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Hatchery culture of European clam species (family Veneridae)

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Abstract
Aquaculture and capture production of clam species have great economic value in Europe. The production is dominated by the introduced species, Manila clam, *Ruditapes philippinarum*, which has displaced some of the native species of clams. In Europe, landings of autochthonous clams have decreased in the recent years due to overfishing, failure in recruitment, abiotic stress and diseases. Due to aforementioned factors, seed production in hatcheries has become essential to ensure the sustainability of the fisheries and aquaculture production of European native clams. Many studies have focused on the different steps involved in hatchery rearing, but the volume of seed of native species produced in commercial hatcheries is still relatively low in comparison to the exotic Manila clam. Presently, in European hatcheries, seeds from only two native species, *Ruditapes decussatus* and *Venerupis corrugata*, are produced at industrial scale. The present review analyses the state of hatchery culture of European native clam species, and the aims are to transfer information to the industry and identify critical gaps that might be impeding the development of hatchery production.

Keywords European native clams · Hatchery · Conditioning · Larvae · Seed · Biotic and abiotic parameters

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Abbreviations

AA	Arachidonic acid
CAS	Closed aquaculture system
DHA	Docosahexaenoic acid
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
FTS	Flow-through system
GABA	Gamma-aminobutyric acid
PVC	Polyvinyl chloride
RAS	Recirculation aquaculture system

Introduction

In 2016, the clam market represented 37% of the global bivalve market (mussels, clams, oysters and scallops) and corresponded to $27.6 billion USD (FAO 2018). In 2014, the Manila clam (*Ruditapes philippinarum*) was the most economically important species belonging to the Veneridae family, representing 95% of worldwide production (captures and aquaculture) and reaching a 4,264,274 t harvest (FAO 2014). In Europe alone, the Manila clam represented 54% of Veneridae production in 2014 (FAO 2014). Manila clam was first introduced to European waters between 1972 and 1975, first in France and later in England, Spain and Italy (Flassch and Leborgne 1992). This clam species has shown a high adaptability to various coastal environments (Humphreys et al. 2007), and it has caused competitive displacement of European native clam species such as the grooved carpet shell, *Ruditapes decussatus* (Marin et al. 2003).

Clam production is of great economic interest in Europe. Among the autochthonous Veneridae clams (see Table 1 for the geographical distribution of clam species), the striped venus clam (*Chamelea gallina*) accounted for 64% of clam production in 2014 and is predominantly produced in Italy, Spain, France and Portugal (FAO 2014). The same year, the clam *R. decussatus* was second in terms of production, with 6155 t of capture in Italy, Portugal, Spain and other countries (FAO 2014). The remaining production is composed of the pullet carpet shell (*Venerupis corrugata*), the smooth callista clam (*Callista chione*), the banded carpet shell (*Polititapes rhomboides*), the warty venus clam (*Venus verrucosa*) and the golden carpet shell clam (*Polititapes aureus*) (FAO 2014).

Worldwide, inconsistent seed supply and drastic annual fluctuations of seed recruitment in wild beds are the major challenges the clam production industry faces (Matias et al. 2009). Clam production is greatly affected by anthropogenic actions and climate change (for instance, temperature increase, low salinity, ocean acidification, etc.; Velez et al. 2016) and diseases (mainly caused by bacteria, viruses and protozoa; Carella et al. 2015). Stocks of the European native clam species have decreased in recent years due to various factors, such as overfishing and recruitment failure in *C. gallina* (Joaquim et al. 2016a) and *V. corrugata* (Joaquim et al. 2016c). Harvesting and aquaculture production of *R. decussatus* are limited by low recruitment, adult mortality caused by *Perkinsus* spp. and the high cost of producing spat in commercial hatcheries (Fernández-Reiriz et al. 1999; Matias et al. 2009; Matias 2013). Clam harvesting and aquaculture support thousands of jobs and numerous companies in coastal areas, representing a source of sustainable local development. Hatchery production of autochthonous clam seed, therefore, is essential to sustain and increase aquaculture, and subsequent capture, of these species.

Table 1 Geographical distribution and type of production in hatcheries of European native clam species

Species	Geographical distribution	Type of production in hatcheries
Ruditapes decussatus	From Southern and Western England to the Iberian Peninsula and into the Mediterranean. South to western Morocco and Senegal, West Africa[a]	Commercial
Venerupis corrugata	From Northern Norway to the Iberian Peninsula and into the Mediterranean. North-West Africa[b]	Commercial
Polititapes rhomboides	From Norway to the Iberian Peninsula and into the Mediterranean. Morocco[c]	Experimental
Polititapes aureus	From Norway to the Iberian Peninsula and into the Mediterranean and the Black Sea. Morocco[c]	Experimental
Callista chione	From Southern British Island to the Iberian Peninsula, Morocco, Canary Islands and the Azores. Throughout the Mediterranean[c]	Experimental
Chamelea gallina	From Northern Norway to Morocco[c]	Experimental
Venus verrucosa	From the British Islands to South Africa. Throughout the Mediterranean and in the Northern Red Sea[c]	Experimental

[a] Poppe and Goto (1991)

[b] Hayward and Ryland (1995)

[c] Carpenter and De Angelis (2016)

Manila clam is the primary clam species produced in European hatcheries due to the simplicity of its production, fast growth rate and high market demand (Robert et al. 2013). Currently, it is produced in commercial hatcheries in France, United Kingdom, Spain, Netherlands and Ireland (Robert 2009; Robert et al. 2013). Among autochthonous species, only *R. decussatus*, in Spain and Netherlands, and *V. corrugata* in Spain are produced in commercial hatcheries in Europe (Robert et al. 2013) (Table 1). Despite the high market demand and high commercial value of these local species, the volume of seed produced is quite low compared to the Manila clam.

Due to its significance in worldwide production, Manila clam represents the largest proportion of all clams reported in the scientific literature. Important knowledge of native clam cultivation, however, has been generated over the last 40 years. Pioneering work concerning the Veneridae family was carried out by Pérez-Camacho et al. (1977) in Spain with regard to the species *R. decussatus* and *V. corrugata.* Unfortunately, since then, most of the available information comes from grey literature (MsC and PhD theses, conference abstracts, reports, etc.) and gaining access to these works tends to be difficult. This information must be readily available to encourage further research in hatchery culture of native clam species. Such access will promote technological development for production in hatcheries at a commercial scale. Therefore, this review aims to (i) compile the available information about hatchery culture of different species of native clams belonging to Veneridae family, (ii) identify areas for improvement of hatchery production yields and (iii) propose new areas with potential for refinement in hatchery rearing protocol.

Broodstock maintenance and conditioning

Broodstock conditioning, preparing shellfish for spawning, is a crucial step in the operation of bivalve hatcheries. Artificial conditioning allows hatcheries to extend their production season,

reducing their reliance on the period of the year when wild beds become naturally mature to spawn. The aim of broodstock conditioning is to achieve maximum fecundity of adults whilst preserving the quality and viability of the gametes (Utting and Millican 1997) as well as the overall health of the bivalve.

Alternatively, wild populations of *V. corrugata* and *P. rhomboides* sampled in Galicia (NW Spain) between 2005 and 2010 exhibited the ability to produce mature gametes year-round (Cerviño-Otero 2011; Cerviño-Otero et al. 2012). If proven to be an annual occurrence, this unique characteristic means that conditioning is not needed in these species, and larvae may be obtained relatively easily in the hatchery throughout the year.

Inadequate conditions lead to a reduction of gamete quality and quantity; therefore, maintaining optimal conditions for broodstock, to minimize stress, must be ensured (Ojea et al. 2008). In bivalves, glycogen and total lipids represent the majority of energy reserves used for gametogenesis, and their variation is linked to the gonad development stage (Ojea et al. 2004; Matias et al. 2016). Maximum glycogen content in bivalves immediately precedes gamete proliferation and is followed by a decrease as gametogenesis advances, reaching minimum values during the spawning period (Ojea et al. 2004). Exhibiting an opposite trend, *R. decussatus* total lipids tend to increase during the course of gametogenesis. This trend is supported by de novo lipid synthesis from stored glycogen (Matias et al. 2016).

Tank volumes typically vary from 20 to 200 L and broodstock is held in flow-through systems (FTS) with a seawater flow of 0.5–1 L min^{-1} per kg of clam biomass (Ruiz-Azcona 2013). Ojea et al. (2007) observed that a renewal flow of 1/5 of the tank volume per hour was most advantageous during *R. decussatus* conditioning. Most clam species do not need to be maintained in substrate (Ruiz-Azcona 2013). One exception can be found in the razor clams, *Solen marginatus*, *Ensis magnus* and *Ensis siliqua*, where substrate is required for burrowing and, consequently, to prevent shell gaping (da Costa and Martínez-Patiño 2009; da Costa et al. 2010, 2011a). Another benefit of substrate use was observed in the smooth callista clam, *C. chione*, where reduced mortality was observed in broodstock buried in sand compared to traditional broodstock maintenance systems, such as trays without substrate (Martínez-Pita et al. 2008). In addition, the use of a sand bed during conditioning benefitted the energy condition of the broodstock, and thus, time to maturation was improved and higher DW of the gonad was observed (Delgado et al. 2016). During clam conditioning without substrate, broodstock are suspended off-bottom in a mesh tray with large apertures in the base so as not to retain faeces and detritus to leave the system (Ruiz-Azcona 2013). Other broodstock holding methods include the use of net bags suspended from PVC rods in broodstock tanks or small volume (2 L) tubes with inverted seawater flow (Ruiz-Azcona 2013). Both systems allow clams to reduce the energetic costs of closing their valves, allowing them to allocate more energy for reproduction, and thus reduce the time necessary for conditioning (Ruiz-Azcona 2013).

For biosecurity reasons, at the outlet of broodstock tanks, sieves should be installed to collect embryos and larvae in case of spontaneous spawning (Ruiz-Azcona 2013). For instance, partial spawning is a common phenomenon observed during *R. decussatus* conditioning since this clam species releases ripe oocytes regularly once a certain level of gonadal development has been reached (Hamida et al. 2004b; Delgado and Pérez-Camacho 2005, 2007; Ojea et al. 2008).

Broodstock maturation is controlled by both endogenous and exogenous factors, such as temperature, salinity, photoperiod and food quantity and quality (Matias et al. 2016). Temperature and food appear to be the most important of these factors (Matias et al. 2016). The

geographic origin of the broodstock is another factor to be considered when selecting broodstock for conditioning in the hatchery (Matias et al. 2009).

The time necessary to artificially bring broodstock to desirable spawning condition depends on the initial gametogenic stage at the onset of conditioning and is affected by the initial temperature and food quality and quantity (Delgado and Pérez-Camacho 2003).

Beginning conditioning when the gonad is in resting stage, for instance, during the fall season, the amount of time needed to effectively condition and spawn *R. decussatus* broodstock was 14 weeks. During the winter and spring seasons, when the gonad stage was more advanced, it took to only 6–7 weeks to reach spawning condition (Ojea et al. 2008). The increased effectiveness of conditioning during the winter months was also confirmed by a study done by Matias et al. (2009).

Temperature

The rate of gonad development is directly related to the increase in temperature, up to a certain temperature threshold, depending on the species (Delgado and Perez-Camacho 2007; Matias et al. 2016). Most of the authors obtained favourable conditioning results for *R. decussatus* with temperatures ranging from 18 to 20 °C (Table 2; Delgado and Perez-Camacho 2007; Ojea et al. 2008; Matias et al. 2009, 2016). Although initiation of gonadogenesis and proliferation of germ cells was reported at temperatures as low as 10 °C, complete gametogenesis did not occur, and ripening of oocytes was not achieved (Blanco 2010). The clam *R. decussatus* can be brought to maturity using artificial conditioning even at 14 °C, though more than 2 months are required for maturation in winter (Delgado and Perez-Camacho 2007). Drastic changes between temperature in wild beds, at the time of broodstock collection, compared to the water temperature in the hatchery may lead to stress and induce high mortality in adults (Ojea et al. 2008). These sudden changes in temperature may also distort cycles of nutrient storage and use of energy reserves, thereby negatively impacting gonad maturation and gamete quality (Ojea et al. 2008). For instance, Ojea et al. (2008) observed that *R. decussatus* broodstock conditioning mortality was reduced by gradually increasing the temperature until reaching the optimal temperature for conditioning (20 °C). A higher condition index was observed in *R. decussatus* clams which underwent a gradual increase of temperature compared to those that faced a constant temperature regime (Ojea et al. 2008). Other studies have shown the same relationship between condition index increments and gonad development (Hamida et al. 2004b; Ojea et al. 2004) suggesting that gradual temperature change is preferable. Moreover, clams conditioned using this gradual increase in temperature regime produced larvae with higher survival through metamorphosis (35%) than larvae obtained from broodstock conditioned at a constant temperature (22%; Ojea et al. 2008).

In *R. decussatus*, optimal conditioning temperature was dependent upon the geographical origin of the broodstock. Matias et al. (2009) reported that *R. decussatus* from the northern Iberian Peninsula required an optimal conditioning temperature of 20 °C, as opposed to broodstock from the southern Peninsula, which were best conditioned at 22 °C (Table 2; Matias et al. 2009).

Food quantity and quality

Food availability and quality have significant effects on bivalve maturation and influence both the gonad development rate and the quality of the gametes produced

Table 2 Broodstock conditioning

Species	Season	Photoperiod	Temperature	Microalgae	Daily ration per clam	Best conditions and time necessary to obtain ripe broodstock	Reference
Ruditapes decussatus	Winter	NC	14 °C; 18 °C	*Tisochrysis lutea*	0.5% algae DW/clam LW	18 °C (57 days)	Delgado and Pérez-Camacho (2007)
R. decussatus	Autumn, winter and spring	NC	20 °C-CT; 20 °C-GT	*T. lutea* + *Diacronema lutheri* + *Skeletonema marinoi* + *Chaetoceros calcitrans* + *Tetraselmis suecica*	4% of meat DW/DW of algae	Spring, 20 °C-GT (337.5 D°)	Ojea et al. (2008)
R. decussatus	nd	NC	14 °C; 18 °C; 22 °C	*T. lutea* + *Tetraselmis chuii*	0.05 mg OW of food/LW of clam per hour	22 °C (41 days)	Blanco (2010)
R. decussatus	Autumn and winter	NC	18 °C; 20 °C; 22 °C	*T. lutea* + *C. calcitrans*	4% of meat DW/DW of algae	Winter, 20 °C for northern and 22 °C for southern population (11 weeks)	Matias et al. (2009)
R. decussatus	Autumn	NC	20 °C-CT; 20 °C-GT	*T. lutea* + *D. lutheri* + *S. marinoi* + *C. calcitrans* + *T. suecica*	LR: 3%; HR: 6% of meat DW/DW of algae	20 °C-CT and 6% ration (8 weeks)	Ojea (2013)
R. decussatus	Winter	NC	20 °C; 22 °C	Unfed; *T. lutea*; *C. calcitrans*;	4% of meat DW/DW of algae	20 °C and *T. lutea* + *C. calcitran* (8 weeks)*s*	Matias et al. (2016)
R. decussatus	Winter	NC	22 °C-GT	Unfed; *C. calcitrans*; *T. suecica*; *Nannochloropsis oculata*; *C. calcitrans* + *N. oculata*; *C. calcitrans* + *T. suecica*; *T. suecica* + *N. oculata*;	1% algae DW/clam LW	*C. calcitrans* and *C. calcitrans* + *T. Suecica* (34 days)	Abbas et al. (2018)
R. decussatus	nd	NC	18 °C	*T. lutea*	1. 2.5%; 2. 5.0% and 3. 10.0%. Percentage of food OW as a proportion of the clam LW	Rations 5% and 10% (35 days)	Delgado et al. (2004)
R. decussatus	nd	NC	18 °C	*T. lutea*	0.96%; 0.48%; 0.24%; 0.05%; 0.10%; 0.025%. Percentage of food OW as a proportion of the clam LW	Rations 0.96% and 0.48% (46 days)	Delgado and Pérez-Camacho (2003); Pérez-Camacho et al. (2003)

Table 2 (continued)

Species	Season	Photoperiod	Temperature	Microalgae	Daily ration per clam	Best conditions and time necessary to obtain ripe broodstock	Reference
R. decussatus	Autumn and spring	8 h light/16 h darkness; 16 h light/8 h darkness	19 °C	*T. lutea* + *D. lutheri* + *S. marinoi* + *C. calcitrans* + *Phaeodactylum tricornutum* + *T. suecica*	5% of meat DW/DW of algae	Spring, 16 h light/8 h darkness (less than 30 days)	Pazos et al. (2003)
R. decussatus	Winter	8 h light/16 h darkness; 16 h light/8 h darkness	19 °C	*T. lutea* + *D. lutheri* + *S. marinoi* + *C. calcitrans* + *T. suecica*	6% and 9% of meat DW/DW of algae	9% ration and 16 h light/8 h darkness (less than 45 days)	Martínez et al. (2005)
Venus verrucosa	Summer	nd	nd	*Cylindrotheca closterium*; *Chaetoceros muelleri*; *T. suecica* + *Isochrysis galbana* + *C. closterium*; *T. suecica* + *I. galbana* + *C. muelleri*	3% of meat DW/DW of algae	*T. suecica* + *Isochrysis galbana* + *C. closterium*	Gavrilovic et al. (2010)
Callista chione	Spring	NC	18 °C	*T. suecica* + *Chaetoceros* sp. + *I. galbana*	3% and 6% of meat DW/DW of algae	6% ration	Martínez-Pita et al. (2011)

CT constant temperature, *GT* gradual temperature, *DD°* degree-days, *DW* dry weight, *LW* live weight, *NC* natural conditions, *nd* no data, *OW* organic weight

(Utting and Millican 1997). Delgado and Pérez-Camacho (2003) and Pérez-Camacho et al. (2003) investigated the effect of food ratio and energy balance on the sexual maturation of *R. decussatus*. The authors fed clams daily food rations of *Tisochrysis lutea* (=*Isochrysis* affinis *galbana* clone T-Iso) with 0.025, 0.05, 0.10, 0.24, 0.48 and 0.96 organic dry weight, supplied as a proportion of the live weight of the clams (Table 2). The results showed that gonad developed under all energy balance conditions, however, less so under negative energy balance.

Another study showed that for *R. decussatus*, optimal conditioning was obtained with a mixed diet of *T. lutea* + *Diacronema lutheri* + *Skeletonema marinoi* + *Chaetoceros calcitrans* + *Tetraselmis suecica* with a ratio of 6% of dry flesh weight in dry weight of food per day (Table 2; Ojea 2013). Similarly, in *C. chione* broodstock, the same diet in a 6% dry weight ration produced higher condition index and speed up maturation when compared to a 3% dry weight ration (Martínez-Pita et al. 2011). Delgado et al. (2004) also investigated food effects in *R. decussatus* conditioning with a ration of 2.5, 5.0, or 10.0% of the organic weight of food (*T. lutea)* supplied as a proportion of the live weight of clams. They found that after 35 days of conditioning, high rations of 5 and 10% showed similar results regarding on gonad ripeness.

Matias et al. (2016) showed that for *R. decussatus* conditioning, the bi-specific diet composed by *T. lutea* and *C. calcitrans* promoted higher condition index, greater gonad development and increased fecundity and spawning success than mono-specific diets of *T. lutea* or *C. calcitrans* (Table 2). More recently, it was shown that *R. decussatus* broodstock matured faster and had a higher response to spawning induction when fed *C. calcitrans* in a mono-specific diet or when fed using a bi-specific diet of *C. calcitrans* and *T. suecica* (Table 2; Abbas et al. 2018). The saltwater microalgae *Nannochloropsis oculata* has poor food value to *R. decussatus* broodstock (Abbas et al. 2018), perhaps due to the low digestibility of its hard cell walls (Payne and Rippingale 2000). In a previous study, it was deduced that the diatom *Cylindrotheca closterium* could enhance the nutritive value of *Isochrysis galbana* and *T. suecica* when added to this multi-species diet during the conditioning of *V. verrucosa* (Table 2; Gavrilovic et al. 2010).

Photoperiod

Despite having a significant effect on gametogenic cycles and maturation in clams (Pazos et al. 2003), literature regarding the effect of photoperiod on clam conditioning is scarce. In *R. decussatus*, the use of a summer photoperiod regime (16 h light/8 h darkness) allowed researchers to obtain sexually mature clams after 75 days in autumn (Table 2; Pazos et al. 2003). Meanwhile, the same clams held in the same conditions but under a winter photoperiod regime (8 h light/16 h darkness) never progressed past the early gametogenic stages. During another experiment, held in the spring months, the summer photoperiod regime again accelerated maturation when compared to the winter photoperiod regime (Pazos et al. 2003). In a later study of *R. decussatus*, it was established that a summer conditioning regime (16 h light/8 h darkness) yielded greater gonad development than the winter photoperiod regime (8 h light/16 h darkness) when combined with a high feed ration (9%) (Table 2; Martínez et al. 2005).

Table 3 Spawning induction methods in Veneridae

Spawning method	Species	Details of the method	Individuals spawned	Eggs per female (millions)	References
Thermal shock	*Ruditapes decussatus*	Temperature rise up to 28 °C. Addition of sperm after 1 h. Duration 3 h.	35%	1.0	Pérez-Camacho et al. (1977)
	Ruditapes decussatus	Clams kept dry at 4 °C for 12 h. Thermal shock (14 to 25 °C). Addition of microalgae and gonad extracts.	18 females out of 79 individuals	1.8	Aranda-Burgos et al. (2014b)
	Ruditapes decussatus	Temperature rise from 20 to 22 °C up to 28 °C. Duration 6 h.	36–67%	0.25–0.87	Matias et al. (2016)
	Ruditapes decussatus	Temperature rise from 20 to 22 °C up to 28 °C. Duration 6 h.	30–60%	0.32–0.77	Abbas et al. (2018)
	Venerupis corrugata	Temperature rise up to 20–22 °C. Addition of sperm after 1 h. Duration 3 h.	23%	2.3	Pérez-Camacho et al. (1977)
	Venerupis corrugata	Temperature rising from 12 to 20 °C. Cycles of 2 h each. Few periods of dryness. Addition of sperm.	–	0.31–0.67	Cerviño-Otero (2011)
	Polititapes aureus	Temperature rising from 15 to 25 °C. Cycles of 30–40 min each. Addition of microalgae.	17 males and 15 females out of 46 individuals (70%)	1.1–2.1	Pereira (2011)
	Venus verrucosa	Temperature rising from 18 to 30 °C. Cycles of 1 h each.	57%	–	Gavrilovic et al. (2009)
Exposure to air	*Venus verrucosa*	Broodstock exposed to dryness for 18–20 h. Then, once in the seawater gradual increase of temperature up to 24 °C.	–	1–1.5	Royo and Gómez Ramblado (2002).
Open circuit	*Chamelea gallina*	Clams held at 22 ± 1 °C in flow-through with a renewal of 20% per h with aeration.	–	–	Joaquim et al. (2016a)
Injection of serotonin	*Ruditapes decussatus*	Injection in the gonad of 0.2 to 0.4 mL of serotonin at concentrations of 0.08 and 0.8 mg cm^{-3}.	–	–	Pérez-Camacho and Román (1987)
	Venus verrucosa	Injection in the adductor muscle of 0.2–0.3 mL of serotonin at a concentration of 0.2, 2 and 4 mM.	7–16%	0.27	Turolla and Rossi (2004)
	Venus verrucosa	Injection in the gonad of 0.3 mL of serotonin at a concentration of 2 mM.	47%	–	Gavrilovic et al. (2009)

Microbiological aspects of broodstock conditioning

Broodstock are one of the main bacterial sources to larval cultures due to the vertical transmission of bacteria from adults to larvae (Schulze et al. 2006). Bivalve broodstock brought into a hatchery are usually cleaned and brushed prior to spawning induction as routine precautionary measure (Helm and Bourne 2004). Broodstock microbiota may include opportunistic pathogens that are harmless to broodstock but harmful to larvae, such as larval pathogens belonging to the genus *Vibrio*. Such *Vibrio* spp. have been isolated from the gonad of broodstock held in shellfish hatcheries (Prado et al. 2014). For instance, Prado et al. (2013) showed that *R. decussatus* and *V. corrugata* broodstock acted as reservoirs of *Vibrio* spp. that were subsequently transferred to gametes during spawning. In bivalve hatcheries, antibiotics are frequently used as a way to reduce the bacterial load in broodstock or larvae (Medhioub et al. 2017). In Europe, chloramphenicol was banned for use in the rearing of animals for human consumption, including aquaculture (EC 1994); however, it has been used for research purposes in experimental hatcheries (Dubert et al. 2016c). The prophylactic use of chloramphenicol for 4 days in experimental cultures (8 mg L^{-1} day^{-1}) of broodstock, combined with larval rearing in a FTS, eliminated the need to treat larvae with antibiotics, significantly reducing vibrio levels and allowing larvae to successfully settle (Mechri et al. 2012). Similarly, a 5-day treatment of florfenicol (8 mg L^{-1} day^{-1}) was effective in reducing *Vibrio* spp. and marine heterotrophic bacteria of *R. decussatus* broodstock (Medhioub et al. 2017). By using the aforementioned procedure, authors obtained *R. decussatus* fertilized eggs free from any *Vibrio* contamination. The use of antibiotics to reduce bacterial load of broodstock represents an additional expense for hatcheries and may have limited effectiveness for ubiquitous pathogens or be a source of resistant bacteria proliferation in the natural surroundings of the hatcheries (Berthe 2005; Dubert et al. 2016c).

Due to the drawbacks of antibiotics, it may be advisable for hatchery operators to reducing bacterial loads in broodstock, and its subsequent vertical transmission to larvae, by alternative means (Dubert et al. 2016a). In fact, Dubert et al. (2016a) developed a conditioning protocol for *R. decussatus* which reduced the *Vibrio* load in broodstock and obtained the best gonadal development (i.e. higher gonad developmental stage using histological techniques) compared to other treatments. This alternative method relied upon conditioning *R. decussatus* broodstock from Galicia (NW Spain) in May, at 20 °C, without food supply for 2 weeks, followed by 2 weeks of feeding before spawning induction.

Spawning induction

Spawning induction allows hatchery workers to carry out controlled fertilization and plan the timing and resource allocation needed for larval culture production in the hatchery (Joaquim et al. 2008). The lack of efficient spawning induction protocols may hinder the production of bivalves in hatcheries (Mouëza et al. 1999).

Thermal shock is a widely used spawning technique for clams, which consists of exposing ripe broodstock to rapid water temperature change, favouring gamete release (Ruiz-Azcona 2013). Temperature differences between cool and warm water cycles may exceed 10 °C and usually increase from 10 °C up to 28 °C, depending on the species (Ruiz-Azcona 2013). The duration of cool and warm water cycles usually varies from 20 to 60 min, depending on the species, degree of ripeness and aquaculturist preference. The number of cool/warm cycles

Table 4 Embryonic and larval development of native species of Veneridae family in Europe

Species	Eggs	D-larvae		Pediveliger			Postlarvae			Temperature (°C)	Reference
	L (μm)	L (μm)	S (%)	L (μm)	S (%)	Day	L (μm)	S (%)	Day		
Ruditapes decussatus	62–68	–	–	–	–	–	205–207	9–40	30	20 °C	Ojea et al. (2008)
Venerupis corrugata	65–75	100–105	84	210–250	24–87	16–18	300–350	56[a]	22–25	18 °C	Cerviño-Otero (2011)
Venerupis corrugata	–	–	–	200–240	40	15–24	300–400	80[a]	27–36	17–20 °C	Costa (2018)
Polititapes rhomboides	70	95	10–35	200–250	15–73	20–25	300–350	60[a]	30–35	19 °C	Cerviño-Otero et al. (2011)
Polititapes aureus	71–75	106	74	182–193	2–4	15	–	–	–	20 °C	Pereira (2011)
Polititapes aureus	–	–	–	201	60	14–16	375	30[a]	28–35	17–20 °C	Costa et al. (2017)
Callista chione	–	119	–	245	31.9	25	404	7	27	19 °C	Delgado et al. (2008)
Callista chione	89.5	–	–	209–213	–	32	–	2–8	50	19 °C and 23 °C	Pérez-Larruscain et al. (2011)
Chamelea gallina	–	–	56	–	–	8–10	–	8–24	13	20 °C and 23 °C	Joaquim et al. (2016a)
Venus verrucosa	78.9	–	–	220–230	–	17	270–310	–	22–24	23 °C	Turolla and Rossi (2004)
Venus verrucosa	–	80	–	209	0–22	16–19	–	–	–	20 °C	Royo and Gómez Ramblado (2002)

L length, *S* survival, "–" no data

[a] Metamorphosis rate

required to induce spawning, and the temperature gradient to which broodstock is exposed, both depend on the species and the readiness of broodstock to spawn. This methodology has been successfully used for spawning *V. verrucosa*, *V. corrugata*, *P. aureus* and *R. decussatus* (Gavrilovic et al. 2009; Cerviño-Otero 2011; Pereira 2011; Ruiz-Azcona 2013) (Table 3). The thermal stimulation technique is often combined with the use of other stimulants such as microalgae, to activate filtration, or gametes (mainly sperm) (Cerviño-Otero 2011; Aranda-Burgos et al. 2014b).

To increase the efficiency of spawning induction by thermal shock, some hatchery operators may keep clams out of the water, at room temperature (18–20 °C), for periods from 1 h up to overnight (Ruiz-Azcona 2013). Clams may also be exposed to air at 4–6 °C, which speeds up gamete release once thermal shock induction starts; however, the number of clams responding to spawning induction is reduced (Ruiz-Azcona 2013). These pre-spawning induction treatments have been shown to have no negative effect on the subsequent larval development (Ruiz-Azcona 2013). For example, exposure to air for 18–20 h successfully induced gamete release in *V. verrucosa* and led to successful larval cultures (Royo and Gómez Ramblado 2002).

Gamete and thermal shock stimulation were tested to induce spawning in *C. gallina* with negative results (Joaquim et al. 2016a). The authors intended to maintain *C. gallina* broodstock in a FTS at 22 °C for three consecutive days, but after 24 h, uncontrolled spawning in the tank yielded fertilized embryos.

Serotonin (5-hydroxy tryptamin), which is a natural neurotransmitter present in the bivalve nervous system, can also be used as a spawning inducer (Table 3; Joaquim et al. 2008). Clams can be injected in either the gonad or the anterior adductor muscle (Joaquim et al. 2008). Serotonin injection showed positive results in *R. decussatus* and *V. verrucosa*, triggering a spawning response from the ripe individuals within a few minutes (Pérez-Camacho and Román 1987; Gavrilovic et al. 2009). This stimulus, however, is intense and frequently provokes the emission of immature oocytes and fragments of the gonad (Pérez-Camacho and Román 1987). Such is the intensity of the reaction in *V. verrucosa* that adults injected with serotonin may shut their valves without withdrawing the foot or siphons, cutting them off (Turolla and Rossi 2004). Serotonin was used to activate *R. decussatus* oocytes obtained by stripping, which are blocked at prophase I, and cannot be fertilized (Hamida et al. 2004a). The results were unsatisfactory, however, because both the percentage of fertilized oocytes obtained through stripping and the percentage development to D-shaped larvae were very low (Hamida et al. 2004a).

Larval culture

Standard embryological and larval development

In the 1970s, embryos and larvae of *V. corrugata* and *R. decussatus* were first described using light microscopy (Pérez-Camacho and Román-Cabello 1973; Pérez-Camacho et al. 1977). Recently, a more detailed description of embryological and larval stages of these species using light microscopy, scanning electron microscopy and transmission electron microscopy was published (Cerviño-Otero 2011; Aranda-Burgos et al. 2014b). Egg diameter ranges between 65 and 75 μm for most species, except for *C. chione* which has substantially larger eggs, averaging 90 μm (Table 4). Comparisons of larval survival rates are difficult since some of the

works provided survival data based on different life stages. There is a general lack of data regarding the survival rate of the D-larval stage (Table 4); one of the few published studies indicates high variability and low survival (10–35%) in *P. rhomboides* (Cerviño-Otero et al. 2011). For most of the clam species included in this review, the larval development duration is around 15–20 days (Table 4). The shortest larval development duration was observed for *C. gallina*, lasting 8 to 10 days when maintained at 20 °C and 23 °C, respectively (Joaquim et al. 2016a), and the longest was observed for *C. chione*, lasting from 25 to 32 days, at 19 °C and 23 °C, respectively (Delgado et al. 2008; Pérez-Larruscain et al. 2011).

Incubation studies

The effect of temperature on the incubation of *V. corrugata* and *R. decussatus* larvae was investigated by Pérez-Camacho et al. (1977). Laboratory-scale incubations were carried out using 2-L beakers, filled with 1-μm filtered seawater treated with UV and antibiotics. Among temperatures tested (14, 18, 22, 26, and 30 °C) for *V. corrugata* egg incubation, the best results were produced at 14, 18 and 22 °C which yielded more than 94% normal D-shaped larvae (Pérez-Camacho et al. 1977). At 26 and 30 °C, the percentage of normal D-shaped larvae decreased below 12% (Pérez-Camacho et al. 1977). Pérez-Camacho et al. (1977) proposed optimal stocking densities of either 500 or 1000 eggs per cm^{-2} in *V. corrugata*, and these densities yielded normal larvae hatching at a rate of 35–44%. A stocking density up to 10,000 eggs cm^{-2} also produced *V. corrugata* larvae with satisfactory results (hatching rate of 29%). Using the same stocking protocols developed for *V. corrugata*, these authors obtained 26% normal D-shaped larvae of *R. decussatus* (Pérez Camacho et al. 1977).

Effect of temperature and stocking density

Temperature is one major factors affecting growth and survival of bivalve larvae (Joaquim et al. 2008). The duration of larval period decreases with increasing temperature, if kept below the critical lethal temperature, which is species-specific (Perez-Camacho et al. 1977). According to Perez-Camacho et al. (1977), rearing temperatures of 18, 22 and 26 °C resulted in the highest growth rate (6–9 μm day^{-1}, with maximal rate obtained at 26 °C). At these three different temperatures, mortality remained below 5% (Perez-Camacho et al. 1977). The effect of temperature on late larval development and metamorphosis, however, was not investigated in this study. Alternatively, *R. decussatus* larvae did not fare well at high temperatures, 22 or 28 °C, exhibiting high mortality in the first week of larval development (Beiras et al. 1994). Subsequently, other authors used lower temperatures, such as 18–20 °C, in both species, when working in the development of rearing protocols in hatcheries (Cerviño-Otero 2011; Matias et al. 2011; Aranda-Burgos et al. 2014b; Joaquim et al. 2016c). While high temperature waters are prone to bacterial outbreaks, the benefit of using lower temperatures is the lower risk of bacterial contamination and mortality outbreaks during larval culture (Joaquim et al. 2008).

Pérez-Larruscain et al. (2011) studied larval development and growth of the smooth clam *C. chione* under two different temperature regimes (19 and 23 °C). They concluded that 19 °C was the best rearing temperature for larvae in terms of growth, reaching 8% survival (2% at 23 °C). A significant difference in length was observed at day 11, however, where larvae

reared at 23 °C reached a length of 175.15 μm and larvae reared at 23 °C reached a length of 137.88 μm (Pérez-Larruscain et al. 2011).

In *C. gallina*, the combined effects of two different temperatures (20 and 23 °C) and three feeding regimes (*T. lutea*, *C. calcitrans* and unfed) on larval growth and survival over the course of 13 culture days (ending with pediveliger stage) was studied by Joaquim et al. (2016a). In this study, it was observed that only temperature significantly affected growth of larvae fed with *T. lutea*, yielding higher growth at 23 °C compared to 20 °C (Joaquim et al. 2016a).

Culture density impacted larval growth and larval stage duration of *V. corrugata* larvae (Pérez-Camacho et al. 1977). It was deduced that optimal density at 20 °C for larval rearing was 5 and 10 larvae ml^{-1}, with larval cultures lasting 20 and 27 days, respectively, and with postlarval yields not differing between densities, ranging between 70 and 80% (Pérez-Camacho et al. 1977). These authors also investigated the effect of larval density on *R. decussatus*, obtaining similar results, suggesting that larva of this species should be reared between 5 and 10 larvae ml^{-1}.

Larval rearing systems in clams

Batch larval culture or closed aquaculture system (CAS) is the most employed method for rearing larval clams (Pérez-Camacho et al. 1977; Joaquim et al. 2016c; Medhioub et al. 2017). For all the advantages that this method can confer, there are also disadvantages. For example, it is labour-intensive, costly, the manipulation involves mechanical stress (sieving), larvae are subjected to water quality changes in each water renewal and microbial contamination risk and disease transmission are high (Joaquim et al. 2016c; Medhioub et al. 2017).

Other culture methods have been developed in recent years, such as FTS and recirculation aquaculture systems (RAS). Larval rearing in a FTS, where regular replacement of water prevents oxygen deficiency and the build-up of organic matter, and allows for a constant food density in culture tanks (da Costa et al. 2015). The use of FTS allows other advantages such as reduced labour demand, high larval stocking density, the minimization of space requirements and improved larval development and metamorphosis (Rico-Villa et al. 2008). Literature investigations of FTS performance with clams are scarce. Medhioub et al. (2017) compared larval performances and bacterial load of *R. decussatus* larvae reared in a CAS at 5 larvae ml^{-1} to FTS at 25 larvae ml^{-1}. When broodstock was treated with florfenicol prior to spawning, they observed similar larval growth in larvae reared in CAS treated with florfenicol and in larvae reared in FTS without addition of antibiotics. Larval survival, however, was slightly higher in larvae reared in CAS with florfenicol compared to those larvae maintained in FTS (Medhioub et al. 2017). Nonetheless, FTS may be a good alternative to increase larval density and to eliminate the need for antibiotic treatments during larval rearing. In other bivalve species, the use of FTS is recommended as an efficient system for larval production at a commercial scale, such as with the mussel *Perna perna* (densities of 45–80 larvae ml^{-1}; Turini et al. 2014) or *Crassostrea gigas* (densities of up to 150 larvae ml^{-1}; Asmani et al. 2017).

The use of RAS consists of a FTS linked into a recirculation system where water is treated and reused (Joaquim et al. 2016c). It may reduce energy cost associated with heating water and provides a higher quality control of the culture (Joaquim et al. 2016c); however, the equilibrium between the physical, chemical and microbiological parameters of water must be maintained in the system to ensure that optimal conditions for larval development are maintained (Joaquim et al. 2016c). Joaquim et al. (2016c) investigated the effect of stocking

Table 5 Nutritional studies in clam larvae

Species	Temperature	Algal species	Rations	Growth rate (μm day^{-1})	Survival (%)	Period	Reference
Ruditapes decussatus	22 °C	*Tisochrysis lutea*	50 cells μl^{-1}	4.69	67.00	Days 2–23	Matias et al. (2011)
R. decussatus	22 °C	*Chaetoceros calcitrans*	50 cells μl^{-1}	4.95	71.98	Days 2–23	Matias et al. (2011)
R. decussatus	22 °C	Unfed	–	0.33	23.21	Days 2–23	Matias et al. (2011)
R. decussatus	21 °C	*Isochrysis galbana* + *Diacronema lutheri* + *Chaetoceros muelleri* (1:1:1)	From 40 to 70 cells μl^{-1}	6.9	27	Days 2–22	Aranda-Burgos et al. (2014a)
R. decussatus	21 °C	*I. galbana* + *D. lutheri* + *C. muelleri* (1:1:2)	From 40 to 70 cells μl^{-1}	6.7	32	Days 2–22	Aranda-Burgos et al. (2014a)
R. decussatus	21 °C	*I. galbana* + *D. lutheri* (1:1)	From 40 to 70 cells μl^{-1}	5.9	8	Days 2–22	Aranda-Burgos et al. (2014a)
R. decussatus	21 °C	*C. muelleri*	From 40 to 70 cells μl^{-1}	7.3	25	Days 2–22	Aranda-Burgos et al. (2014a)
R. decussatus	22 °C	*T. lutea*	100 cells μl^{-1}	4.51	~30	Days 2–21	Matias et al. (2015)
R. decussatus	22 °C	*C. calcitrans*	100 cells μl^{-1}	3.54	~20	Days 2–21	Matias et al. (2015)
R. decussatus	22 °C	*T. lutea* (T) + *C. calcitrans* (C)	50 cells μl^{-1} (T) + 50 cells μl^{-1} (C)	4.05	~32	Days 2–21	Matias et al. (2015)
R. decussatus	22 °C	*T. lutea* (T) + *C. calcitrans* (C)	60 cells μl^{-1} (T) + 40 cells μl^{-1} (C)	5.52	48	Days 2–21	Matias et al. (2015)
R. decussatus	22 °C	*T. lutea* (T) + *C. calcitrans* (C)	40 cells μl^{-1} (T) + 60 cells μl^{-1} (C)	4.13	~18	Days 2–21	Matias et al. (2015)
R. decussatus	22 °C	Unfed	–	0.37	10	Days 2–21	Matias et al. (2015)
Venerupis corrugata	18 °C	*T. lutea*	100 cells μl^{-1}	6.33	~95	Days 11–22	Fernández-Reiriz et al. (2011)
V. corrugata	18 °C	*Tetraselmis suecica*	10 cells μl^{-1}	4.84	~60	Days 11–22	Fernández-Reiriz et al. (2011)
V. corrugata	18 °C	*T. lutea* + *T. suecica*	*T. lutea* (50 cells μl^{-1}) + *T. suecica* (5 cells μl^{-1})	5.88	~95	Days 11–22	Fernández-Reiriz et al. (2011)

Table 5 (continued)

Species	Temperature	Algal species	Rations	Growth rate (μm day^{-1})	Survival (%)	Period	Reference
Chamelea gallina	20 °C	*T. lutea*	50 cells μl^{-1} (days 2–4), 75 cells μl^{-1} (days 5–10), 100 cells μl^{-1} (days 11–13)	5.15	21.1	Days 1–13	Joaquim et al. (2016a)
C. gallina	20 °C	*C. calcitrans*	68 cells μl^{-1} (days 2–4), 100 cells μl^{-1} (days 5–10), 135 cells μl^{-1} (days 11–13)	1.40	7.5	Days 1–13	Joaquim et al. (2016a)
C. gallina	20 °C	Unfed	–	0.78	8.7	Days 1–13	Joaquim et al. (2016a)
C. gallina	23 °C	*T. lutea*	50 cells μl^{-1} (days 2–4), 75 cells μl^{-1} (days 5–10), 100 cells μl^{-1} (days 11–13)	7.96	24.2	Days 1–13	Joaquim et al. (2016a)
C. gallina	23 °C	*C. calcitrans*	68 cells μl^{-1} (days 2–4), 100 cells μl^{-1} (days 5–10), 135 cells μl^{-1} (days 11–13)	1.28	~18	Days 1–13	Joaquim et al. (2016a)
C. gallina	23 °C	Unfed	–	1.42	7.5	Days 1–13	Joaquim et al. (2016a)

density on *V. corrugata* larval culture in a RAS when compared to a batch system. The authors showed that *V. corrugata* larvae can be reared at higher densities in a RAS, 40 larvae ml^{-1} than in batch at 10 larvae ml^{-1}, without negatively affecting survival. Joaquim et al. (2016c) reported an increase in shell growth and a decrease in larval development until the settling stage, when comparing the RAS to the batch system. Later, Asmani et al. (2017) compared FTS and RAS for oyster larval culture (*C. gigas*) showing that at a density of 50 larvae ml^{-1}, growth and survival were similar in both culture systems, whereas the percentage of metamorphosis was lower in RAS. Also, promising results using RAS for larval culture were reported in the oyster *Crasostrea angulata* (Qiu et al. 2017) and the scallop *Argopecten purpuratus* (Merino et al. 2009).

Nutrition

Food quantity and quality are considered the main factors affecting larval bivalve survival and growth (Joaquim et al. 2008). Pérez-Camacho et al. (1977) investigated the effect of food density on the growth of *V. corrugata* larvae with a mono-specific diet of *D. lutheri*. The authors established that the best feeding strategy for *V. corrugata* larvae reared at 8 larvae ml^{-1} was 25 cells μl^{-1} for the first week, followed by an increase to 50 cells μl^{-1} at the second week and another increase to 100 cells μl^{-1} until the end of the larval culture. This feeding strategy allowed for more efficient stock management of the microalgae produced in the hatchery. For *R. decussatus* larvae, Pérez-Camacho et al. (1994) investigated the effect of food concentration on growth rate from a physiological energetics perspective. The authors fed larvae microalgal concentrations of *I. galbana* ranging from 10 to 300 cells μl^{-1}, observing that ingestion rate of microalgae by the different larval sizes increased with food concentrations up to 200 cells μl^{-1}, and then remained constant. From a practical point of view, it was shown that 4- and 21-day-old *R. decussatus* larvae consumed approximately 2000 and 14,000 *I. galbana* cells per day, respectively (Pérez-Camacho et al. 1994).

Food value is determined by the biochemical composition of microalgae, especially lipid quality composition (da Costa et al. 2016). Essential fatty acids (EFA), particularly 20:5n-3 (Eicosapentaenoic acid, EPA) and 22:6n-3 (docosahexaenoic acid, DHA) and sterols (mainly cholesterol), are important for larval survival and growth (Rico-Villa et al. 2006). The knowledge base for larval nutrition of clams is limited. Nóvoa et al. (2002) showed that *V. corrugata* can incorporate arachidonic acid (20:4n-6, AA) when this fatty acid is delivered in gelatin-acacia microcapsules. However, the role of this EFA for clam larvae is still unclear. *V. corrugata* larvae exhibited low growth and high mortality when fed *T. suecica* from day 11 of rearing (umbonate stage) until day 34 (postlarval stage), which is a DHA-deficient diet (Table 5; Fernández-Reiriz et al. 2011). For the same species, Fernández-Pardo et al. (2016) showed the positive effects of *I. galbana* on the growth of early *V. corrugata* larvae (until day 10–12 post-fertilization). During the early larval phases, *V. corrugata* showed low preference for the diatom *Chaetoceros neogracile*. This could be explained by the relatively larger size and volume of *C. neogracile* cells compared to other sources of food such as flagellates, or by the presence of silica rods o at the surface of this diatom (Fernández-Pardo et al. 2016). Late larvae and young postlarvae *V. corrugata* grew more quickly using a combination of *I. galbana* and *C. neogracile* at concentration ranges of 23–30 cells μl^{-1} and 43–48 cells μl^{-1}, respectively (Fernández-Pardo et al. 2016). These results suggest the importance of providing *V. corrugata* larvae with the appropriate diet during each developmental stage.

The diatom *Chaetoceros muelleri*, and likely other *Chaetoceros* sp., may have a beneficial effect on *R. decussatus* larval development (Table 5; Aranda-Burgos et al. 2014a). *R. decussatus* larvae fed with *C. calcitrans* alone exhibited slightly higher growth rates and survival, and lower percentage foot development when compared to larvae fed with *T. lutea* alone (Matias et al. 2011). In a later study, Matias et al. (2015) found that *R. decussatus* early larvae did not efficiently assimilate *C. calcitrans*. The same authors proposed that the optimal diet for *R. decussatus* larvae consisted on a mixture of *T. lutea* and *C. calcitrans* (60/40 cells μl^{-1}; Table 5).

The embryological development of *R. decussatus* is fuelled by lipid reserves, mainly neutral lipids, while the energy necessary for metamorphosis is mainly obtained via the proteins stored during larval development (Matias et al. 2011). Aranda-Burgos et al. (2014a) showed that increasing lipid and carbohydrate total content in the diet may reduce *R. decussatus* larval growth and survival. Regarding the influence of EFAs, it has been shown that EPA should be preferentially used over DHA for increased larval performance in *R. decussatus* (Aranda-Burgos et al. 2014a). This observation was linked to the importance of feeding *Chaetoceros* to *R. decussatus.* This study also reported the positive effect of increasing dietary levels of AA on larval growth and survival (Aranda-Burgos et al. 2014a).

Larvae of the species *Chamelea gallina* exhibited higher growth rate and survival when fed with *T. lutea* than with *C. calcitrans* (Table 5; Joaquim et al. 2016a). In fact, in the same study, it was demonstrated that unfed *C. gallina* larvae exhibited similar growth to larvae fed with *C. calcitrans.* Consequently, this evidence suggested the inadequacy of this diatom species to the nutrition of *C. gallina* larvae.

Effect of broodstock on larval quality

Bivalve eggs and larvae exhibit inherent biochemical and physiological variability affecting production success in hatcheries (Joaquim et al. 2016b). The first stages of larval development, embryogenesis and early larval development, rely on endogenous energy reserves supplied to the egg by the broodstock, and any energetic deficiency can have serious consequences in the subsequent larval success (Ojea et al. 2008; da Costa et al. 2011b, 2013). The effect of broodstock condition on oocyte and larval quality of wild broodstock of *V. corrugata* was studied by Cerviño-Otero (2011) and Joaquim et al. (2016b). Cerviño-Otero (2011) investigated the combined effect of the seasonality and broodstock geographic origins on the number of eggs released and larval performance of *V. corrugata*. The author reported significant differences in the biochemical composition of broodstock gonad during the different seasons among of all the populations studied. Broodstock from different Galician populations (NW of Spain) of *V. corrugata* spawned throughout the year in the hatchery (Cerviño-Otero 2011), whereas broodstock from Ria de Aveiro (N of Portugal) only spawned during the first half of the year, with two distinct spawning periods, in February–March and June–July (Joaquim et al. 2016b). Alternatively, Cerviño-Otero (2011) did not find any significant differences in larval success among in the different broodstock, regardless of origin. No relationship between biochemical contents of broodstock gonad and the quantitative response to spawning stimulation or larval survival were found in any *V. corrugata* populations studied (Cerviño-Otero 2011). Survival of *V. corrugata* D-shaped larvae was related to broodstock condition index and to oocyte quality, estimated by total lipids, organic matter and total energy (Joaquim et al. 2016b). The authors, therefore, proposed these three parameters as indicators of early larval success in hatcheries. Alternatively, Cerviño-Otero (2011) did not find any significant

Table 6 Feeding experiments in clam seed

Species	Initial size or weight	Type of diet	Diets tested	Daily ration per clam	Best conditions	Reference
Ruditapes decussatus	0.17–1.25 mg ind^{-1} LW	Live algae	*Tisochrysis lutea*; *Chaetoceros calcitrans*; *Thalasiosira* pseudoenana; *Phaeodactylum tricornutum*	nd	*T. lutea*	Laing et al. (1987)
R. decussatus	0.77 mm (0.15 mg ind^{-1} LW)	Live diets	*T. lutea*, *T. suecica* and *P. tricornutum*	2% of algae OM/LW of seed	*Tetraselmis suecica*	Albentosa et al. (1996b,c)
R. decussatus	nd	Live and spray-dried algae	Spray-dried *T. suecica*, live *C. calcitrans* and *T. suecica*	Live *C. calcitrans* (100 cells µl^{-1} initially); live *T. suecica* (10 cells µl^{-1} initially); the dried *T. suecica* diet (0.3 g day^{-1})	No significant differences among diets	Laing and Gil Verdugo (1991)
R. decussatus	nd	Live algae, flours and powdered fish	Live *T. suecica*, *Dunaliella bardawil* and *Nitzschia acicularis* grown under autotrophic or mixotrophic conditions; and inert diets (rye and soy flours and powdered fish)	nd	Live microalgae grown in autotrophic conditions	Lamela et al. (1996)
R. decussatus	0.48–1.38 mm (0.04–0.96 mg ind^{-1} LW), 4 experiments	Live and freeze-dried algae	Live *T. lutea* and freeze-dried *T. lutea*, *T. suecica* and *P. tricornutum*	2%, 4% and 6% of algae OM/LW of seed	Live *T. lutea*	Albentosa et al. (1997)

Table 6 (continued)

Species	Initial size or weight	Type of diet	Diets tested	Daily ration per clam	Best conditions	Reference
R. decussatus	1.76–2.10 mm (1.34–2.46 mg ind^{-1} LW)	Live algae, cornstarch and cornmeal	*I. galbana*, cornstarch and cornmeal	0.5%, 1%, 2% of algae OM/LW of seed	Live *I. galbana* 2% ration and 75% *I. galbana* and 25% cornmeal	Pérez-Camacho et al. (1998)
R. decussatus	2.10 mm (2.46 mg ind^{-1} LW)	Live and wheatgerm diets	Live *T. lutea* and wheatgerm	0.5%, 1%, 2% of algae OM/LW of seed	Live *T. lutea* (2% ration) and live *T. lutea* (50%) + 50% wheatgerm (2% ration)	Albentosa et al. (1999)
R. decussatus	4.0–5.8 mm (16.18–37.47 mg ind^{-1} LW)	Live algae and single cell detritus (SCD)	Live *I. galbana* and single cell detritus (SCD) from *Laminaria saccharina*	20 mg DW of *I. galbana* per g LW of the clams	Diet 1. Live *I. galbana* and diet 2. Single cell detritus (SCD) from *L. saccharina* (80–90%) + *I. galbana* (10–20%)	Pérez-Camacho et al. (2007)
R. decussatus	Experiment 1: 4.3 mm (24.3 mg ind^{-1} LW) and experiment 2: 3.7 mm (13.1 mg ind^{-1} LW)	Live algae and cheese whey	Live *T. suecica* + *P. tricornutum* and cheese whey	2.5% of algae DW/clam LW	25% (*T. suecica* + *P. tricornutum*) + 75% cheese whey	Enes and Borges (2003)
V. corrugata	1.36 mm (0.47 mg ind^{-1} LW)	Live diets	*T. lutea*; *T. suecica*; *T. lutea* + *T. suecica*	5% of algae DW/clam LW	*T. lutea* + *T. suecica* and *T. lutea*	Albentosa et al. (1993); Albentosa et al. (1994b)
Chamelea gallina	3.7 mm (16.2 mg ind^{-1} LW)	Live diets	*T. lutea*, *C. calcitrans*, *T. lutea* + *C. calcitrans*	2% of algae DW/clam LW	*T. lutea*	Joaquim et al. (2016a)

DW dry weight, *LW* live weight, *nd* no data, *OM* organic matter, *WW* wet weight

relationships between the number of eggs released or the success of larval development in *V. corrugata* when compared to the condition index, biochemical composition or maturity index of broodstock.

Oocyte quality can be analysed using a microarray-based analysis to identify differentially expressed genes in *R. decussatus* according to oocyte quality (de Sousa et al. 2015). In this study, de Sousa et al. (2015) postulated that DnaJ (Hsp40) homologue and Hsp70 were linked to high-quality oocytes in *R. decussatus*, whereas tumour necrosis factor receptor and caspase 8 were more abundant in poor quality oocytes (de Sousa et al. 2015).

Microbiological studies of clam larval cultures

Pathogenic species belonging to genus *Vibrio* are the main aetiological agents leading to the rapid loss of larval batches in hatcheries, representing the main bottleneck in the bivalve production process (Dubert et al. 2016b). Major sources contributing to *Vibrio* contamination are broodstock, phytoplankton and seawater (Prado et al. 2014; Dubert et al. 2015, 2016a). In addition to the aforementioned sources, the persistence of *Vibrio* in different areas and equipment in bivalve hatcheries may be a potential source of contamination to healthy cultures and may cause disease outbreaks (Badreddine et al. 2013). Badreddine et al. (2013) investigated the adhesive properties of 54 *Vibrio alginolyticus* strains transferred from *R. decussatus* to various inert materials typically used in hatchery facilities in Tunisia. They found that adhesion ability depends on the *V. alginolyticus* strains and on the tested materials. For example, *V. alginolyticus* isolates exhibited a high potential to adhere to glass and most of them were strongly adhesive to PVC, polystyrene and polyethylene (Badreddine et al. 2013).

Several species of *Vibrio* have been identified as pathogenic for clam larvae of the species included in this review. For instance, Gómez-León et al. (2005) isolated *V. alginolyticus* and *Vibrio splendidus* biovar II from moribund larvae and spat of *R. decussatus*, during two disease outbreaks in a commercial hatchery in Spain. The authors found bacillary bacteria in moribund larvae causing "bacillary necrosis" disease, with lesions in the vellum, necrosis of soft tissues and pale colour of the digestive tract. In a *R. decussatus* experimental hatchery in Tunisia, the *Vibrio* strains found most frequently in the larvae were *V. alginolyticus*, *V. fluvialis* and *V. parahemolyticus* (Mechri et al. 2012). Among these strains, *V. alginolyticus* was identified as the causative agent of high mortalities in *R. decussatus* veliger larvae and juveniles (Mechri et al. 2015). These *V. alginolyticus* isolates showed total resistance to several antibiotics, such as ampicillin, erythromycin, tetracycline, streptomycin and chloramphenicol (Mechri et al. 2015). Flumequine was identified as an effective antibiotic against *V. alginolyticus*, suggesting its potential as a therapeutic molecule in shellfish hatcheries (Mechri et al. 2015).

Other *Vibrio* strains causing mortality in clams are *Vibrio tubiashii* subsp. and *europaeus* subsp. nov. (Prado et al. 2015, 2016), which were initially isolated from disease outbreaks observed in *Ostrea edulis* larvae and *R. philippinarum* spat in two Galician hatcheries. The pathogenicity of six isolates of this *Vibrio* strain were evaluated in an experimental challenge using *R. decussatus* larvae. The *Vibrio* caused 100% mortality after 48 h, whereas the survival rates were higher than 95% in the negative controls (Prado et al. 2015). *Vibrio tubiashii* subsp. and *europaeus* subsp. nov. have been recently re-classified as a new species named *Vibrio europaeus* (Dubert et al. 2016d). This species was confirmed as the pathogen responsible for the disease outbreak of *R. decussatus* larvae in both untreated and chloramphenicol treated larval cultures (Dubert et al. 2017a). High mortality observed during early development of

R. decussatus larvae suggested that eggs might be the source of infection due to vertical transmission from broodstock (Dubert et al. 2017a).

Dubert et al. (2016e) described the pathogen *Vibrio bivalvicida*, which was initially isolated from *R. decussatus* broodstock, moribund larvae and in the seawater belonging to the larval tanks of a Spanish bivalve hatchery. Experimental challenges using *R. decussatus* larvae infected with *V. bivalvicida* proved the pathogenicity of this *Vibrio* sp. to clam larvae (Dubert et al. 2016e).

Settlement and metamorphosis

Some of the limiting factors of bivalve hatchery culture are settlement and metamorphosis. These have proven to be linked to variables such as survival, low developmental synchronicity, and delayed metamorphosis (Joaquim et al. 2008). Limitations on the success of competent larvae to metamorphose can be overcome with the use of chemical inducers, such as gamma-aminobutyric acid (GABA) and epinephrine (Joaquim et al. 2008). García-Lavandeira et al. (2005) showed, in *V. corrugata*, that the highest rates of metamorphosis were obtained after 24 h of exposure to 10^{-4} M GABA and 10^{-5} M epinephrine. Furthermore, a 48-h exposure to 10^{-2} M GABA effectively induced metamorphosis in *R. decussatus* (Galley et al. 2013). GABA treatments, however, induced higher mortality compared to controls, thus suggesting the toxicity of this compound to *R. decussatus* (Galley et al. 2013). Alternatively, epinephrine was identified as a highly effective inducer of metamorphosing behaviour at a concentration of 10^{-6} M, producing a 2-fold increase in settlement rate, without affecting mortality (Galley et al. 2013).

Seed culture

Postlarval and seed rearing systems in clams

Ready-to-settle clam pediveliger larvae are reared in containers in down-welling systems until they reach 0.5–1 mm in length (Ruiz-Azcona 2013). This stage can last up to 3–4 weeks until settlement and metamorphosis are completed (Ruiz-Azcona 2013). Seed of larger sizes are reared in upwelling systems, in tanks of larger volumes (Ruiz-Azcona 2013). The data reported by Martínez-Patiño (2014) showed that the growth rate calculated for *R. decussatus*, *V. corrugata* and *P. rhomboides* was 20–25 μm day^{-1} for the first 30 days of postlarval life and it increased up to 30–35 μm day^{-1} until seed reached 3–4 mm length.

Clam postlarvae are reared in closed system and FTS, whereas seed of greater size is grown in FTS (Ruiz-Azcona 2013). The drawback of FTS is the high cost of pumping and heating large amounts of seawater needed in nurseries (Kamermans et al. 2016). This cost can be considerably reduced by linking the FTS to a recirculation system, thus reusing a considerable volume of water through continuous treatment (Kamermans et al. 2016). Kamermans et al. (2016) reported negligible *R. decussatus* seed growth in an experimental RAS in comparison to a FTS. Poor results in experimental RAS have been associated with the challenge of supplying a sufficient amount of food (Kamermans et al. 2016). In the same study, however, the experimental RAS system proved to be successful in achieving similar seed growth in

comparison to a FTS for other bivalve species, such as oysters (*Crassostrea gigas*), scallops (*Pecten maximus*) and mussels (*Mytilus edulis*).

Temperature and salinity

Temperature is a major factor affecting juvenile clam growth due to its impact on metabolic rates and feeding activity (Laing et al. 1987). Exposure to temperatures below 6 °C for longer than 3 weeks can cause mortality in fed and unfed seed of *R. decussatus* due to the loss of weight, decrease of biochemical reserves and decrease of condition index (i.e. the ratio of dry meat weight to dry shell weight) (Laing and Child 1996). In Europe, low temperatures can routinely occur in northern locations such as the United Kingdom and Ireland. Optimal temperature for efficient growth of *R. decussatus* spat was demonstrated to be between 15 and 20 °C (Laing et al. 1987), and significant growth was recorded at temperatures up to 28 °C (Laing et al. 1987). A similar optimal temperature of 20 °C was proposed for growth of *V. corrugata* juveniles, given enough food is provided (Albentosa et al. 1994a).

Exposure to sub-optimal salinities may lead to a reduction in the feeding activity of bivalve seed, thereby reducing spat growth and causing mortality (Pérez-Acosta et al. 2014). In estuarine areas, highly variable salinity fluctuations may have a significant impact in seed production in bivalve nurseries. One study investigated the effect of different salinities (0, 5, 15 and 25 ppt) at a temperature of 14 °C on seed survival and activity (in terms of siphon presence after a rest period of 30 min) of the clams *V. corrugata*, *R. decussatus* and *P. rhomboides* (Pérez-Acosta et al. 2014). Clams were subjected to different salinities for a period of 1 week, followed by 1 week of recovery at a room temperature of 23 °C and salinity of 33 ppt. No activity was recorded in any of the species when exposed to salinities below 15 ppt (Pérez-Acosta et al. 2014). The species *P. rhomboides* exhibited a low tolerance to salinities below 25 ppt, causing mortality greater than 90% (Pérez-Acosta et al. 2014). Alternatively, *R. decussatus* had a high tolerance to low salinity, exhibiting a survival rate greater than 90% at the end of the experiment (14 days), even when exposed to salinity of 0 ppt (Pérez-Acosta et al. 2014).

Seed nutrition

Food quantity and quality have a significant effect on clam seed growth (Albentosa et al. 1993, 1996b). Little is known about the ideal ration for clam spat, since, so far, only two studies have focused on this topic. Albentosa et al. (1996b) showed that, in *R. decussatus* seed of 1.6 mm in length, the rates of ingestion and absorption efficiency were the primary physiological processes governing seed growth, and ingestion rates increased with food concentration, up to a maximum of 100 cells μl^{-1}. Maximum growth occurred when clams were supplied with a diet of *T. lutea* at a concentration of 50 cells μl^{-1}. The authors discovered that the pseudofaeces production threshold was found at a concentration of 100 cells (*T. lutea*) μl^{-1}. In a FTS, Beiras et al. (1993) studied the growth rate of 2-mm-long *V. corrugata* seed using a physiological approach with metrics such as gross growth efficiency (K_1). Growth rate (GR) was calculated, as $GR = (\ln LWt - \ln LW_0) \times 100 / t$, where LW_0 and LW_t are initial and final live weights and t is time in days. Authors calculated K_1 as follows: K_1 = organic biomass increase / total organic weight of algae (*I. galbana*) consumed, showing that optimal growth in terms of K_1 was observed at a concentration of 100 cells μl^{-1} (Growth rate = 8.2 day^{-1}), whereas maximum growth rate was found at a concentration of 300 cells μl^{-1} (drowth rate = 11.9 day^{-1}).

V. corrugata seed showed increased growth rates compared to *R. decussatus* seed due to the greater ingestion rate of *V. corrugata* seed (Beiras et al. 1993; Albentosa et al. 1996b). At similar ingestion rates, however, analogous growth rates were observed in both species. Unfortunately, these studies only investigated food ration on a single size group of clam seed (1.6–2 mm long). The nutritional value of different microalgae species to bivalve seed has important effects on culture performance (Albentosa et al. 1993). The literature dealing with the value of single microalgal species as food is difficult to compare since most of the studies tested microalgal strains grown under different culture conditions, and such this variability may influence biochemical composition. Comparisons between studies are also difficult, since different investigations do not share microalgal species that can be used as a reference diet or control. In addition, nutritive quality of microalgal strains cannot be explained by a single factor, but rather by the contribution of various properties, such as algal size, diet acceptability, digestibility and biochemical composition (Albentosa et al. 1993), metrics which are not always reported.

The algae species most frequently used in European bivalve hatcheries have invariably been tested in nutritional studies of clam seed. For instance, a mono-specific diet of *T. lutea* showed the species had high nutritional value for *V. corrugata* and *R. decussatus* seed (Table 6; Laing et al. 1987; Albentosa et al. 1993). Alternatively, the algae *T. suecica* promoted decreased growth for the spat of *V. corrugata* (Albentosa et al. 1993). A mixed diet of *T. lutea* and *T. suecica*, when fed together induced greater growth of *V. corrugata* spat than if either species were fed as single diets (Albentosa et al. 1993). The authors explained that, for *V. corrugata* spat, the poor nutritive value of *T. suecica* likely came from its low digestibility, low protein, low lipid and low DHA content (Albentosa et al. 1993, 1994b). Alternatively, *T. suecica* was an excellent food for *R. decussatus* spat (Table 6; Albentosa et al. 1996c); therefore, Albentosa et al. (1996c) suggested that the nutritional requirements of *V. corrugata* and *R. decussatus* spat must be different. The authors concluded that *R. decussatus* seed may have lower protein and lipid requirements than *V. corrugata* seed (Albentosa et al. 1993, 1996c), and *R. decussatus* seed may have the ability to compensate energy requirements more effectively when fed a diet low in proteins and lipids as long as carbohydrates were present in the diet (Albentosa et al. 1996c). The diatom *Phaeodactylum tricornutum* has a poor nutritional value to *R. decussatus* spat (Table 6; Laing et al. 1987; Albentosa et al. 1996c), which may be due its relative indigestibility (Epifanio 1983). The absence of EPA or DHA in the diet did not limit growth of *R. decussatus* seed (Albentosa et al. 1996a). Other diatoms, such as *C. calcitrans* and *Thalassiosira pseudonana*, had greater value as food for *R. decussatus* spat than the diatom *P. tricornutum*, but lower than the flagellate *T. lutea* (Table 6; Laing et al. 1987). In agreement with the results reported by Laing et al. (1987), *T. lutea* promoted greater growth in length and weight in *C. gallina* juveniles than in *C. calcitrans* (Table 6; Joaquim et al. 2016a).

Hatchery rearing of bivalves is highly dependent on live microalgae production, where it is considered a potential limiting factor, and averages about 30% of the total seed production cost (Coutteau and Sorgeloos 1992). To reduce the high cost of microalgal production, several studies have investigated the use of inert diets to partially or totally replace the use of live microalgae for bivalve spat nutrition (for review, see Robert and Trintignac 1997; Knauer and Southgate 1999; Aji 2011). Research for the development of live microalgae substitutes has focused mostly on microalgae concentrates, dried algae, lipid emulsions and microcapsules, yeasts, carbohydrate-rich cereal flours and derived products of macroalgae (Robert and Trintignac 1997; Knauer and Southgate 1999). These spat nutrition studies have focused on

the nutritional effects in more "established species", such as Manila clam (*R. philippinarum*), but only a few of these alternative diets have been tested in native Veneridae clam species from European waters (Albentosa et al. 1997; Pérez-Camacho et al. 1998).

Concentrated and dried microalgae are two alternative substitutes to live microalgae. Laing and Gil Verdugo (1991) demonstrated that *R. decussatus* juveniles fed spray-dried *T. suecica* exhibited similar growth rates to those fed live *T. suecica* and *C. calcitrans* (Table 6). These results contrast with those obtained by Albentosa et al. (1997) pertaining to *R. decussatus* seed. In a series of experiments, the authors investigated the effect of various freeze-dried microalgae (*T. lutea*, *T. suecica* and *P. tricornutum*) on small seed (less than 1.5 mm). Among the freeze-dried microalgae tested, *T. lutea* microalgae induced greater growth in weight (Table 6; Albentosa et al. 1997). The use of frozen or freeze-dried *T. lutea* as food for *R. decussatus* seed, however, significantly reduced growth compared to concentrated or live *T. lutea* (Albentosa et al. 1997). The authors postulated that the lower nutritional value of the freeze-dried diet may be explained by altered digestibility of the microalgae due to the freezing process. A substitution of freeze-dried microalgae with 20% live *T. lutea* significantly increased growth rate to that of the lower levels of growth achieved with the live diet (Albentosa et al. 1997).

Other inert diets that were tested as food for *R. decussatus* seed are carbohydrate-rich cereal flours (Table 6; Lamela et al. 1996; Pérez-Camacho et al. 1998; Albentosa et al. 1999). Cornmeal and cornstarch can be used to replace 50% of *I. galbana* live diet (ration 2%) in *R. decussatus* seed, promoting similar growth rates to that of a purely live microalgal diet (Pérez-Camacho et al. 1998). Examining *R. decussatus* spat, Albentosa et al. (1999) also found similar growth response between full live diets of *T. lutea* and a diet in which 50% of live microalgae was substituted by wheat germ flour. Other studies of *R. decussatus* spat have reported negative effects of flour diets on growth due to the high bacterial contamination of water promoted by these flour diets (Lamela et al. 1996).

Few studies have investigated other alternatives to live microalgal diets. One example, Lamela et al. (1996) reported that the use of protein-rich fish powder as food for *R. decussatus* spat promoted greater weight gain than other artificial diets (soy and rye flour), but less weight gain compared to live microalgae (Table 6). Another study showed that *R. decussatus* spat growth, measured in length and weight, could be improved by replacing 75% of the live diet (*P. tricornutum* and *T. suecica*) with cheese whey diet when compared to a full live diet (Table 6; Enes and Borges 2003).

Biotransformed products from macroalgae offer promising alternatives for replacement of high percentage live microalgae diets. Pérez-Camacho et al. (2005) developed a method to prepare single cell detritus (SCD) from *Laminaria saccharina* using enzymes and two bacteria to produce a suspension of algal cells and detritus of less than 20 μm in diameter, which can easily be filtered and digested by bivalves. The replacement of a live microalgal diet by 80–90% SCD from *L. saccharina* promoted similar or higher growth rates than the control live diet in *R. decussatus* seed (Table 6; Pérez-Camacho et al. 2007).

Areas for improvement and future prospects

Among the species considered in this review, *R. decussatus* and *V. corrugata* constitute the vast majority of the literature available. This fact is likely due to the considerable commercial value of *R. decussatus*, its wide distribution within European waters and the great interest in

several European countries in this species. Despite also having a high commercial value, *V. corrugata* is only harvested and cultured in Spain and Portugal. Both species, however, are produced in commercial hatcheries (Table 1). Regarding the other species considered herein, little is known about cultivation protocols in hatcheries, and their cultivation is only performed on an experimental scale (Table 1).

The effects of various abiotic and biotic parameters on clam conditioning is only relatively well studied in *R. decussatus* and literature is scarce for the rest, with only two references available on *C. chione* conditioning (Martínez-Pita et al. 2008; Delgado et al. 2016) and one in *V. verrucosa* (Gavrilovic et al. 2010). Broodstock conditioning protocols developed for *R. decussatus* at an experimental scale are ready to be transferred to commercial hatcheries. Autumn broodstock conditioning techniques, however, need to be improved, since the current process takes too long (up to 14 weeks) to obtain ripe clams. This means that clams are ready to spawn nearly at the same time as if conditioning had begun in winter. Thus, the cost of feeding clams during autumn conditioning makes the current procedure not profitable for industrial production. Pazos et al. (2003) and Martínez et al. (2005) reported promising results by using the summer photoperiod for autumn and winter conditioning in *R. decussatus*. Photoperiod may be a key factor for mastering clam conditioning during most months of the year, combined with temperature changes to reproduce conditions necessary for energy storage and allocation towards maturation that occurs in clams in the wild. This must be investigated not only in *R. decussatus* but also in other European native clam species, in order to allow the extension of the period when larvae production is possible in hatcheries. Thus, removing reliance on the relatively brief period of the year when adults of these species naturally bear mature gametes in the wild would greatly benefit the industry.

Clam broodstock nutrition is largely dependent on the production of live microalgae, which represents high operational costs in hatcheries (Coutteau and Sorgeloos 1992). Future research should focus on the partial or total replacement of clam broodstock microalgal diets with inert alternatives, such as macroalgae biotransformates or cereal flours, among others (for review, see Knauer and Southgate 1999). The use of formulated feeds in clam commercial hatcheries, however, is still limited and production relies almost completely on the use of microalgae (Muller-Fuega 2000). Such alternatives would reduce production costs whilst meeting the nutritional requirements of broodstock for the production of high-quality clam larvae.

Spawning induction is a crucial step in bivalve hatchery production. As mentioned before, in clam hatchery culture, the main method used to obtain gametes for larval production is thermal shock. Unfortunately, this method has yielded variable results in some clam species, such as *R. decussatus* in Southern Portuguese populations (Matias et al. 2009), or even negative results, which may limit the production of larvae at industrial scale. One attempt to solve this problem was the use of serotonin to activate *R. decussatus* oocytes (Hamida et al. 2004a). The results were unsatisfactory, in terms of the percentage of fertilized oocytes obtained through stripping and the percentage development to D-shaped larvae (Hamida et al. 2004a). Injection of serotonin in the abductor muscle as a spawning induction method caused a very intense stimulus causing the emission of immature gametes and fragments of gonads. Therefore, alternative methods to obtain gametes ready for fertilization should be investigated if the production of larvae is to take place on a large scale. One alternative may be the immersion of clams in baths containing dissolved serotonin, as already tested in other bivalve species (Gibbons and Castagna 1985). Other chemicals, such as hydrogen peroxide, have been proven to induce spawning in other bivalves species (Beckvar 1981).

The low development rate of embryos to D-shape larvae observed in *P. rhomboides* (10–35%) may limit the production of spat in this species. Embryos were incubated in 500-l tanks at 19 ± 1 °C at a density of 5–10 larva ml^{-1}, using the standard methods used for other clam species at the same laboratory (Cerviño-Otero 2011). In this study, after spontaneous spawning, embryos were collected at the outlet of the tanks using sieves; therefore, neither the spawning induction nor fertilization procedure may have impacted the development of embryos. Factors that may explain the poor results reported in the study by Cerviño-Otero et al. (2011) may be due to poor microbiological and/or biochemical quality of the embryos, among other issues. Future research on larval production of *P. rhomboides* should focus on incubation protocols and on the determination of the critical aspects impeding the production of adequate quantities of D-shaped larvae. The development of conditioning techniques for this species may also improve egg quality, fertilization rates and larval yields.

Larval culture systems in clams should be optimized to reduce production costs and improve yields, since experimental and commercial hatcheries rearing clams mainly use closed (batch) system. As aforementioned, studies dealing with FTS and RAS in clam larvae are scarce and limited to one clam species for each system. Implementation of these new rearing methods could directly contribute to more stable and reliable production of better-quality larvae by reducing costs and, therefore, boosting clam aquaculture and restocking programs. Further research should focus on the optimization of flow rates and larval densities for FTS. Further studies of RAS may focus on renewal rate (i.e. rate and ratio of fresh seawater replacement in the system), the rate of circulation through the RAS rearing tanks, stocking densities, improving the stability of water quality, etc. FTS and RAS should be refined for current species and implemented for larval rearing of other species of clams, while also being scaled up from small volumes of experimental scale to large volumes of industrial scale.

The implementation of FTS and RAS for larval culture at commercial scale may also help to reduce the use of antibiotics. Antibiotics are generally used in bivalve hatcheries that rear larvae in closed circuits. Antibiotic use must be avoided due to its release of residues and its ability to yield resistant bacteria, the latter which may be released into the aquatic environment (Dubert et al. 2017b). Improvement of the microbiological quality of larval culture must be assured for a sustainable and profitable production of clams in hatcheries. Eco-friendly alternatives to the use of antibiotics in bivalve hatcheries may consist of probiotics, quorum quenching and phage-therapy, which were extensively reviewed by Dubert et al. (2017b). These alternative treatments have not yet been applied to clam larvae, and future research may be devoted to such.

Postlarval and small seed culture in clams is generally carried out in containers in down-welling and, for larger seed sizes, in upwelling systems. A system referred to as a "bottle system" is also used for oysters (Duthie 2012) and Manila clam (Jones et al. 1993). This system consists of tubes or bottles in which seawater, mixed with food, flows up through the rearing system, and oyster seed are fluidized in the system. Bivalve seed are retained by a marble or balls at the inflow, preventing seed from escaping if the water inflow is stopped. This system allows high rearing densities inside the tubes or bottles. Another advantage is that, contrary to traditional containers, the seed does not need to be cleaned on a regular basis, thus reducing labour for hatchery operators. To our knowledge, despite the fact that this system has been utilized for quite a few years, no studies describing rearing densities or flow rates for clam seed culture have been performed. Thus, transfer of this knowledge to commercial hatcheries has not taken place.

To date, only a few studies have tested bivalve seed culture in RAS (Widman 1998; Blanco Garcia and Kamermans 2015; Kamermans et al. 2016). Implementation of new methods of spat rearing could directly contribute to more stable and reliable production of better quality spat, reducing costs, and therefore boosting clam aquaculture and restocking programs (Ruiz-Azcona 2013).

Stocks of native European clam species have suffered continuous decrease in recent decades. For instance, *R. decussatus* has progressively reduced in population size due to abnormal adult mortalities caused by *Perkinsus* spp. Selective breeding techniques may offer a way to produce improved bivalve varieties with faster growth and resistance to disease and abiotic stress conditions. By implementing selective breeding, new combinations of genes can be obtained by crossing selected stocks with new genotypes possessing traits of interest (e.g. greater survival rate, faster growth rate, among others). Bivalves are ideal candidates for selective breeding programs because of their high economic value, the ease with which one can manipulate their biological cycle, their high levels of genetic variability, and their high fecundity (Gosling 2003). In bivalves, available data on response to selection in breeding programs reveals that growth rate can be improved by 10% per generation, and disease resistance by 15% per generation with the implementation of individual or family-based selection (Hollenbeck and Johnston 2018). Therefore, rapid gains in growth rate and disease resistance in bivalves may also be obtained in native European clam species. Currently, several selective breeding programs at experimental and commercial scale are run in the United States, Australia, New Zealand, France and China with oysters (*C. gigas*, *C. virginica* and *Saccostrea glomerata*), mussels (*Perna canaliculus*) and some species of clams (*R. philippinarum* and *Sinonovacula constricta*) (Camara and Symonds 2014; Dégremont et al. 2015; Hollenbeck and Johnston 2018). Concerning European native clams, there is an experimental breeding program with *R. decussatus* in Andalusia (Spain) led by Dr. Jose Ignacio Navas Triano at Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA). To our knowledge, however, there is no commercial breeding program. In the future, breeding programs implemented by commercial companies may contribute to sustainable and stable aquaculture production of clams within Europe.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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