ISSN: 0001-5113
AADRAY

ACTA ADRIAT.,
57(1): 93 - 106, 2016

ORIGINAL SCIENTIFIC PAPER

Broodstock conditioning and gonadal development of the smooth clam *Callista chione* (Linnaeus, 1758) (Mollusca: Bivalvia) on the Catalan coast (NE Spain)

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In order to select the most suitable broodstock conditions for Callista chione*, a conditioning experiment was performed and the gametogenic cycle of a natural population on the coast of Arenys de Mar (Catalonia, NE Spain) was studied. The influence of food availability and sand bed presence on energy balance and gonadal development was analyzed, also the proliferation of intracellular bacteria in gills was monitored.*

The gametogenic cycle started between August and September. In November, most of the gonads were ripe and spawning began in December. The higher abundance of spawning clams occurred between January and April (with sea surface temperature 13-15ºC). Three experimental conditions were tested in September at a constant temperature of 14ºC:T1 (0.10% of organic weight of microalgae as a proportion of the live weight of the clams per day and a sand bed); T2 (0.05% and a sand bed); T3 (0.10% without a sand bed). Gonadal maturity was a priority for this species and was reached at the end of all trials. Food availability and sand bed presence benefit survival and gonadal development. The best results were obtained in clams from trial T1. Nevertheless, broodstock conditions seemed to trigger the proliferation of intracellular bacteria in gills, and as the experiment advanced, prevalence and intensity of infection increased for all trials reaching values of 100% and between 9-40 intracellular bacteria/pair of gill plica, respectively.

Key words: *Callista chione*, reproduction, temperature, food availability, sand, gills, histopathology

INTRODUCTION

The smooth clam *Callista chione* (Linnaeus, 1758), is distributed on sandy bottoms throughout the Mediterranean Sea and European Atlantic coasts over 0-180 m depth range (SALAS, 2011). This species is of great commercial interest in Spain, and is intensively exploited along the Catalan coast (DOGC, 2008). Catches have dramatically decreased over the recent years, threatening the biological and economic sustainability of this fishery along the natural beds of the Catalan coast (DOGC, 2008). This decline in catches and the interest in diversifying aquaculture, have led to the development of a program of investigation into the management in hatch-

eries of this species through projects carried out in different Spanish geographical locations (JACUMAR).

Most of the biological information currently available, proceeds from studies carried out in the natural environment, and several studies have already described the reproductive cycle of this species (VALLI *et al.*, 1994; TIRADO *et al.*, 2002; MOURA *et al.,* 2008). These studies reported a prolonged reproductive cycle with three spawning peaks and the absence of a resting period in SE Spain and southern Portugal. However, despite commercial interest, no detailed information about reproductive aspects of *C. chione* has been reported in the NW Mediterranean Sea. This information is not only necessary to implement fisheries management measures but also to select the most appropriate period of the year for carrying out broodstock conditioning.

Environmental parameters determine to a great extent the dynamics of growth and reproduction of bivalves (GABBOTT & BAYNE, 1973). Temperature and food availability have been identified as playing a major role in the evolution of the reproductive cycle (SASTRY 1979; BAYNE & NEWELL, 1983). Temperature is normally responsible for initiating gonadal development and an increase in temperature speeds up most of the physiological processes, including clearing and ingestion rates as well as respiration and growth (GRIFFITHS & GRIFFITHS, 1987). On the other hand, previously stored food reserves or food supply also plays an important role in the success of reproduction determining the extension of the reproductive process (LUBET, 1959; NAVARRO *et al.*, 1989) or the rate of gonadal development (RUIZ *et al.*, 1992; DELGADO & PÉREZ-CAMACHO, 2003). Other authors have described the negative effects of food shortage on the reproductive output of certain species such as *Mytilus edulis* (GABBOTT & BAYNE, 1973). In all cases, both variables interact and determine the acquisition and expenditure of energy available for reproduction and/or somatic growth (DELGADO & PÉREZ-CAMACHO, 2007).

Broodstock conditioning experiments on venerid species usually test the influence of temperature or diet on gonadal development (PÉREZ-CAMACHO *et al.*, 2003; DELGADO & PÉREZ-CAMACHO, 2007; OJEA *et al.*, 2008). The influence of other environmental variables on energy balance or gonadal development has not been studied. Specifically, the burrowing in substratum of bivalves has not yet been widely studied or related to physiological aspects despite the fact that this is the normal condition of most of these organisms in the natural beds. Growing burrowing molluscs under non-natural conditions could potentially cause physiological stress (BOSCOLO *et al.*, 2003), shell deformities and/or behavioral changes (LAVALLEY, 2001). In contrast, EPELBAUM *et al.* (2011) suggest that the addition of substratum may have an indirect effect on clams through increasing the three-dimensional space available to them which, in turn, could increase growth and survival by reducing physiological stress.

In spite of commercial interest, only two conditioning experiments at 18ºC have been carried out (DELGADO *et al.*, 2007; MARTÍNEZ-PITA *et al.*, 2011). DELGADO *et al.* (2007) described the first conditioning experiment of adults and reported severe mortality events associated with the presence of branchial intracellular bacteria, histologically designated as rickettsia-like organisms. The presence of these intracellular bacteria was detected in the sample from the natural beds but strengthened under broodstock conditions. The level of intensity of the infection probably affected respiration and ingestion rates and, consequently, increased mortality rates.

In order to select the most suitable broodstock conditions for *C. chione*, our main objective was to study the influence of food availability and sand bed presence, at a lower conditioning temperature (14ºC), on energy balance and gonadal development as well as to determine their influence on the proliferation of intracellular bacteria in gills. The reproductive cycle in the natural beds was also studied in order to adjust the beginning of the conditioning trials to a suitable gametogenic stage and define the proper conditioning temperature.

MATERIAL AND METHODS

Analyses and visualizations of remote-sensing data observations of sea surface temperature and *chlorophyll-a* concentration used in this study were produced with the Giovanni online data system, developed and maintained by the NASA GES DISC (Ocean color radiometry online visualization and analysis).

Samples of *C. chione* were collected by dredging off the coast of Arenys de Mar (Catalonia, NE Spain; 41º34'N, 2º35'E) (Fig. 1). For each individual, the shell length (maximum distance along the anterior-posterior axis of the shell, SL) was measured to the nearest 0.1 mm with a digital caliper. Live weight (LW) was also recorded for each specimen to the nearest 0.001 g.

Reproductive cycle

The reproductive cycle in the natural beds of Arenys de Mar was studied to adjust the beginning of broodstock conditioning trials to a suitable gametogenic stage and to define the proper conditioning temperature. A natural population of *C. chione* was monitored over one year (January–December 2008). Samples were collected monthly in the natural beds off the coast of Arenys de Mar at a depth of 5-30 m (Fig. 1). Given that species size at first maturity is estimated to 30 mm SL for the littoral area of Catalonia (GALIMANY *et al.*, 2006), approximately 20 individuals (> 38 mm SL) were collected monthly in order to study the gametogenic cycle by means of histological techniques.

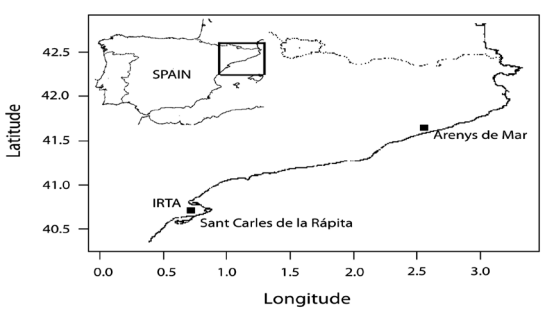

Fig. 1. Map of the Catalan coast (NE Spain) and location of Arenys de Mar and IRTA hatchery

Broodstock conditioning: Design and experimental conditions

The conditioning experiment was performed in September in the laboratory (IRTA hatchery; Fig. 1). In this month most of the clams in the natural bed on the Catalan coast (NE Spain) (> 70%) were in suitable stages of gonad development to start a new gametogenic cycle (stages 1 and 2) Due to the fact that a temperature of 18ºC could be a stress factor (DELGADO *et al.*, 2007), and the coincidence of the maturation period with temperatures around 14ºC in these natural beds (see Results section), our objective was to test using 14ºC as the conditioning temperature. Moreover, the effects of two contrasting conditions of food availability were examined at this temperature as well as the presence of sand substrate that would allow clams to burrow. A histopathological monitoring of gills was also carried out for all experimental conditions.

The experiments were performed in a flow-through system containing seawater filtered through 1 μm mesh and maintained at a constant temperature (14ºC) and salinity (33%). The initial number of specimens was 70 for each experimental condition (45.13 ± 7.37 mm SL). As a consequence of the large number of individuals used in each experiment and the long duration (58 days) of the surveys, clams were kept in large groups in 25 liter plastic tanks. In this way, food concentration was more stable and equal for all clams at each experimental stage and was closer to natural conditions. The design of this experiment was similar to the one used by DELGADO & PÉREZ-CAMACHO (2003) and PÉREZ-CAMACHO *et al.* (2003).

Two different concentrations of daily food rations of microalgae were assayed: 0.10% (T1 and T3) and 0.05% (T2) (percentages corresponded to the organic weight of food supplied as a proportion of the LW of the clams). The food was introduced into the circulating water with a variable-flow peristaltic pump. The microalgae diet was composed of *Isochrysis galbana clone T-ISO* (25%), *Tetraselmis suecica* (25%), *Chaetoceros calcitrans* (25%) and *Phaeodactylum tricornutum* (25%). The microalgae

were initially cultured in 6 l flasks and then transferred to 100 l tanks. The microalgae were harvested during the stationary growth phase.

For the highest food ration (0.10%), two conditions were also assayed: presence (T1) or absence of sand bed (T3) on the bottom of conditioning tanks. The sand bed was also present in T2 (0.05% food ration). The sand bed was composed of sand grains with a diameter of between 500 μm and 1 mm, similar to the ones found in the natural habitat of this species on the coast of Arenys de Mar (VIDAL *et al.*, 2006). Fresh sterilized sand was used at the beginning of the experiment and replaced every 10 days.

The total conditioning period was 58 days, with sampling being performed at the start of the experiment (day 0), at days 30 and 58. At each interval, ten individuals were selected to determine the dry weight of soft tissues and ten were sampled for histological study of gonadal development and histopathological monitoring of gills. The number of specimens per sample was increased in order to obtain a minimum of four specimens for each sex.

Histology

A piece of visceral mass and a piece of gills were taken from each individual and a conventional histology protocol was followed: tissues were fixed in formaldehyde (4%), dehydrated (through a series of graded ethanol: 70%, 90% and 100%) and subsequently embedded in paraffin (56ºC). Sections of 6 μm were prepared and stained following the Harris hematoxyline and eosin protocol (BANCROFT & STEVENS, 1996).

Identification of the gametogenic stages in *C. chione* was achieved by observing the histological preparations under a light microscope (Nikon Eclipse 90i) and using the criteria shown in Table 1 and Fig. 2. Since one peculiarity observed in the gametogenic cycle of *C. chione* is the coexistence of two or more stages in the same gonad, stage assignation was carried out using the most representative stage.

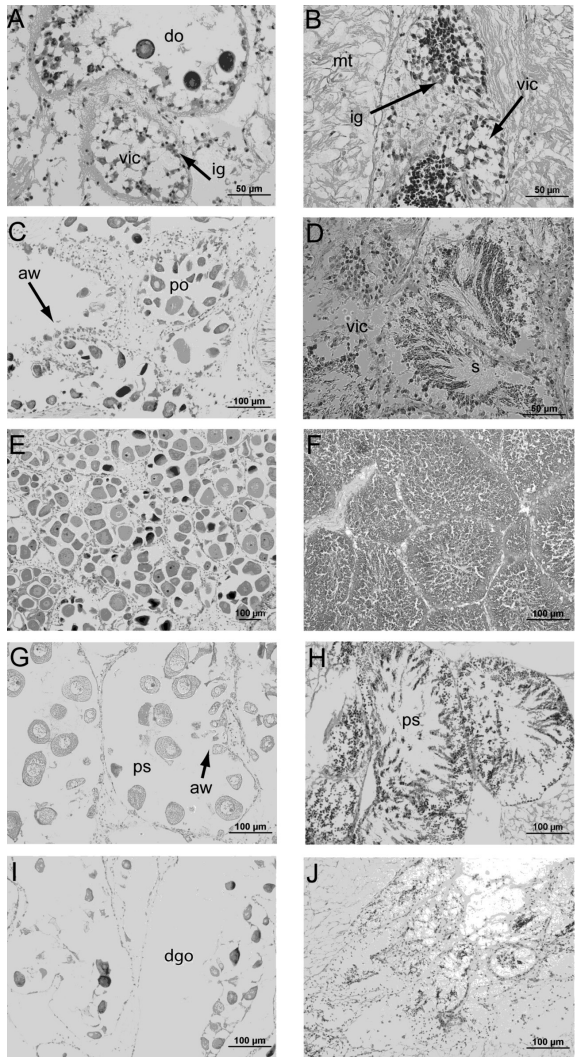

Fig. 2. Gonadal development in females and males of Callista chione*. A and B. Stage 1: Initiation of gametogenesis with presence of vesicular intrafollicular cells and immature gametes. C and D. Stage 2: Active gametogenesis. E and F. Stage 3: Totally mature female and male. G and H. Stage 4: Individuals after a partial emission of gametes. I and J. Stage 5: Spent. Abbreviations: aw: active walls; dgo: degenerative oocyte; do: developing oocyte; ig: immature gametes; mt: muscular tissue; ps: signs of partial emission; po: pedunculated oocyte; s: spermatozoa; vic: vesicular intrafollicular cells*

Soft-tissue dry weight with reference to a standard clam (DWs)

The adductor muscles of each individual were cut and the clams were placed on their ventral surface, allowing them to drain for 5 min. The soft tissues were separated and dried at 100°C for 24 h and then weighed to obtain

Table 1. Scale of gametogenic developmental stages for Callista chione *females and males*

Stages	Name	Females	Males
0	Sexual rest or indeterminate	Gonadal follicles are absent and connective and muscular tissue occupy the entire zone from the digestive gland to the foot. There is no evidence of gonadal development and sex determination is not possible. Beginning of formation of intrafollicular vesicular cells (reserve tissue)	
1	Initiation of gametogenesis	Appearance of follicles isolated in the abundant muscular tissue. They increase in size and appear covered with oocytes in growth phase. Abundance of intrafollicular vesicular cells	Appearance of gonadal acini isolated in the abundant muscular tissue They increase in size and appear covered with immature gametes (spermatogonia and spermatocytes). Abundance of intrafollicular vesicular cells
2	Advanced gametogenesis	Follicles occupy a greater part of the visceral mass. At the end of this stage, characterized by intense cellular growth in females, vitellogenic oocytes protrude at the centre of the lumen remaining attached to the wall via the peduncule. Free oocytes started to appear in the lumen of the follicle	Acini occupy a greater part of the visceral mass. The majority of the acini appeared full of spermatids and spermatozoids
3	Ripe	Most of the oocytes are mature. The rupture of the peduncule occurs freeing oocytes inside follicles	Most of the gametes are mature. More than two-thirds of the acini filled with spermatozoids
4	Partially spawned	Throughout this period partial emissions occur. Some zones show signs of gametogenic recovery after a partial spawning and immature cells are detected in acini or follicle walls. Signs of atresia	
5	Spent	Total emission of gametes. Disorganization of tissue structure of the gonad. Presence of residual gametes. Signs of atresia	

the soft-tissue dry weight (DW). The relationship between DW and SL was estimated for each sampling by fitting data to the function $DW = a\ SL^b$, where a is the ordinate and b the slope. The temporal variation of soft-tissue dry weight (DWs) with reference to a specimen of constant size (44.74 mm SL), should normally correspond to the accumulation or loss of reproductive tissue (PÉREZ-CAMACHO *et al.*, 2003).

Prevalence and infection intensity by intracellular bacteria

Prevalence was estimated as the proportion of the population (sample) found to be infected by the branchial intracellular bacteria. The intensity of infection in each clam was rated similarly to the method described by VILLALBA *et al.* (1999). The mean infection intensity for intracellular bacteria colonies was calculated by recording the number of colonies occurring in 5 pairs of gill plica present in a histological section. Colonies were also measured microscopically using the software NIS-Elements AR 3.2 (Nikon) at day 58.

Statistical analysis

The analysis of variance (ANOVA) was used to compare trials and samplings. Cochran's test was used to check the homogeneity of the variances. For multiple comparisons, the differences between trials or samplings were analyzed by means of a Tukey's HSD multiple-rank test. These statistical analyses were performed according to the methods described by SNEDECOR AND COCHRAN (1980). The statistical analyses and plots were performed with the R language and environment for statistical computing (R DEVELOPMENT CORE TEAM, 2012).

RESULTS

Seasonal variations in sea surface temperature (SST) and *chlorophyll-a* concentrations during 2008 on the coast of Arenys de Mar is shown in Fig. 3. Sea surface temperature displayed minimum levels in winter (between 13 and 15ºC). Temperature increased from April and temperatures above 24ºC were recorded in summer. From September the temperature decreased reaching 15ºC in December. *Chlorophyll-a* concentrations showed an inverse pattern and the highest levels (0.6 mg.m^{-3}) were observed in winter whilst minimum levels were recorded between July and October (0.15-0.22 mg.m^{-3}). From November *chlorophyll-a* concentration increased and in December a mean value of 0.5 mg.m^{-3} was reached.

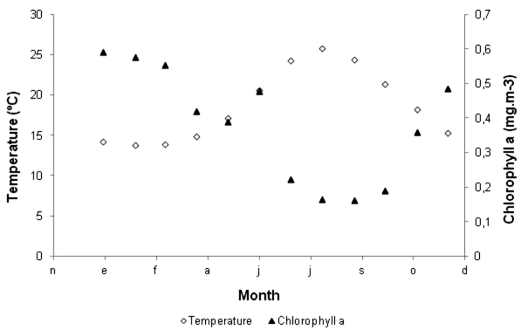

Fig. 3. Monthly variations on remote-sensing data observations in sea surface temperature (ºC) and chlorophyll-a *concentration at Arenys de Mar coast from January to December 2008*

Gametogenic cycle

The gametogenic development of *C. chione* was characterized by the simultaneous presence of different stages in the same month or even in the same gonad. Partially spent or spent individuals (stages 4 and 5) were observed throughout most of the year indicating a prolonged reproduction period of this species (Fig. 4).

In January and February 2008, all gametogenic stages were present except stage 0. The most representative stages were stage 4 and 5 (partially spent and spent) indicating a previous maturation followed by the release of gametes. Gamete emissions took place from January to

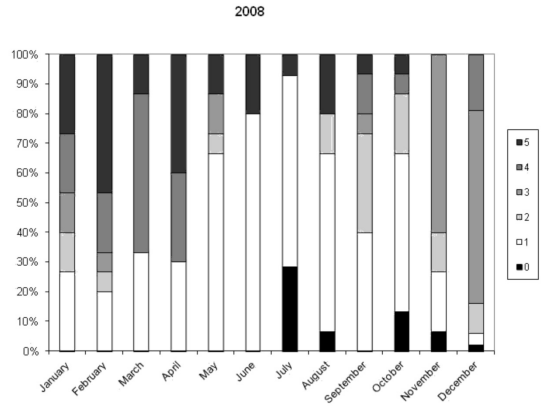

Fig. 4. Monthly variation of the proportion of gonadal development stages (0-5) of Callista chione *from January to December 2008 in the natural beds of Arenys de Mar (Catalan coast, NE Spain)*

April since only individuals in stages 1, 4 and 5 could be observed in these months. In stage 5 follicles and acini were totally emptied or had a few residual gametes (Figs. 2G and H) and significant atretic phenomena were observed in stages 4 and 5. In May a small group of clams in stages 2 and 3 were detected, and probably, a spawning peak of smaller intensity occurred between May and June. In stage 3, males had clearly visible acini full of spermatozoids which had organized themselves in rosettes (Fig. 2F). Females showed follicles full of oocytes, thus taking on a polygonal contour in stage 3 (Fig. 2E). It was not possible to delimit a resting period, but during summer (June and July), only inactive (stage 0), pre-active (stage 1) or spent individuals (stage 5) could be observed indicating a probable "partly-resting" period. In fact, in June the highest proportion of individuals in stage 1 was observed. This stage is characterized by the presence of storage reserve cells: vesicular intrafollicular cells (Figs. 2A and B). The new gametogenic cycle probably started between August and September since the maximum proportion of individuals in stage 2 (33%) was recorded in September. As gametogenesis progresses, follicle diameter increases, while the thickness of muscular tissue and the presence of vesicular intrafollicular cells decreased. Follicles appeared covered with oocyte in growth phase in females (Fig. 2C) and acini were covered with immature gametes in males (Fig. 2D).

In the period between November and December the highest proportion of clams in stage 3 were found (60-65%).

Broodstock conditioning: Effects of food availability and sand bed presence

Soft-tissue dry weight with reference to a standard clam (DWs)

Fig. 5 shows the DWs variation during the experimental period of each trial. DWs from individuals on experimental conditions T1 and T2 displayed an increase throughout the experimental period, emphasized more clearly in the case of T1. However, DWs from T3 individuals remained constant for the first 30 days and thereafter underwent a significant decrease reaching a minimum value on day 58. These differences between samplings or trials were statistically significant (Table 2).

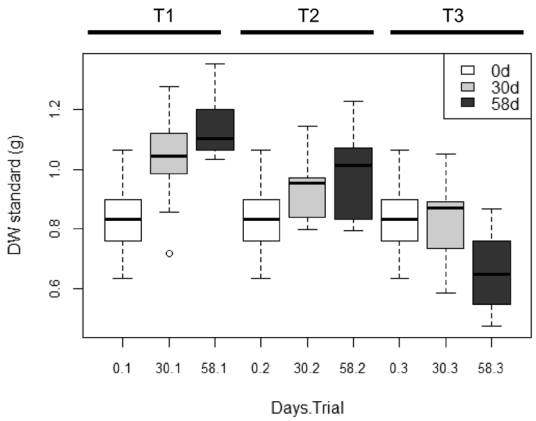

Fig. 5. Changes in soft-tissue dry weight (DW, g ind-1) of a standard clam of 44.74 mm SL along the experimental period for all trials. X-axis nomenclature Days. Trial: number of sampling days (0 days, 30 days, 58 days). number of trial (1:T1, 2:T2, 3:T3)

Gonadal development

At the start of the experiment, approximately 70% of individuals were in stage 1 (initiation of gametogenesis) (Fig. 6). As can be seen in this figure, gonads developed in all trials with very slight differences. After 30 days, the percentage of individuals in stage 2 (advanced gametogenesis) reached values around 40% (T1 and T2) or 30% (T3), and in ripe individuals, signs of partial emission or spent individuals could also be observed. At the end of the experimental period most individuals were emitting gametes (between 50-70% depending on trial).

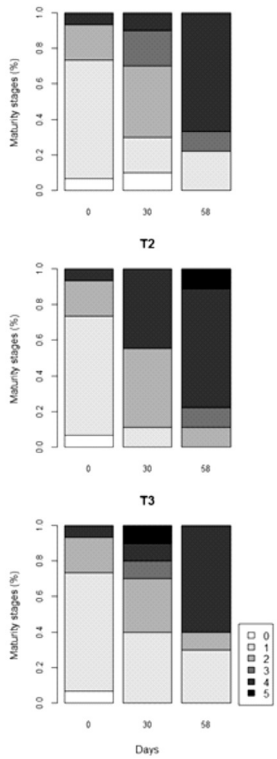

Fig. 6. Variation of the proportion of gonadal development stages during the experimental period for all trials (T1, T2 and T3)

Table 2. Analysis of variance of dry weight of a standard clam (DWs)

ANOVA comparison	Selected data	F-value	P-value	Tukey's HSD test, homogeneous groups
Trial	Day 30	5.60	<0.01	T1>T3
Trial	Day 58	32.38	<0.001	(T1=T2)>T3
Sampling	Trial 1	17.05	<0.001	Day 0< (Day 30=Day 58)
Sampling	Trial 2	5.69	<0.01	(Day 0=Day 30)<Day 58
Sampling	Trial 3	7.00	<0.01	(Day 0=Day30)>Day 58

Prevalence and infection intensity of intracellular bacteria

Prevalence of intracellular bacteria was 14.3% in the initial sampling of clams from natural beds in Arenys de Mar. The mean intensity of infection in this case was 0.09 intracellular bacteria/pair of gill plica. As the conditioning period advanced prevalence and mean intensity of infection increased (Table 3). This infection involved a pronounced disorganization of branchial architecture. In sampling at day 30, prevalence oscillated between 55% and 67% depending on trial. However, these differences were not statistically significant. At day 58, 100% of clams were infected by intracellular bacteria with variable intensity. Clams from T2 showed the highest mean infection intensity level (40.52) and this was significantly different from T1 and T3 (9.07-12.85). The maximum diameter of the colonies at day 58 ranged between 7.2-85.8 µm and the mean value was around 25 µm for all trials.

Regarding to mortality data (%) in the first week around 3-4% of clams from T1 and T3 died, probably as a result of adaptation to the broodstock conditions in the hatchery. This rate increased progressively in the case of T3 reaching 8.57% by the end of the experiment, whereas in trial T1 it did not increase. In trial T2 no mortality was recorded.

*Table 3. Prevalence, mean intensity of infection of intracellular bacteria colonies and accumulated mortality data along conditioning period. *Statistical significant differences between trials from the same sampling ($p<0.05$)*

Sampling	Trial	Prevalence (%)	Intensity	Mortality (%)
0 days		14.3	0.09	
30 days	T1	55.5	0.48	2.86
	T2	66.7	0.37	0
	T3	60.0	0.33	7.14
58 days	T1	100	9.07	2.86
	T2	100	40.52*	0
	T3	100	12.85	8.57

DISCUSSION

Reproductive cycle on the Catalan coast

The gametogenesis of *Callista chione* followed a seasonal pattern contrary to the SST pattern in Arenys de Mar. The seasonal SST pattern in this area was characterized by low temperatures (13-15ºC) during winter, a progressive increase during spring until reaching maximum in summer (24ºC), and a decrease in autumn. The reproductive cycle started at the end of the summer coinciding with this temperature decline. A high percentage of ripe individuals or those in advanced gametogenesis stage were found in November, and spawning started in December when temperatures were below 15ºC. A negative correlation between SST and the reproductive cycle has also been described for other species such as *Venerupis senegalensis* (JOAQUIM *et al.*, 2011). On the other hand, the most important events in the reproductive cycle of *C. chione* (onset of gametogenesis and spawning) were closely related with high *chlorophyll-a* concentration at sea. This behavior seems to be a useful reproductive strategy because it ensures food availability for the energy supply needed to support the beginning of the gametogenesis and larvae, respectively.

In general terms, MOURA *et al.* (2008) in relation to the SW coast of Portugal, and TIRADO *et al.* (2002) in relation to the SE coast of Spain, have described similar reproductive cycle for *C. chione* except for the onset of gametogenesis and spawning, respectively. These geographical differences in the timing of gonadal development could be related to temporal variations in environmental conditions. In this sense, NAVARRO *et al.* (1989) suggested that the inter-annual differences in the timing of gametogenesis and spawning of *Cerastoderma edule* were related to inter-annual differences in environmental parameters values.

According to this study *Callista chione* showed spawning stages throughout the year indicating a prolonged reproductive period. Due to the extended reproductive period, the asynchronic development and the difficulty in iden-

tifying a unique event of gamete emission, *C. chione* could be considered, in terms of fecundity, as a multiple partial spawner species, as other venerids such as *Chamelea gallina* (DELGADO *et al.*, 2013). TIRADO *et al.* (2002) and MOURA *et al.* (2008) also reported the absence of the resting period. In our study, it was not possible to define this resting period. However, during June and July, only inactive, pre-active or spent individuals (stage 5) could be observed thus indicating a "summer inactivity" period. In fact, in June the majority of individuals were in stage 1 showing a high presence of storage tissue (vesicular intrafollicular cells and muscular tissue). This is a typical characteristic before or parallel to the beginning of a new gametogenic cycle in other bivalves such as *Ruditapes philippinarum* (MEDHIOUD & LUBET, 1988), *Ruditapes decussatus* (RODRIGUEZ-MOSCOSO & ARNAIZ, 1998) or *Venerupis senegalensis* (JOAQUIM *et al.*, 2008). These cells are important for glycogen storage together with muscular tissue. RODRIGUEZ-MOSCOSO & ARNAIZ (1998), RODRÍGUEZ-MOSCOSO *et al.* (1992) and PÉREZ-CAMACHO (2003) observe that the glycogen is stored during the resting period in vesicular cells and muscular tissue and is important for the beginning the gametogenesis and gamete ripening. In our case, the presence of these storage cells in June coincided with high levels of food availability (chlorophyll a concentration) at sea. This storage phase could help to overcome the low levels of food availability displayed between July and September, and could be used in the next gametogenic cycle.

Broodstock conditioning: Gonadal development

Differences in conditioning, spawning success or larval viability depend on the initial condition of the broodstock and the phase of gametogenesis the adults are in when conditioning starts (OJEA *et al.*, 2008; MATIAS *et al.*, 2010). As explained in the results section and discussed previously, the broodstock conditioning was performed in the most suitable month (September), when clams were initiating or already were in advanced gametogenesis stages.

This study represents the second experiment focused on the study of the influence of food availability on gonadal development of *C. chione*. Previously MARTÍNEZ-PITA *et al.* (2011) described the effect of diet in this species in an experiment carried out at 18ºC. In the present study the influence of food quantity was evaluated at 14ºC. This temperature seemed to be more suitable according to reproductive cycle in the natural beds and SST pattern. In fact, 14ºC was not a temperature threshold for *C. chione*, in terms of reproductive activity, since gonadal development occurred in natural beds and for all experimental conditions. Temperature is one of the main factors affecting the gametogenic cycle in bivalves and defines the starting point and the rate of gonadal development (LUBET, 1959). In addition, temperature does not appear to be a limiting factor in the reproductive activity of *R. philippinarum* within the range 10-27ºC (TOBA & MIYAMA, 1995; DELGADO & PÉREZ-CAMACHO, 2007) or *Crassostrea gigas* within the range 16-25ºC (CHÁVEZ-VILLALBA *et al.*, 2002). The effect of lower temperature on gametogenesis for both species only caused a slower process of sexual ripening; to a large extent, possibly the result of reduced food intake due to the reduction of temperature.

Concerning the influence of food availability, gonadal development occurred in all trials (T1 and T3:0.10%; T2:0.05%) and maturity was reached. MARTÍNEZ-PITA *et al.* (2011) reported that gonadal developmental stages did not differ between clams fed with a high or low diet at 18ºC. In accordance with this finding our results showed a similar gonadal developmental rate for all experimental conditions. Nevertheless, this gonadal development corresponded to two different energy balances. In T1, when clams were fed with 0.10% (and kept with a sand bed) a significant accumulation of reproductive or somatic tissue (increase of DWs) took place. In T2, with 0.05% diet (and sand bed presence) a moderate increase of DWs was recorded. Whilst in T3, the DWs remained constant until day 30 and afterwards decreased with diet 0.10% (and absence of sand bed). Therefore, T1 and T2 conditions provided clams with a positive energy balance.

Food availability only affected the gonadal extension, as described for the venerid *Ruditapes decussatus* (DELGADO & PÉREZ-CAMACHO, 2003), while clams kept under T3 conditions provoked a negative energetic balance. According to UTTING & MILLICAN (1997) most bivalve species do not need to be maintained in substrate, even though scallops are generally held in sand or gravel. But in our case, a sand bed seemed to benefit the energy condition of *C. chione* in the hatchery. In relation to this, ORTMANN & GRIESHABER (2003) described how the metabolic rate of *Corbicula fluminea* was reduced when valves closure occurred and this enabled the clam to save energy with a depressed metabolism. The presence of sand could help to maintain valves closed or semi-closed and probably reduced the energy expenditure channeled to this effort.

Broodstock conditioning: Branchial infection by intracellular bacteria

Conditioning temperature was reduced (to 14ºC) in an attempt to minimize the proliferation of intracellular bacteria in branchial tissues. Mortality associated with this occurrence, had been observed in a previous experiment carried out by our group on the Catalan coast (DELGADO *et al.*, 2007). In that study, clams were conditioned at 18ºC and without a sand bed, and after 55 days 70% of the population was dead. The new broodstock conditions reduced the mortality rate noticeably compared to the aforementioned. Both variables, lower conditioning temperature (14ºC) and sand bed presence, probably played a beneficial role in the clam's survival since the mortality rate was kept to under 10% throughout the whole experimental period and for all trials tested.

Nevertheless, in the present study husbandry conditions again triggered the proliferation of intracellular bacteria, to a certain extent. *C. chione* individuals from a natural population (at the beginning of the experience) displayed prevalence and mean infection intensity values around 14% and 0.09 intracellular bacteria/pair of gill plica, respectively. As the experiment advanced, prevalence and intensity of infection increased for all trials reaching values of 100% and 9-40 intracellular bacteria/pair of gill plica. Some differences could be detected and after 58 days clams from the T2 trial showed the highest mean value of infection intensity despite the absence of mortalities during the experimental condition period.

It is likely that, a longer experimental period would have allowed for the detection of a higher mortality rate in this trial as the infection level was severe (up to 50 colonies in a pair of gill plica were counted in some individuals). There are precedents of mass mortalities caused by branchial rickettsiae in venerids such as *Venerupis rhomboides* (VILLALBA *et al.*, 1999) with similar infection intensity values; in scallops, *Pecten maximus* (LE GALL *et al.*, 1988) and razor clams, *Siliqua patula* (ELSTON & PEACOCK, 1984). Additionally, it must be noted that the response of the bivalves to infection by branchial intracellular bacteria could influence respiration or food ingestion rates and consequently, determine the amount of energy assimilated by the animal for somatic and reproductive growth, with adverse effects on gonadal development.

The results obtained during this study could be used to improve conditioning procedures for *C. chione*. In general terms, the broodstock conditions here studied improved survival and allowed the clams to reach gonadal maturity at the end of the experiment. Gonadal development took place at 14ºC and after 58 days a high percentage of clams showed signs of gamete emissions. Food availability and sand bed presence benefit gonadal production and the best results were obtained in clams kept at 14ºC, 0.10% diet and sand bed presence (trial T1). On the other hand, our results showed that gonadal development is a priority for this species and occurred even under negative energy balance situations. Nevertheless, further investigations should be undertaken to investigate the intracellular bacteria proliferation in gills and study the effects that other factors, such as pollutants, could have in gill condition. The influence of this pathology on physiological rates, and consequently on gonadal output, should also be studied.

ACKNOWLEDGEMENTS

This study was funded by the Spanish government project "Cultivo de nuevas especies de moluscos bivalvos de interés en hatcheries. JACUMAR, PCM-06-002". The authors thank the staff working in IRTA-Sant Carles de la Rápita; especially to Olga BELLOT for her technical support in carrying out the histological work. We thank Dr. Karl ANDREE and Lesley CREWDSON for the English language revision of the manuscript and Javier GAMERO for his help with image analysis techniques. We are also grateful to the members of the "Cofradía de Pescadores de Arenys de Mar" especially to Mauricio PULIDO.

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Received: 15 December 2013
Accepted: 27 October 2015

Uvjeti mriještenja i razvoja gonada školjke rumenke *Callista chione* (Linnaeus, 1758) (Mekušci: Školjke) na katalonskoj obali (sjevernoistočni dio Španjolske)

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SAŽETAK

Kako bi se odabrali najprikladniji uvjeti za mriještenje vrste *Callista chione*, proveden je eksperiment kojim se pokušao utvrditi gametogeni ciklus prirodne populacije na obali Arenys de Mar (Katalonija, SI Španjolska). Analiziran je utjecaj dostupnosti hrane i prisutnosti pješčanog morskog dna na energetsku ravnotežu i gonadni razvoj, također je praćena proliferacija intracelularne bakterije u škrgama.

Gametogeni ciklus započeo je između kolovoza i rujna. U studenom je većina gonada zrela i mrijest je započeo u prosincu. Veći broj mriještenja školjki dogodio se između siječnja i travnja (uz površinske temperature mora 13-15ºC). Tri eksperimentalna uvjeta su ispitana u rujnu na konstantnoj temperaturi od 14 stupnjeva: T1 (0.10% organske mase algi, udjela žive vage školjki po danu i pješčanog dna); T2 (0,05% pješčano dno); T3 (0.10% bez pješčanog dna). Gonadna zrelost je prioritet za ovu vrstu, a postignuta je na kraju svih ispitivanja. Dostupnost hrane i pješčano morsko dno pogoduju opstanku i razvoju gonada, a najbolji rezultati dobiveni su u školjkama u ispitivanju označenom T1. Ipak, činilo se da uvjeti mriještenja aktiviraju proliferaciju intracelularne bakterije u škrgama, a kako je eksperiment napredovao, učestalost i intenzitet zaraze se respektivno povećao kod svih ispitanih primjeraka dosežući vrijednosti od 100% i između 9-40 intracelularnih bakterija po paru škržnih nabora.

Ključne riječi: *Callista chione*, reprodukcija, temperatura, dostupnost hrane, pijesak, škrge, histopatologija