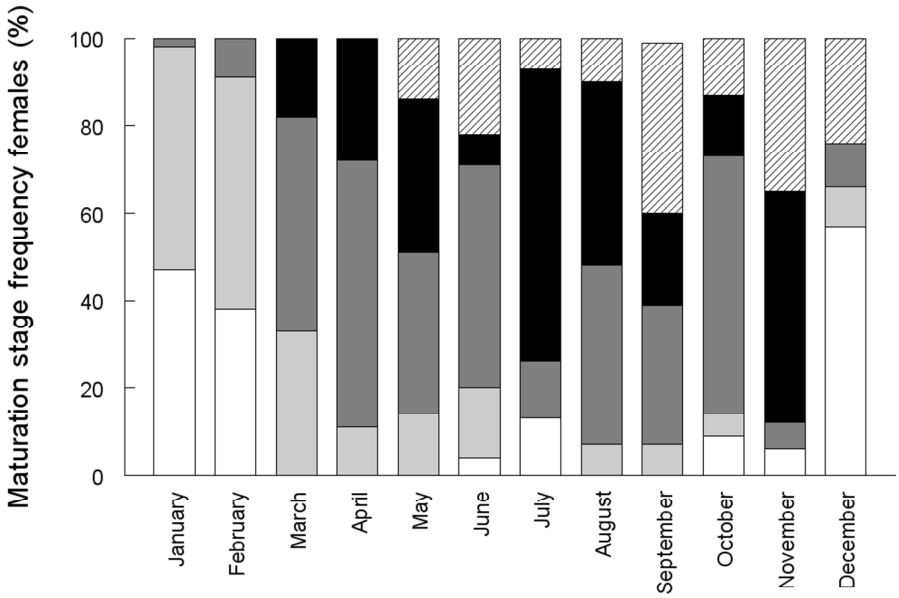
Rodríguez-Jaramillo C, Hurtado M, Romero-Vivas E, Ramírez JL, Manzano M, Palacios E. 2008. Gonadal development and histochemistry of the tropical oyster, *Crassostrea corteziensis* (Hertlein, 1951) during an annual reproductive cycle. J. Shellfish Res. 27:1129-1141.

Saout C, Paulet YM, Duinker A. 1999. Histological study on the early stages of oogenesis in *Pecten maximus*: a new approach with quantitative semithin histology. Bergen, Norway, 12th Interational Pectinid Workshop. pp. 129-130.

Van Der Molen S. 2003. Estudio sobre la reproducción de Notothenioidei (Pisces: Perciformes) del Océano Austral. PhD Thesis. Universidad Autónoma de Barcelona.

West G. 1990. Methods for assessing ovarian development in fishes: a review. Aust. J. Mar. Fresh. Res. 41:199-222.

Whalan S, Battershill C, de Nys R. 2007. Sexual reproduction of the brooding sponge *Rhopaloeidesodorabile*. Coral Reefs 26: 655–663.



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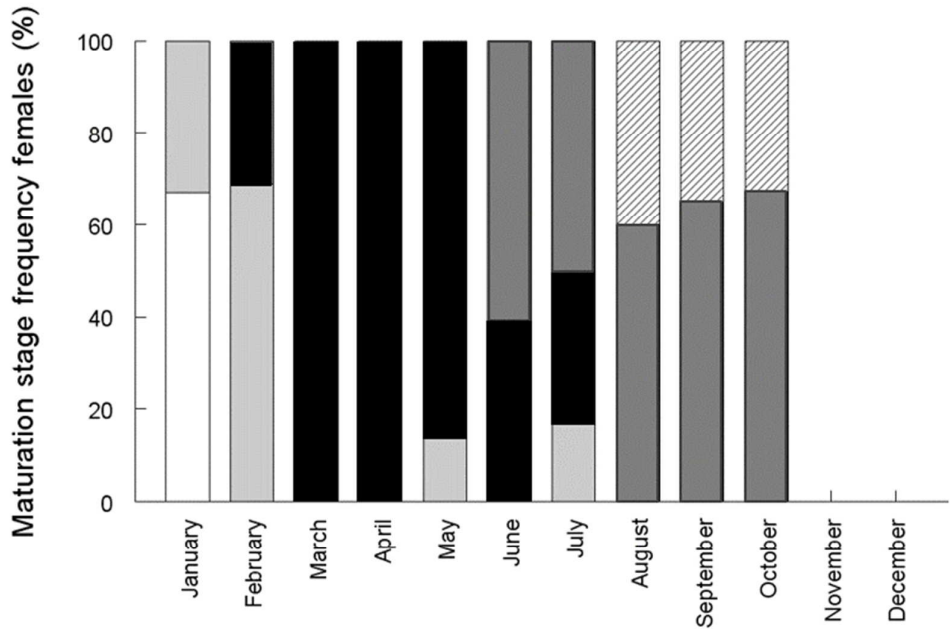


583
584 Fig. 1A) Frequency of gonad developmental stages determined by histology in females
585 of A) *Crassostrea corteziensis* and; B) *C. gigas*, sampled from Ceuta Lagoon System,
586 Sinaloa, Mexico. White bars = Immature or stage 0; light gray bars = Previtellogenesis
587 or stage I; dark gray bars = Vitellogenesisor stage II; black = Postvitellogenesis or stage
588 III; hatched bars = Spawned or stage IV.

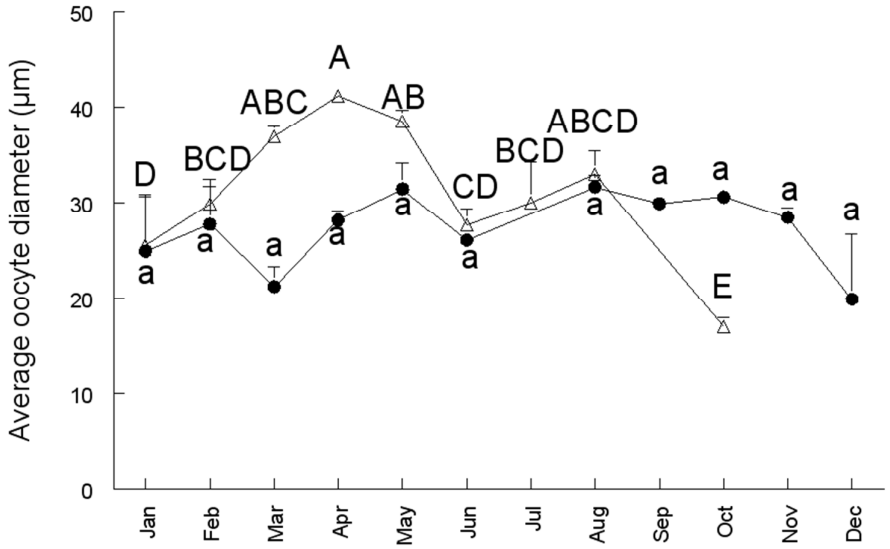
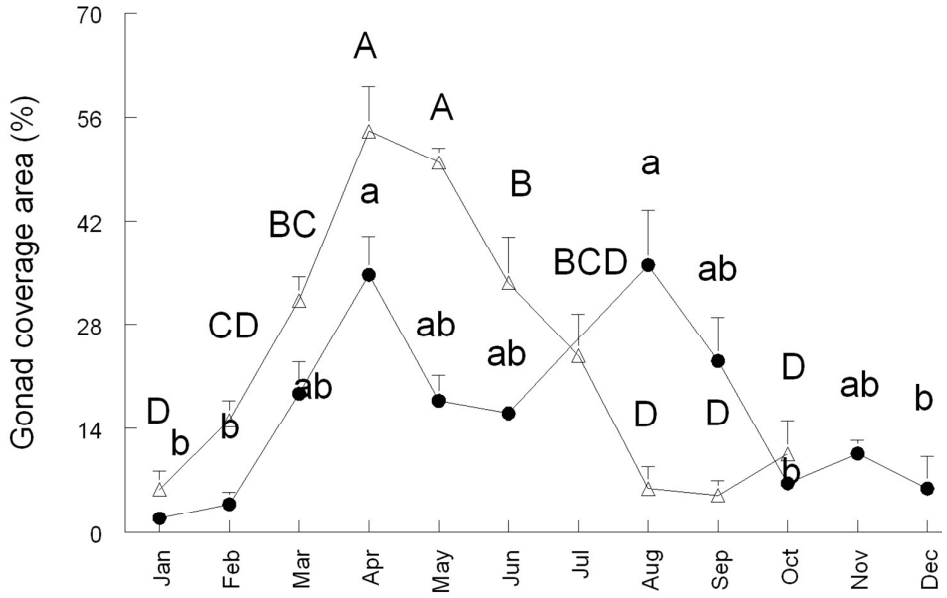


Fig. 2. Average oocyte diameter (µm) in *C. gigas* (white triangles) and *C. corteziensis* (Black circles). Data were analyzed using sampling month as the independent variable in a unifactorial ANOVA ($P<0.05$). Results are reported as mean ± standard error. Different letters indicate significant differences; each species were analyzed separately ($P<0.05$).



596

597 Fig. 3. Gonad Coverage Area (%) for *C. gigas* (white triangles) and *C. corteziensis*
598 (Black circles) by month. Data were analyzed using sampling month as the
599 independent variable in a unifactorial ANOVA (P<0.05). Results are reported as
600 mean ± standard error. Different letters indicate significant differences; each specie
601 was analyzed separately (P<0.05).

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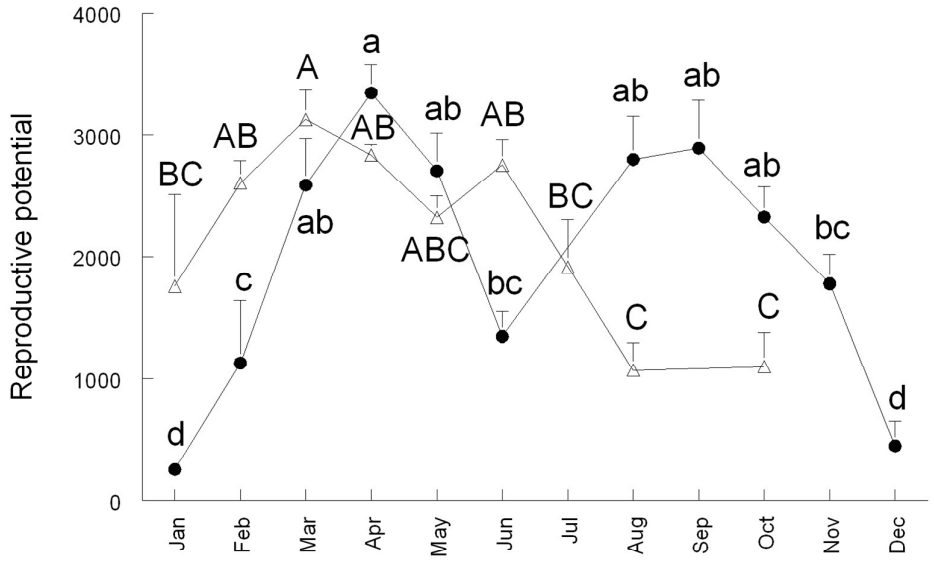

Fig. 4. Reproductive potential for *C. gigas* (white triangles) and *C. corteziensis* (Black circles) by month. Data were analyzed using sampling month as the independent variable in a unifactorial ANOVA ($P<0.05$). Results are reported as mean ± standard error. Different letters indicate significant differences; each specie was analyzed separately ($P<0.05$).

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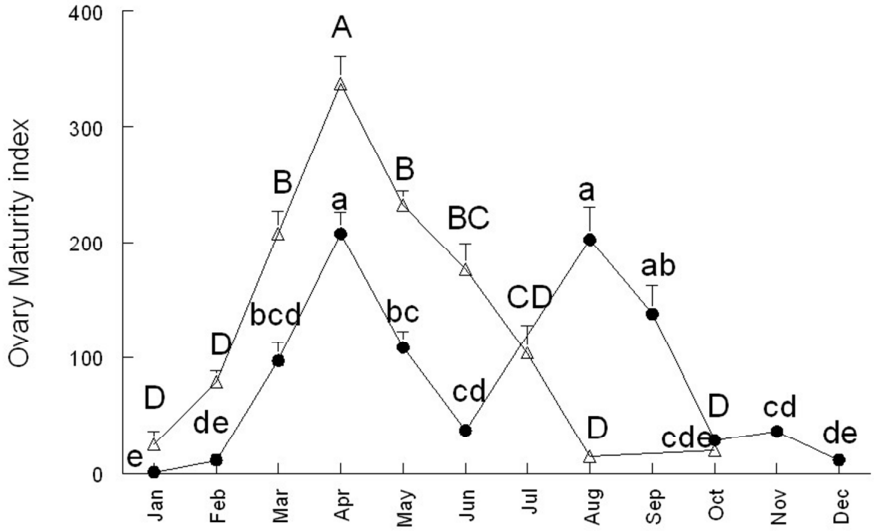

613

614 Fig. 5. Ovary Maturity Index for *C. gigas* (white triangles) and *C. corteziensis*
615 (Black circles) by month. Data were analyzed using sampling month as the
616 independent variable in a unifactorial ANOVA (P<0.05). Results are reported as
617 mean ± standard error. Different letters indicate significant differences; each species
618 was analyzed separately (P<0.05).

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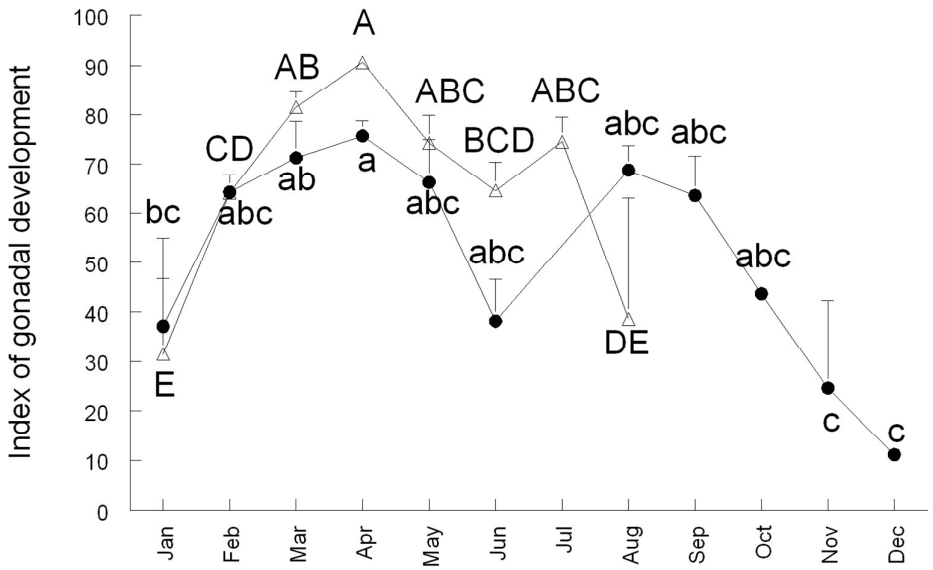

Fig. 6. Index of Gonadal Development for *C. gigas* (white triangles) and *C. corteziensis* (Black circles) by month. Data were analyzed using sampling month as the independent variable in a unifactorial ANOVA ($P<0.05$). Results are reported as mean ± standard error. Different letters indicate significant differences; each species was analyzed separately ($P<0.05$).

Table 1. Female morphometric and maturation data classified by stage of female gonad development of *Crassostrea corteziensis* and *Crassostrea gigas* were sampled from Bahía Ceuta, Sinaloa, Mexico from April 2005 to April 2006.

		Stage I Previtellogenesis	Stage II Vitellogenesis	Stage III Postvitellogenesis	Stage IV Spawned
Average oocyte diameter (µm)	*C. gigas*	27.5±2.0 b	37.4±0.7 a	27.1±2.0 b	26.2±5.0 b
	C. corteziensis	22.8±2.2 b	29.3±1.1 a*	28.5±2.4 ab	30.7±3.7 a
Maximum oocyte diameter (µm)	*C. gigas*	45.8±2.6 ab	51.0±0.9 a	39.2±2.5 bc	37.6±2.6 c
	C. corteziensis	33.9±3.1 b *	46.0±1.9 a *	42.8±2.1 ab	42.2±2.3 ab
Maximum cytoplasm area (µm^2)	*C. gigas*	1202±127 ab	1490±55 a	863±88 bc	595±55 c
	C. corteziensis	730±98 b *	1138±56 a *	1031±54 ab	1021±60 ab
Nucleus:Cytoplasm ratio	*C. gigas*	0.52±0.04 b	0.45±0.01 b	0.55±0.04 b	0.75±0.08 a
	C. corteziensis	0.60±0.07 a *	0.44±0.01b	0.50±0.03b	0.48±0.03 b*
Gonad Coverage Area (GCA, %)	*C. gigas*	14.6±3.7 b	41.3±2.9 a	16.4±2.7 b	6.5±3.9 b
	C. corteziensis	9.6±2.5 b	29.7±2.8 a*	11.3±2.1 b	8.8±4.1 b
Reproductive potential [1]	*C. gigas*	2337±253 ab	2877±91 a	1876±188 b	558±130 cJan
	C. corteziensis	1945±297 b	3059±166 a	1843±162 b	430±263 b
Ovary Maturity Index [2]	*C. gigas*	63.9±9.9 b	241.2±11.1 a	59.4±8.2 b	4.4±1.3 b
	C. corteziensis	52.7±8.9 b	172.0±10.9 a*	52.7±8.9 b	0.2±0.04 b
Index of Gonadal Development [3]	*C. gigas*	8.04±1.5 c	63.06±3.9 b	81.20±1.9 a	56.66±6.3 b
	C. corteziensis	13.75±1.2 b	59.10±5.6 a	68.45±3.6 a*	48.66±11.5 ab

Data were analyzed using stage as the independent variable (4 levels) in a unifactorial ANOVA (P<0.05). Results are reported as mean ± standard error. Different letters indicate significant differences; species were analyzed separately (P<0.05). For each maturation stage, significant differences between species are shown with an *. [1]The Reproductive potential is based on fecundity = 4/3π × radius of oocyte^3× total number of oocytes described by Heffernan and Walker (1989). [2] The Ovary Maturity Index = GCA x Oocyte area x total number of oocytes, as described by Arcos et al. (2005). [3] Index of gonadal development calculated as the ratio of postvitellogenic oocytes in relation to the total number of oocytes present in the gonad, as described by Lannan (1980).

Table 2. Frequency distribution (%) of hits for proposed visual maturation stage or the reproductive scale determined by oocyte average diameter, in relation to the ordinal stage of gonad development assigned by histology of *C. corteziensis* sampled from Bahía Ceuta, Sinaloa, Mexico.

		Stage 0 Immature	Stage I Previtel.	Stage II Vitel.	Stage III Postvitel.	Stage IV Spawned
Visual maturation	Immature	16.7	11.1	8.3	38.9	25.0
	Early maturation	29.5	22.7	18.2	27.3	2.3
	Late maturation	11.9	21.4	50.0	14.3	2.4
	Spawning	4.8	33.3	52.4	4.8	4.8
Reproductive scale	Early maturation	0	71.4	0	0	28.6
	Growing	0	0	50.0	0	50.0
	Mature	0	2.2	22.2	31.8	43.2
	Degenerating	0	23.1	7.7	42.9	28.6

Data were analyzed using a Table of contingency:

Visual maturity for females: $X^2 = 67.2$, Degrees of freedom = 15, $P<0.01$

Reproductive scale based on oocyte diameter: $X^2 = 46.1$, Degrees of freedom = 12 $P<0.01$