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Comparative study of gonadal development of *Ruditapes philippinarum* (Adams and Reeve) and *Ruditapes decussatus* (L.) (Mollusca: Bivalvia): Influence of temperature

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SUMMARY: Laboratory experiments were used to study the influence of temperature on the reproductive behaviour of two species of clam, *Ruditapes decussatus* and *Ruditapes philippinarum*, during their adaptation to the temperature conditions of Galician coastal waters. In both species the rate of gonadal development was directly related to the increase in temperature. At 14ºC the reproductive behaviour was similar, both species needing over 2 months to mature. At 18ºC, the rate of gonadal development of *R. philippinarum* was greater than that of *R. decussatus*. Nevertheless, the results of this study confirm that both species have adapted perfectly to the temperature conditions of the Galician Rias, though certain differences between the reproductive behaviour of these species were detected. *R. philippinarum* accumulates oocytes prior to their partial or total emission, while in the case of *R. decussatus* gametes are liberated continuously. In the early phases of its development, the gonad of *R. philippinarum* is highly heterogeneous in nature, with up to 3 reproductive states being present at the same time, while in *R. decussatus* gonadal development is much more uniform. Another major difference concerns the phenomenon of reabsorption, common in *R. philippinarum* but very rare in *R. decussatus*. These characteristics may well result in a greater reproductive activity in *R. philippinarum* than in *R. decussatus*, and a longer reproduction period in the former. They may also represent a certain advantage for the adaptation of the foreign species (*R. philippinarum*) over the native species (*R. decussatus*) to the temperature conditions of the Galician Rias.

Keywords: gonadal development, temperature, histology, *Ruditapes decussatus*, *Ruditapes philippinarum*, hermaphroditism.

RESUMEN: ESTUDIO COMPARATIVO DEL DESARROLLO GONADAL DE *RUDITAPES PHILIPPINARUM* (ADAMS AND REEVE) Y *RUDITAPES DECUSSATUS* (L.) (MOLLUSCA: BIVALVIA): INFLUENCIA DE LA TEMPERATURA. – Se llevaron a cabo experiencias de laboratorio para estudiar la influencia de la temperatura en el comportamiento reproductivo de dos especies de almeja, *R. philippinarum* y *R. decussatus*, en su adaptación a las condiciones térmicas en las costas de Galicia. En ambas especies la tasa de desarrollo gonadal está directamente relacionada con el incremento de temperatura. A 14ºC el comportamiento reproductivo es similar y ambas especies necesitan cerca de 2 meses para madurar. A 18ºC, la tasa de desarrollo gonadal de *R. philippinarum* es mayor que en *R. decussatus*. No obstante, los resultados de este estudio confirman que ambas especies se han adaptado perfectamente a las condiciones térmicas de las rías de Galicia, si bien, se detectan ciertas diferencias en su comportamiento reproductivo. Así, mientras *R. philippinarum* acumula ovocitos antes de su emisión parcial o total, en el caso e *R. decussatus* los gametos se liberan continuamente. En las primeras fases de su desarrollo, el aspecto de la gónada de *R. philippinarum* es muy heterogéneo, presentándose hasta 3 estados reproductivos simultáneamente, mientras que en *R. decussatus* el desarrollo gonadal es bastante más uniforme. Otra diferencia concierne al fenómeno de la reabsorción, común en *R. philippinarum,* pero muy raro en *R. decussatus*. Estas características pueden sustentar una mayor actividad reproductiva en *R. philippinarum* comparado con *R. decussatus*, y un mayor periodo reproductivo en el caso de la primera. Ello puede también representar una cierta ventaje adaptativa, de la especie foráneas (*R. philippinarum*) frente la especie nativa (*R. decussatus*), a las condiciones térmicas de las rías de Galicia.

Palabras clave: desarrollo gonadal, temperatura, histología, *Ruditapes decussatus, Ruditapes philippinarum*, hermafroditismo.

INTRODUCTION

Ruditapes philippinarum is a species native to the Indian-Pacific region, but due to its high growth rates and its ability to tolerate a wide range of environmental conditions it was introduced into European Atlantic and Mediterranean coastal waters during the twentieth century for commercial cultivation, occupying a habitat that overlaps that of the native species *R. decussatus*.

Many studies have been published on the reproductive cycle of *R. philippinarum* in the natural environment both in the areas of origin of this species, like Japan (Ohba, 1959; Toba *et al*., 1993), and in parts of the world in which it has been introduced: the East Pacific (Holland and Chew, 1974), European coasts (Sarasquete *et al*., 1990; Ponurovsky and Yakovlev, 1992; Rodríguez-Moscoso *et al*., 1992; Meneghetti *et al*., 2004; Drummond *et al*., 2006) and African Atlantic coasts (Shaffee and Daouidi; 1991). The bibliography on *R. decussatus*, however, is not so extensive (Pérez-Camacho, 1980; Villalba *et al*., 1993; Rodríguez-Moscoso and Arnaíz, 1998).

Some studies have compared the reproductive behaviour of the two species in the natural environment (Beninger and Lucas, 1984; Laruelle *et al*., 1994; Xie and Burnell, 1994), but only very few have looked at the effects of temperature on the gonadal development of these species under controlled conditions, and then only with *R. philippinarum* (Mann, 1979; Toba and Miyama, 1995).

The purpose of this study was to compare the influence of temperature on the gonadal development of *R. philippinarum* and *R. decussatus* in the natural environment of the Galician coast, where the range of temperature is 13-18ºC during the reproduction period of these two species. A particular aim was to determine the repercussion of any differences in the reproductive behaviour of the species with regard to temperature on their adaptation to the environmental conditions of Galician coastal waters.

MATERIALS AND METHODS

Design and experimental conditions

The experiments were carried out using adult specimens of *Ruditapes decussatus* (37.9 ± 1.0 mm) and *R. philippinarum* (36.3 ± 2.2 mm) collected from the intertidal environment from Ría de Arousa (Galician coast, Spain) in February of 2003.

The clams were placed in 12 l plastic tanks in a flow-through circuit containing natural sea-water filtered through a 1 μm mesh. Each of the two species was conditioned at each of two temperatures: 14 and 18ºC. The experiments were carried out with groups of 50 individuals.

A peristaltic pump was used to add food to the circuit. The food ration consisted of 0.5% dry weight of the microalga *Isochrysis galbana* clone T-ISO with regard to clam live weight. The microalgae were initially cultured in 6 l jars and then transferred to 1000 l tanks. Walne medium (Walne, 1966) and industrial fertiliser were used for the jar and tank cultivation, respectively. The microalgae were harvested during the stationary growth phase.

The total conditioning period was 78 days, with sampling being performed at the start of the experiment (0 days) and at days 32, 57 and 78. At each interval (including the initial sample at 0 days), groups of 10-12 individuals were sampled for histological study of gonadal development. A minimum of 4 specimens of each sex were taken in each sampling.

Histology

A conventional histology protocol was followed. The soft tissues were fixed with Bouin's fixative (Bancroft and Stevens, 1996), sealed in paraffin, and 4 μm slices were taken. Para-Pak® Trichrome stain was used (Meridian Bioscience, Inc.). From each clam we obtained 3 slices corresponding to 3 different depths in the body.

The identification of the phases of gametogenic development in *R. philippinarum* was done using a modified scale from that proposed for this species by Holland and Chew (1974): Post-spawning and gonadal regression (Period of sexual rest) (Phase I), Initiation of gametogenesis (Phase II), Advanced gametogenesis (Phase III) and Reproduction period (Ripening and spawning period) (Phase IV). Gametogenic developmental phases in *R. decussatus* were identified as a function of the phases proposed for this species by Delgado and Pérez-Camacho (2005). The most relevant characteristics of the different phases of gonadal development of the two species are shown in Table 1.

TABLE 1. – Phases of gonadal development of *R. philippinarum* and *R. decussatus*. Phase I (Post-spawning and gonadal regression; period of sexual rest), Phase II (Initiation of gametogenesis), Phase III (Advanced gametogenesis) and Phase IV (Ripening and spawning period; reproduction period).

Phases	Common characteristics	Specific characteristics	
		R. philippinarum	*R. decussatus*
Phase I	Absence of gametes Sex determination is no possible Follicle walls are broken	Severe infiltration of haemocytes (reabsorption)	Absence of haemocytes infiltration
Phase II	The follicle walls are covered with developing germinal cells Abundance of vesicular cells and intragonadal muscle tissue (reserve tissue)	Infiltration of haemocytes (reabsorption)	Absence of haemocytes infiltration
Phase III	Intense growth of oocytes (high presence of pedunculated oocytes) in females Increase of spermatozoids in the lumen of acini in males First signs of partial emission of gametes	Highly heterogeneous development. Coexistence of developing areas with fully mature, half-empty and reabsorption areas	Homogeneous development.
Phase IV	Generalised maturity of the whole gonad Disappearance of reserve tissue This phase ends with the total emission of gametes	Weak reabsorption	

Oocyte diameter frequency

Gametogenic development in females is characterised by a considerable increase in oocyte size. Image analysis techniques (MicroImage software, Olympus) were used to measure the maximum diameter of the oocytes. Measurements of over 500 oocytes per specimen were obtained, corresponding to 3 different depths in the body of the clam, and size distribution frequency was determined for each specimen, each species and each experimental condition.

Statistical analysis

Statgraphics software (5.0) was used to analyse the experimental results. Analysis of oocyte size frequency distribution gave an indicator of the symmetry of the distribution (skewness) and a descriptor of the form of the distribution (kurtosis). In the case of a normal distribution, i.e. one in which the data are symmetrically distributed, skewness is zero; a positive skewness indicates that the left-hand tail of the distribution is greater, while a negative skewness implies that the distribution is heavier to the right, indicating a greater oocyte diameter. Kurtosis tells us whether the distribution of the data is more or less flat in comparison with a normal distribution. Kurtosis is zero for a normal distribution, positive in the case of a peaked distribution and negative if the distribution is flat.

To compare the effect of temperature on mean oocyte diameter, we used a t-test, at a minimum significance level of $p<0.05$. An F-test was used to check the homogeneity of the variances. All statistical analyses were performed according to the methods described in Zar (1974) and Snedecor and Cochran (1980).

RESULTS

Histological study

Ruditapes philippinarum

The experiment with *R. philippinarum* started with 60% of the individuals showing clear signs of gonadal regression, characteristic of a post-spawning phase (phase I; Figs. 1, 2 and 6). In these individuals, the majority of follicles showed the invasion of haemocytes that enfolded residual gametes corresponding to a previous reproduction period (Fig. 1b). The follicle walls were often very thin and broken. The remaining individuals (40%) were in the initial phases of gametogenesis (phase II), and in some areas there were gonadal follicles in whose walls the new germinal lines were coming through.

Generally speaking, it is important to note the asynchronous state of gonadal development between individuals. This asynchrony is also expressed within the gonad of each individual, and areas with different degrees of maturity usually coexist in the same individual. Nevertheless, in accordance with the description given by Drummond *et al*. (2006) in their study of this species, each individual has been

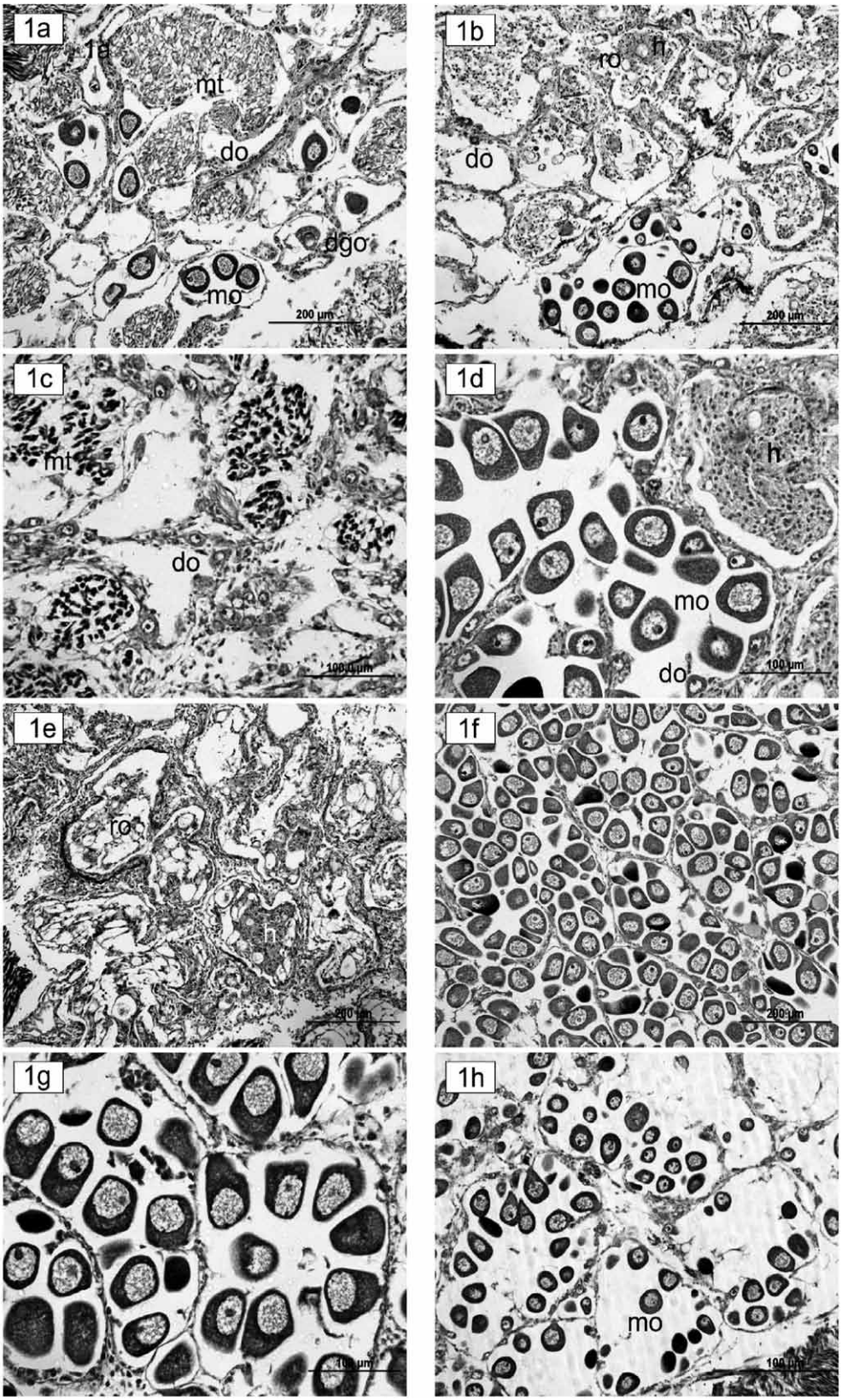

FIG. 1. – Gonadal development in females of *R. philippinarum*. 1a and 1b beginning of the experiment. Experiment 14ºC: 1c) 32 days; 1e, 57 days; 1g, 78 days. Experiment 18ºC: 1d, 32 days; 1f, 57 days; 1h, 78 days. Abbreviations: do, developing oocyte; dgo, degenerative oocyte; h, haemocytes; mo, mature oocyte; mt, muscle tissue; ro, reabsorbing oocyte. Scale bar: 100 µm (photos: 1c, 1d, 1g, 1h) and 200 µm (photos: 1a, 1b, 1e, 1f).

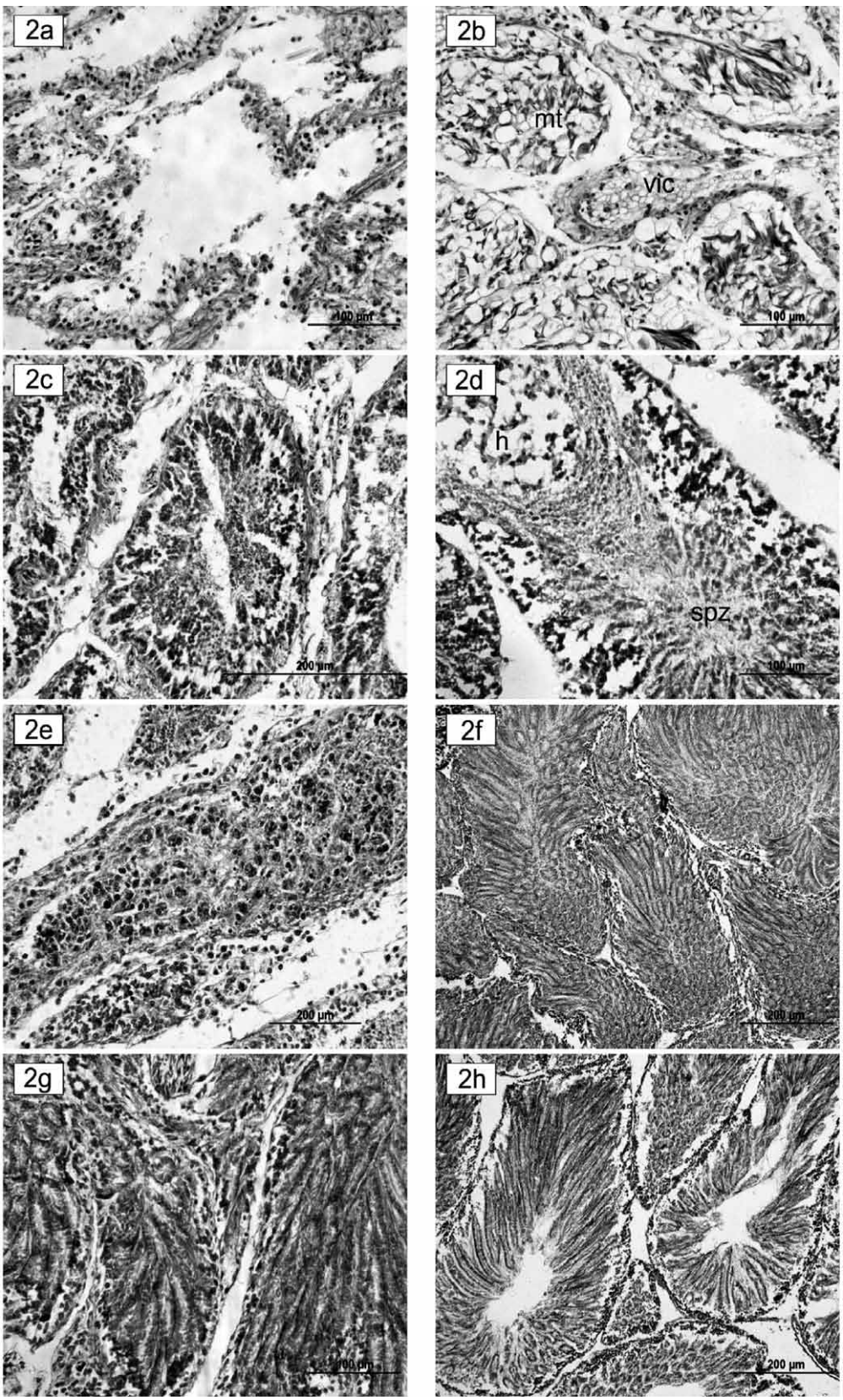

FIG. 2. – Gonadal development in males of *R. philippinarum*. 2a and 2b beginning of the experiment. Experiment 14ºC: 2c, 32 days; 2e, 57 days; 2g, 78 days. Experiment 18ºC: 2d, 32 days; 2f, 57 days; 2h, 78 days. Abbreviations: h, haemocytes; mt, muscular tissue; spz, spermatozoids; vic, vesicular intrafollicular cells. Scale bar: 100 µm (photos: 2a, 2b, 2d, 2g) and 200 µm (photos: 2c, 2e, 2f, 2h).

assigned the reproductive phase that corresponds to that of the greatest number of follicles.

Results show how a temperature increment accelerates the gonadal development of *R. philippinarum*. In this sense, we observe that 32 days after the start of the experiment at 14ºC 70% of the individuals were still in phase II, showing no mature gametes (Figs. 1c, 2c and 6). However, at 18ºC over 50% of the individuals had already reached phase IV and 36% showed the following features of phase III (Figs. 1d and 2d) in the same individual: empty follicles with walls showing intense gametogenic development, follicles full of fully mature gametes and spermatozoids in males, and areas in which partial spawning had taken place, characterised by very thin and broken follicle walls, loose mature oocytes and haemocyte infiltrations reabsorbing residual gametes.

After 57 days, 45% of the individuals kept at 14ºC reached phase III but 28% of the sample were either still in the initiation of the gametogenesis phase (II), or even in that of sexual rest (9%: phase I) (Figs. 1e and 2e). In the experiment at 18ºC, the overall appearance of the population indicated that 90% of the individuals were by then in the reproductive phase (IV).

By the 78th day of the conditioning period at 14ºC the majority (64%) of the individuals were in phase III. However, 36% of the individuals were well into phase IV (Figs. 1g and 2g). Nevertheless, at 18ºC the females were fully mature (100% in phase IV), this state of maturity applying to the complete gonad. In females, the follicles were bigger, and the mature oocytes filled them completely, giving them a polygonal shape (Fig. 1f). The males showed acines of considerable size and full of spermatozoids arranged in rosettes (Fig. 2f). Asynchronicity between individuals had almost disappeared in this final phase of maturation. All the individuals sampled showed clear signs of having suffered partial spawnings (1 and 2 h), and occasional invasions of haemocytes were observed in areas where partial spawning may have occurred, although the frequency with which reabsorption phenomena appeared was noticeably lower than that of previous samplings.

Although *R. philippinarum* is a gonocoric species, we should nevertheless draw attention to the presence of 1 hermaphroditic individual. The individual, with a size of 36.2 mm, was histologically examined after 78 days of conditioning. The female follicles, in an advanced phase of gametogenesis, occupied almost the entire gonad, in which there was an abundance of mature oocytes. In the central part of the gonad a mature male acine was detected, full of spermatozoids arranged in rosettes (Fig. 3), gametes of both sexes therefore being produced simultaneously.

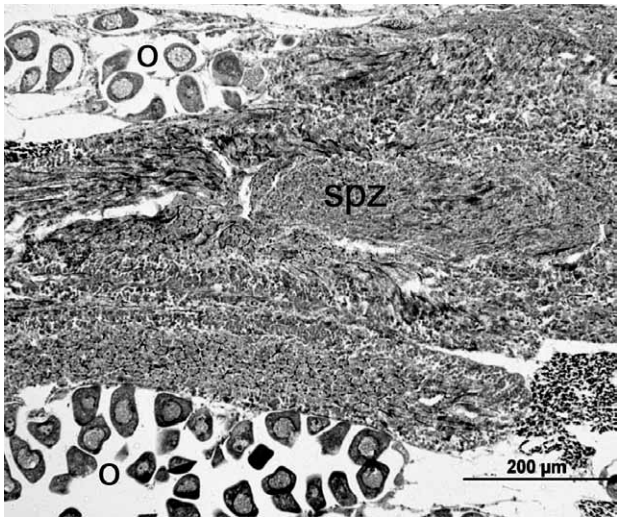

FIG. 3. – Detail of the gonad of the hermaphroditic individual of *R. philippinarum* after 78 days of conditioning. Abbreviations: o, oocytes; spz, spermatozoids. Scale bar: and 200 µm.

Ruditapes decussatus

Initially 60% of the individuals were still in the period of sexual rest (phase I) and showed no evidence of gonadal development, so it was impossible to determine their sex (Figs. 4, 5 and 6). Totally empty areas appeared in some individuals, with an obvious post-spawning appearance in which the follicles, with no gametes either inside them or on their walls, were of great size and had thin and occasionally broken walls (Fig. 4a). In the remaining individuals (40% in phase II), gonadal follicles were starting to make their way through the muscle fibres and the connective tissue that occupied the area from the digestive gland to the foot. In the females, the follicles were still not very large and their walls were both very active and full of immature cells. In the males, the germinal layers were very thick and showed the complete gametogenic process. No mature oocytes or spermatozoids were seen (Figs. 4b, 5a and 5b).

Differences in the velocity of gonadal development were related to temperature increase with time. At 14ºC, 32 days after the start of the experimental period, 70% of the specimens were at phase II, with

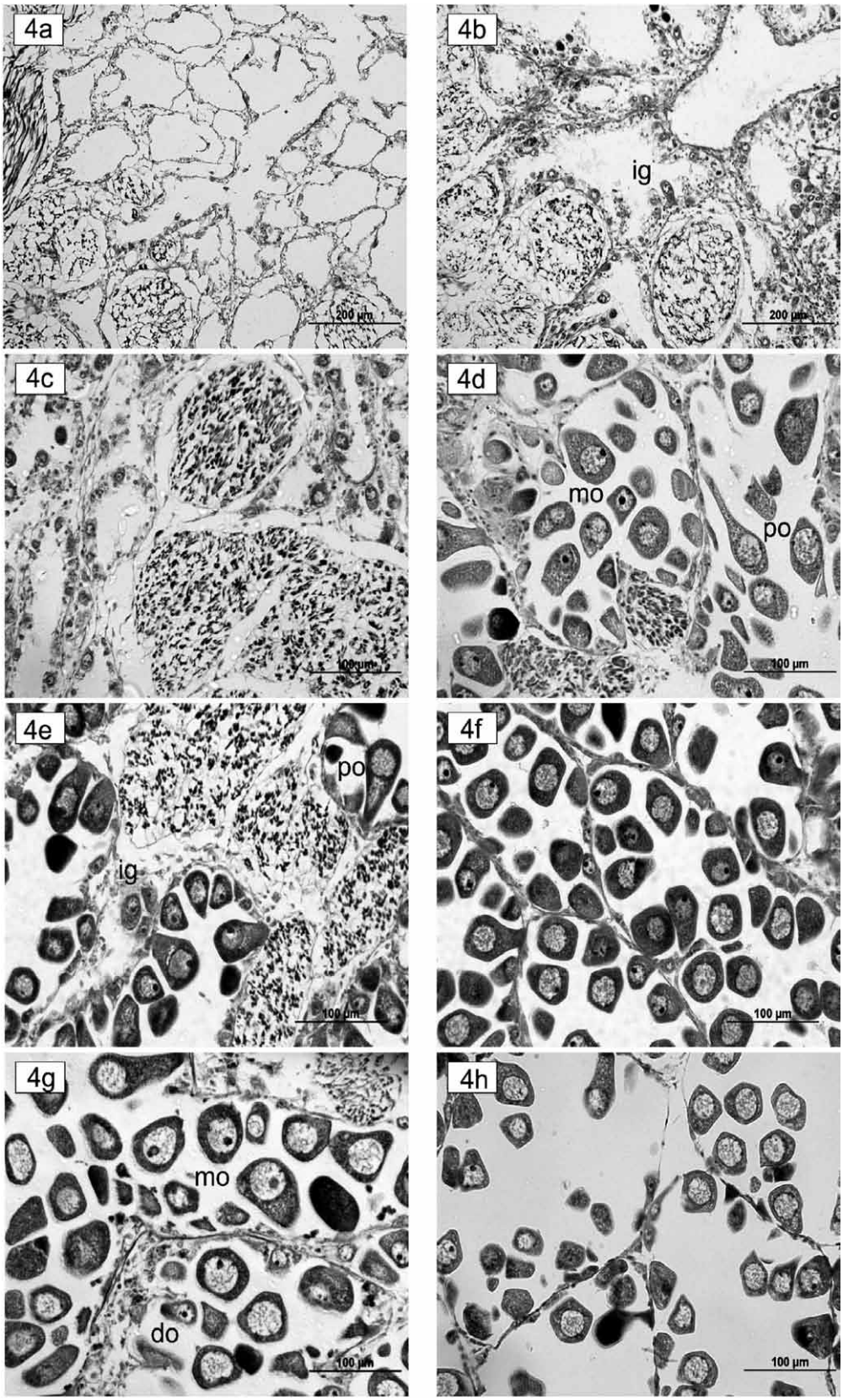

FIG. 4. – Gonadal development in females of *R. decussatus*. 4a and 4b beginning of the experiment. Experiment 14ºC: 4c, 32 days; 4e, 57 days; 4g, 78 days. Experiment 18ºC: 4d, 32 days; 4f, 57 days; 4h, 78 days. Abbreviations: do, developing oocyte; ig, immature gametes; mo, mature oocyte; po, pedunculated oocyte. Scale bar: 100 µm (photos: 4c, 4d, 4e, 4f, 4g, 4h) and 200 µm (photos: 4a, 4b).

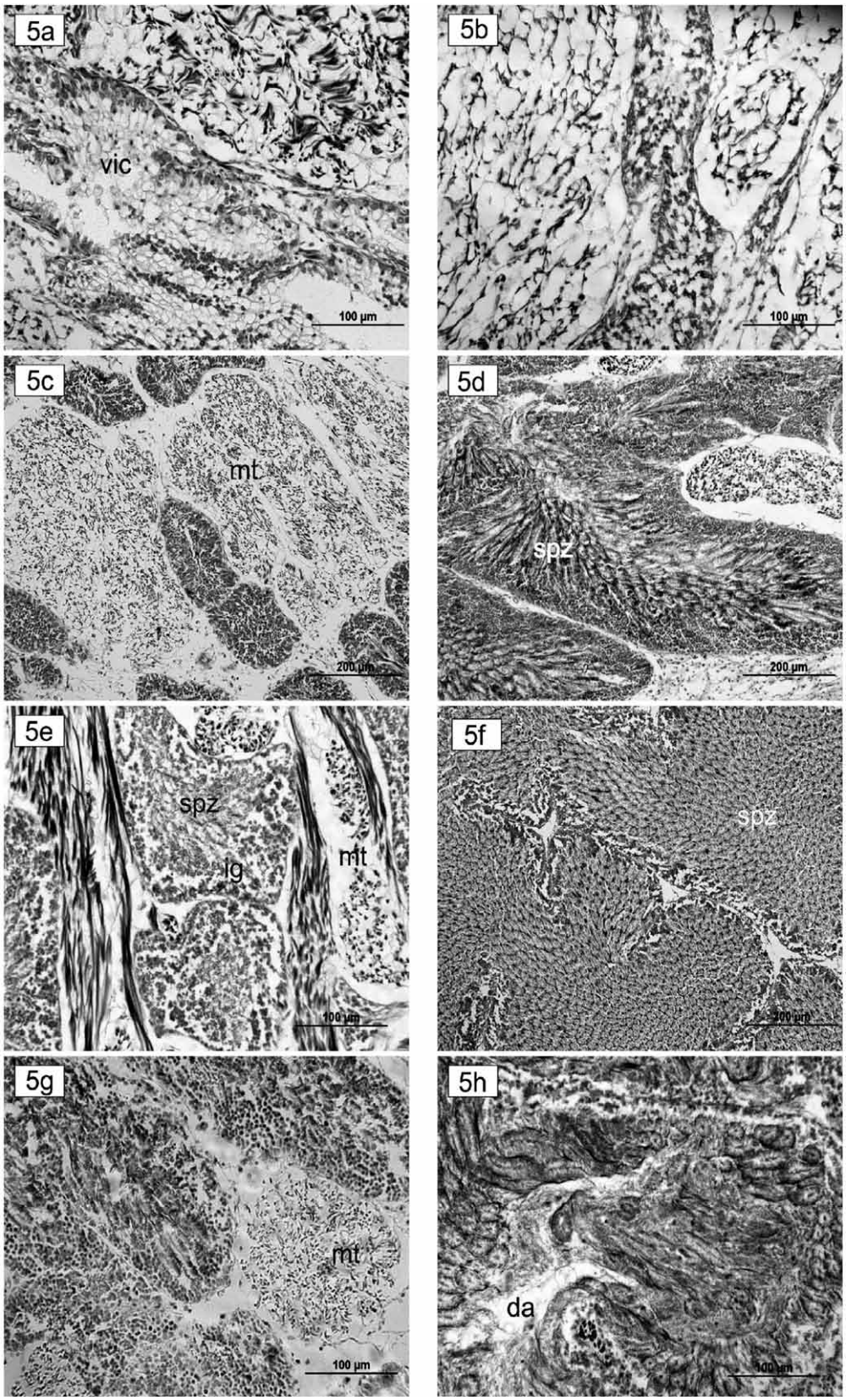

FIG. 5. – Gonadal development in males of *R. decussatus*. 5a and 5b beginning of the experiment. Experiment 14ºC: 5c, 32 days; 5e, 57 days: 5g, 78 days. Experiment 18ºC: 5d, 32 days; 5f, 57 days; 5h, 78 days. Abbreviations: da, disorganised acine; ig, immature gametes; mt, muscular tissue; spz, spermatozoids; vic, vesicular intrafollicular cells. Scale bar: 100 µm (photos: 5a, 5b, 5e, 5g, 5h) and 200 µm (photos: 5c, 5d, 5f).

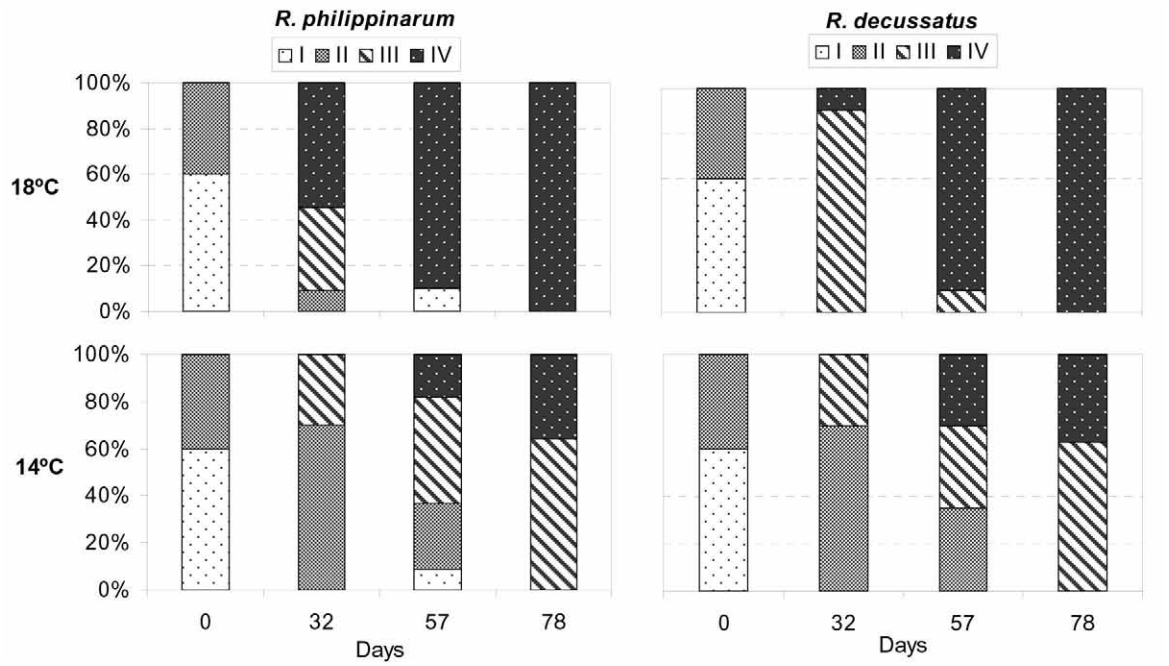

FIG. 6. – Distribution of the percentage of individuals at different phases of gonadal development (phases I, II, III and IV), for each species, experimental temperature and sampling.

the rest (30%) being at phase III (Fig. 6 and 4c). However, at 18ºC, the great majority of individuals (90%) had reached a phase of advanced gametogenesis (phase III), and some were fully mature (phase IV: 10%; Fig. 6). The follicle walls were very active, showing a great proportion of pedunculated oocytes and some free mature oocytes in the lumina of the follicles (Fig. 4d). In males, the germinal layer was thick and also very active, showing spermatogonias and spermatocytes, with the interior of the acine being full of spermatozoids that had started to organise themselves in rosettes (Fig. 5d). The occasional male started to show signs of partial spawning.

After 57 days 35% of the population maintained at 14ºC still remained at phase II of development, while 35% were at phase III and only 30% had reached phase IV (Figs. 4e and 5e). However, at 18ºC 90% of the individuals were at phase IV. The follicles were full of oocytes obliging them to take on a polygonal outline, while in males the germinal layer was thinner and the acines were full of spermatozoids (Fig. 4f and 5f).

After 78 days of conditioning at 14ºC, over 60% of the individuals were still at phase III (Fig. 4g and 5g) and only 37% of the experimental population had reached full maturity (phase IV). On the other hand, at 18ºC, *R. decussatus* was clearly mature, with 100% of the clams at phase IV. In females, the follicle walls were thin and there were commonly signs of partial emission of gametes (Fig. 4h). In males, the spermatozoids were arranged in rosettes and disorganised acines, the result of partial spawning, were easily recognisable (Fig. 5h).

At no time during the experiment, and for none of the conditions assayed with *R. decussatus*, did we observe any relevant reabsorption of haemocytes. Only sporadically could some degenerate oocytes (cytolysis) be seen, with no major haemocyte infiltration being apparent in the surrounding area.

In both species, reserve tissue formed by interfollicular muscle and vesicular cells (polygonal cells with an eccentric nucleus) was easily identified in the interior of the female follicles and male acines at the start of the experiments (Fig. 2a, 2b, 4b, 5a and 5b). This tissue disappeared as the gonadal development advanced, and the velocity of disappearance was a direct function of temperature.

Oocyte size-frequency distribution

The data obtained confirm our histological observations, showing differences in gonadal maturation related to temperature in both species. Thus, after 32 days of conditioning the mean oocyte diameter in *R. philippinarum* at 14ºC was 32.23 μm ± 4.84, while at a temperature of 18ºC it was 38.93 μm ± 3.89, these differences being statistically significant (t-test, $p<0.05$) (Fig. 7). A similar phenomenon

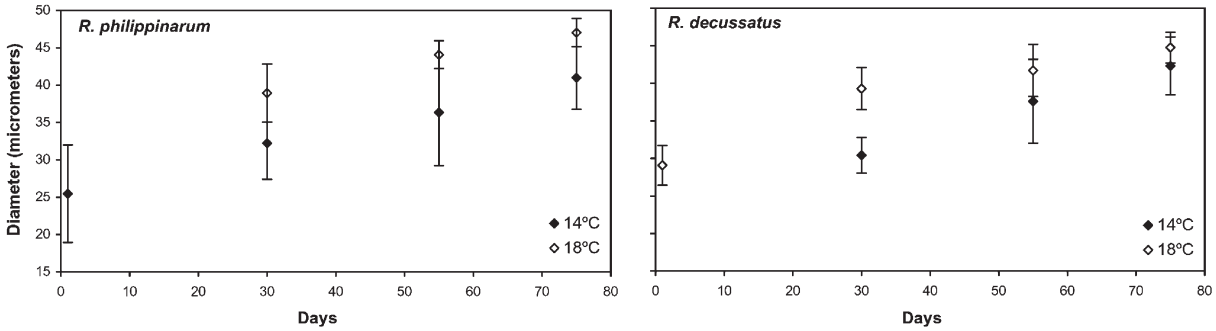

FIG. 7. – Variation in mean maximum diameter throughout the experiments at 14 and 18ºC for *R. philippinarum* and *R. decussatus*.

occurred in the case of *R. decussatus*, mean oocyte diameters being 30.42 µm ± 2.37 and 39.29 µm ± 2.80, at 14 and 18ºC respectively (t-test, $p<0.05$) (Fig. 7).

In the case of *R. philippinarum*, the skewness index (S), which initially stood at 0.73, decreased over the conditioning period, showing a gradual increase in the proportion of oocytes of greater size

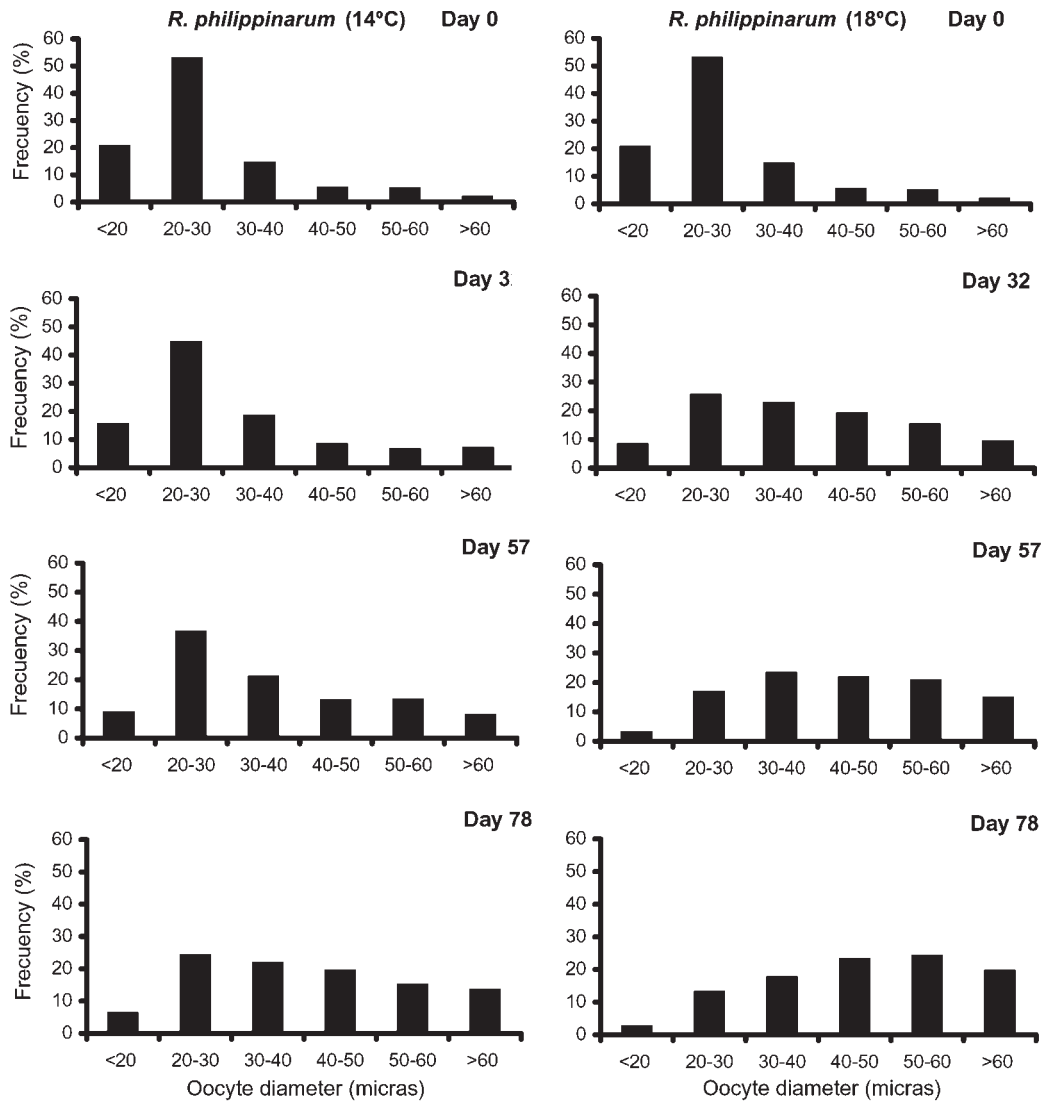

FIG. 8. – Frequency distribution of maximum oocyte diameters in *R. philippinarum* throughout the experiment at 14 and 18ºC.

TABLE 2. – Descriptive parameters of size-frequency distributions of oocyte diameter for *R. decussatus* and *R. philippinarum* experiments at 14 and 18ºC. S: Skewness; K: Kurtosis.

	R. philippinarum				*R. decussatus*			
	14ºC		18ºC		14ºC		18ºC	
	S	K	S	K	S	K	S	K
0 days	0.73	3.57	0.73	3.57	1.21	2.34	1.21	2.34
32 days	1.4	5.41	0.66	0.42	0.86	3.27	0.76	4.7
57 days	0.79	0.99	0.41	-0.21	0.81	5.96	0.88	4.3
78 days	0.54	1.15	0.13	-0.65	0.54	5.33	0.63	1.7

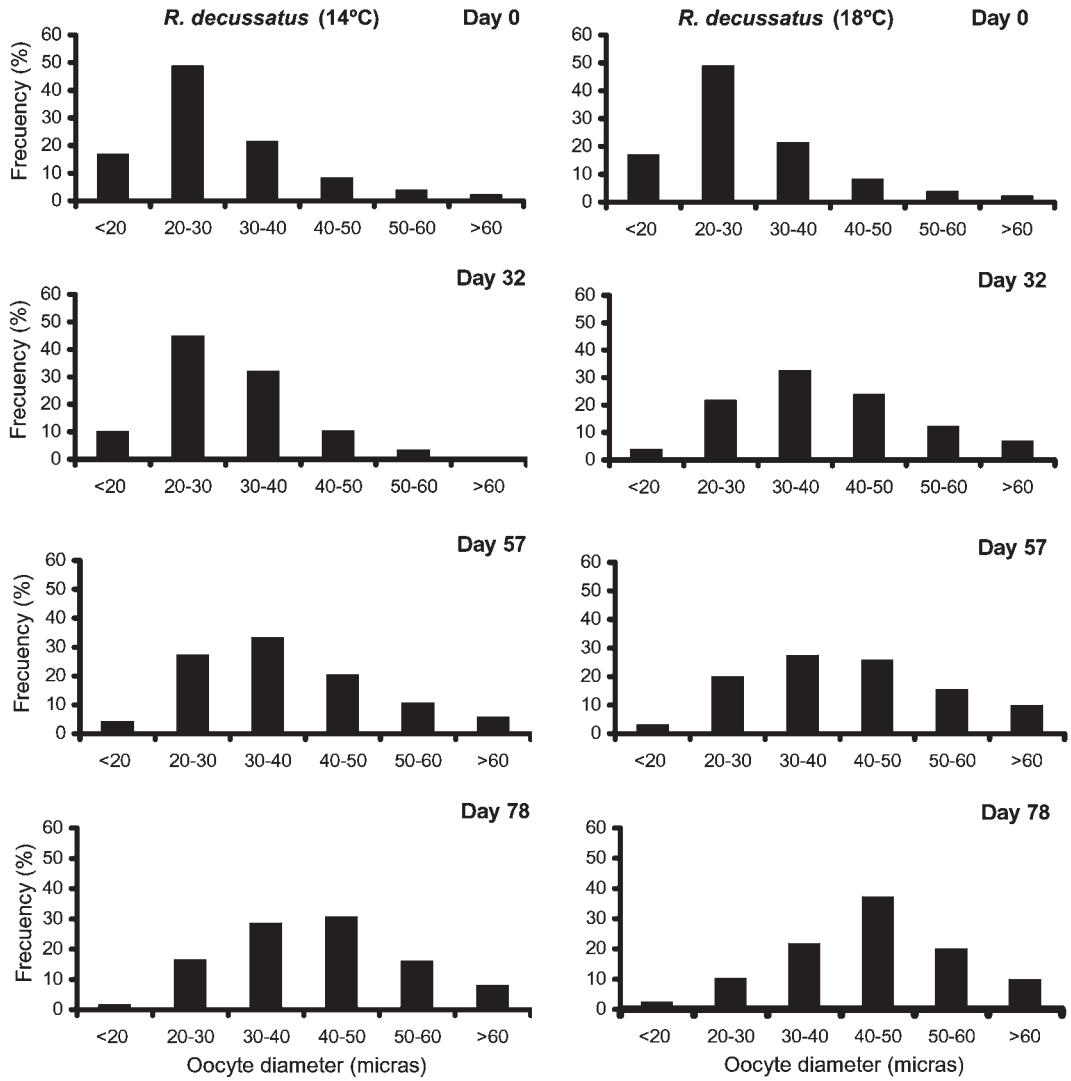

FIG. 9. – Frequency distribution of maximum oocyte diameters in *R. decussatus* throughout the experiment at 14 and 18ºC.

(Table 2). In fact, at 18ºC, the proportion of oocytes with mean diameter >60 μm came close to 20%, with a skewness value of 0.13 (Fig. 8). Furthermore, the effect of temperature was clear, because although the index fell at 14ºC, it did so to a lesser extent than at 18ºC (0.54).

In *R. decussatus*, initial skewness was 1.21 (Table 2), descending over the conditioning period and the accompanying maturation process to values ranging from 0.54 (14ºC) to 0.63 (18ºC). These levels were not so low as those for *R. philippinarum*, but there were no observable temperature-related differences that corresponded to those detected in the degree of maturation of the gonad during the histological study. This may possibly be related to the partial emissions of gametes, with the subsequent

decrease in the percentage of larger oocytes, as is shown by the fact that at 18ºC, after 78 days of conditioning, the proportion of oocytes with a mean diameter >60 µm was no greater than 10% (Fig. 9).

The kurtosis values (K), which indicate whether the distribution of data is more or less flat in comparison with a normal distribution, were initially high in *R. philippinarum* and then decreased as gonadal development progressed (Table 2). Values that approach zero, or are on occasions even negative, show that we have a more even size distribution with no single oocyte size predominating over the rest, i.e. there is a wide range of sizes and both young and mature oocytes are present in very similar proportions. K values for *R. decussatus* were greater than 1, indicating the existence of steeper distribution curves in which certain size classes were predominant (Table 2).

If, at the end of the 78-day experimental period, we compare the distributions of the experiment performed at 18ºC for both species, we can see that in *R. philippinarum* no single size class is particularly predominant (K: -0.65), while in *R. decussatus*, the 40-50 µm class is present in greater proportion than the remaining size classes (K: 1.7) (Table 2, Fig. 8 and 9).

DISCUSSION

In both *R. philippinarum* and *R. decussatus* the gonadal development rate is directly related to the increase in temperature. At low temperatures (14ºC), such as those prevailing in spring on the Atlantic coast of northwest Spain (i.e. the Galician Rias) (Pérez-Camacho, 1980), the histological study performed indicates a very similar reproductive behaviour in both species, which take more than two months to reach reproductive maturity (phase IV). At 18ºC, a common summer temperature for the waters off the northwest seaboard of the Iberian Peninsula (Pérez-Camacho, 1980), *R. philippinarum* initially shows a higher gonadal development rate than *R. decussatus*. These differences, however, gradually decrease over time.

Despite the different origin and geographical distribution of the species in this experiment, the data reflected in the study reveal that both *R. decussatus* and *R. philippinarum* are perfectly adapted to the thermal conditions of the Galician Rias, allowing them to commence gonadal development in spring and reach full sexual maturity in summer. Indeed, the foreign species *R. philippinarum* may even possess a certain reproductive advantage over the native species *R. decussatus* as a result of its higher gonadal development rate.

The ability of *R. philippinarum* to produce mature oocytes at 14ºC (as does *R. decussatus*), and the evidence of partial spawnings in our experiments performed at this temperature, enable it to adapt to cold water environments, and our findings in this regard coincide with those of several authors for the natural environment (Beninger and Lucas, 1984; Rodríguez-Moscoso *et al*., 1992). Some authors, in their studies of *R. philippinarum* in the natural environment, have pointed out that the lowest temperature limit at which spawning is possible in this species is 14ºC, a point which has also been proved in the present study. The low limit for maturation of gametes would be 12ºC, while 8ºC would be the lowest temperature at which gametogenesis could commence (Ohba, 1959; Holland and Chew, 1974; Mann, 1979; Xie and Burnell, 1994).

The native species *R. decussatus* is also well adapted to a wide range of temperatures, its geographical distribution reaching the Mediterranean and Adriatic Seas and stretching in the eastern Atlantic from the British Isles to the coast of Senegal (Vilela, 1950; Tebble, 1966).

If we consider the statistics that describe oocyte size-frequency distribution, at the beginning of the experimental period skewness values were 0.73 in *R. philippinarum* and 1.21 in *R. decussatus*, thus indicating a greater proportion of small oocytes (25-27 µm; Figs. 8 and 9) in both species as compared with the rest of the experiment. As the experiment continued and gonadal maturation progressed, mean oocyte diameter increased and skewness values dropped to almost zero, as the proportion of larger oocytes rose. These indices are similar to those given by Meneghetti *et al*. (2004) in their study of the reproductive cycle of *R. philippinarum* in the Lagoon of Venice.

When the gonad is mature, *R. philippinarum* shows a highly homogeneous distribution of oocyte size classes, with no single size standing out above the rest, whereas *R. decussatus* shows a more heterogeneous oocyte size-frequency distribution and its most numerous size class is that of 40-50 µm.

These data support the interpretation given by Laruelle *et al*. (1994) in their comparative study of the same two species in their natural environment in Brittany (France), according to which *R. philip-*

pinarum undergoes an accumulation of oocytes prior to partial or total emissions, while in *R. decussatus* gametes appear to be released continuously. This behaviour may also explain the absence of differences in skewness values between the two experimental temperatures in the case of *R. decussatus*.

As has been described by various authors (Xie and Burnell, 1994; Delgado, 2002; Meneghetti *et al*., 2004; Drummond *et al*., 2006), there is a high degree of asynchronicity between individuals regarding gonadal development in the two species, and it can therefore be hard to assign a specific reproductive phase to a given population of either one. In the case of *R. philippinarum* this asynchronicity is transferred to the gonad itself, with three different reproductive phases frequently being found within the same individual. This heterogeneity of gonadal development is more obvious in the earliest phases of development and tends to disappear as the gonad matures. When an individual is fully mature it shows a single reproductive phase, with all follicles then being at phase IV. The gonadal development between individuals of *R. decussatus* is more homogeneous than in the former species.

A further major difference between the two species is the phenomenon of reabsorption, which occurs to a high degree in *R. philippinarum* but hardly ever in *R. decussatus* (Delgado and Pérez-Camacho, 2005). The reabsorption process is particularly intense in the period of sexual rest, where we found a multitude of haemocyte infiltrations that cleaned and reabsorbed unemitted gametes from a previous gonadal maturation. Once these follicles have been cleaned, their walls start to develop new germinal lines, temperature permitting, or else accumulate reserves (vesicular cells) if food and temperature conditions so determine.

Some authors confine reabsorption phenomena, or a state of gonadal restoration, to post-total spawning phases at the end of the gonadal cycle (Drummond *et al*., 2006). Medhioub (1986) locates this phenomenon after the final spawnings and detects invasions of granulocytes in the gonadal tubules surrounding the residual gametes, which atrophy and return the gonad to a state of sexual rest. In our case, however, this phenomenon was observed throughout the entire maturation process at both 14 and 18ºC, disappearing only when the individual was fully mature. The clams at the beginning of the experimental period, given the degree of reabsorption and number of residual gametes observed, may well be in a transition phase between post-spawning, or a state of sexual rest, and the start of a new gametogenic cycle. Along these lines, Rodríguez-Moscoso *et al*. (1992), in his study of the reproductive cycle of the *R. philippinarum* clam, cites the appearance of haemocyte infiltrations and gamete reabsorption in some individuals of this species at times that do not coincide with full gonadal maturation (January, February, May, July and October for males; December-March and November-December for females).

Medhioub (1986) and Sarasquete *et al*. (1990) are of the opinion that the vesicular cells that appear at the commencement of gametogenesis originate from cells resulting from haemocyte differentiation. Lysis of the vesicular cells, together with the atrophy and degeneration of the smooth muscle cells adjacent to the gonadal tubules, liberate metabolites for the purpose of covering the energy needs of gametogenesis. In both species the vesicular cells disappear completely while lysis of the muscle cells is never total, as mentioned by authors such as Delgado and Pérez-Camacho (2005), Medhioub (1986), Rodríguez-Moscoso *et al*. (1992) and Rodríguez-Moscoso and Arnaíz (1998). Furthermore, the disappearance of vesicular cells occurs earlier in females than in males. These vesicular cells liberate glucose and products of lipid catabolism (Medhioub, 1986). Atrophied and degenerate muscle cells supply protein and glucide metabolites, which may be used directly by the cells of the germinal line or absorbed by granulocytes and supplied at a later moment to maintain the reproductive effort (Medhioub and Lubet, 1988). As mentioned above, the persistence of reabsorption figures in *R. philippinarum* both in the phase of sexual rest, where this is more apparent, and throughout the process of gonadal maturation, is evidence of the high capacity for gametogenic regeneration in this species and its ability to recover the energy invested in the production of unemitted gametes.

This greater capacity for gonadal regeneration in *R. philippinarum*, coupled with its higher rate of gonadal development, may account for this species' greater reproductive activity and longer reproductive period than *R. decussatus*, as described by Beninger and Lucas (1984), Rodrigues-Carballo *et al*. (1992) and Laruelle *et al*. (1994), and this may even represent a certain adaptive advantage of the foreign species (*R. philippinarum*) over the native species (*R. decussatus*) in the temperature conditions of the Galician Rias.

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