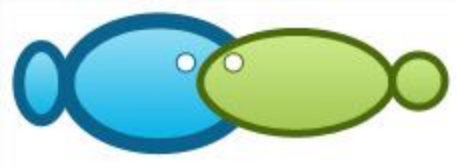

First insight into nutrition effect on spawning and larvae rearing of the clam *Ruditapes decussatus* L. from Dakhla Bay

[1]Yassine Ouagajjou, [2]Touryia El Aloua, [3]Mohamed El Moussaoui, [1]El Mustafa Ait Chattou, [4]Adil Aghzar, [3]Younes Saoud

[1] National Institute of Fisheries Research, INRH, Casablanca, Morocco; [2] Faculty of Science, Chouaib Doukkali University, El Jadida 24000, Morocco; [3] Faculty of Science, Abdelmalek Essaadi University, Tétouan 93002, Morocco; [4] Higher School of Technology, Sultan Moulay Slimane University, Khénifra, Morocco. Corresponding author: Y. Ouagajjou, ouagajjou@inrh.ma; yassinewag200@gmail.com

Abstract. The local clam *Ruditapes decussatus* has been a widespread bivalve along the natural beds in Dakhla Bay (Morocco). But, nowadays its overexploitation during many years has prompted the depletion of its occurrence in many extents and also has limited the availability of natural spat. In this study, the influence of nutrition on spawning, fertilization and larvae rearing was assessed for the first time. The potential of oocytes accumulation and release was highly influenced by the quality of microalgae combination ($F = 20.7$, $p < 0.001$) rather than their availability ($F = 4.092$, $p < 0.05$). Nevertheless, the eggs fertilization was also significantly affected by diet and ration respectively ($F = 7.347$, $p < 0.01$ and $F = 4.645$, $p < 0.05$). For larvae rearing, as regards to physiological responses, small size strains *Isochrysis galbana* and *Pavlova lutheri* are the most suitable regimen during early larvae phase, while diatom *Chaetoceros calcitrans* and large size strain *Tetraselmis chui* are more appropriate during late larvae phase. Therefore, the mixture of the four microalgae was the only diet that led to metamorphosis and settlement throughout this experiment. Many studies are presently undertaken in the basis of these outcomes and improvements are achieved in order to promote production of local seeds in hatcheries.

Key Words: *Ruditapes decussatus*, diets, spawning, larvae rearing, Dakhla Bay.

Introduction. Dakhla Bay comprises a naturally exceptional littoral in which the principal exploitable species belong to crustacean, fish, cephalopods and shellfish. Certainly, the natural conditions that mark it, namely physico-chemical and climate conditions have generated an appropriate ecosystem for either growth and reproduction of several marine species. Hence, the actual rising of species invasion (Cohen et al 1998) alongside the depletion of resources by overfishing have promoted the ecosystems management in order to provide control measures from scientists (Bax et al 2001). Although, assessment for conservation of native species is often hindered by lack of basic ecological information and it is even difficult when the target species are commercially important (Bidegain et al 2015).

The grooved carpet shell clam *Ruditapes decussatus* is one of the oldest molluscs used in fishery practices and was one of the European's most important marine resources until the 1960's (Gosling 2002). It is globally distributed from the British Isles coastlines to the Mediterranean Sea and along the Atlantic coasts of Morocco and Senegal (Roméo et al 1995; Puigcerver 1996). In Morocco, it is distributed all across the marine ecosystems (bays, lagoons and estuaries) from Alboran Sea to the South Atlantic coasts (Shafee & Daoudi 1991; Kamara et al 2005). This species was widespread in the natural beds and was distributed along the quasi-totality of the Dakhla Bay but, because of its considerable economic importance, it has been heavily overexploited in all its habitats along the Moroccan coastlines (Shafee & Daoudi 1991). The over-exploitation of both young and adult individuals has greatly depleted the occurrence of this species in its

natural habitat and has imposed a management strategy for stock rehabilitation and shellfishery guideline. Few studies have been carried out on the local species and based typically on the biological and morphometric aspects (Kamara et al 2005; Amane et al 2019). This work was carried out in order to optimize the process of spat production in hatchery by screening the most appropriate practical process espacially during broodstock feeding and larvae rearing. The main goal of this work is to highlight the crucial status of local clam and to promote the hatchery efficiency and further rehabilitation strategy.

Material and Method

Clam sampling and broodstock conditioning. Two hundred and sixteen adult specimens of the grooved clam *R. decussatus* (mean shell length = 46 mm) were hand-collected on April 8th, 2016 from natural populations in Dunablanca (23°47'26" N/15°44'26" W, Dakhla Bay). Adults taken from the wild population were brought into the hatchery; their shells thoroughly scrubbed and rinsed to remove epifaunal organisms and sediment. Individuals were then placed in 20 L plastic tanks (8 individuals $tank^{-1}$) with a flow through circuit containing natural filtered seawater to 1 µm. The natural seawater has been pretreated using sand filter then filtred successively at 10, 5 and 1 µm. This study was performed under natural conditions of seawater (temperature: 21.5±0.6°C; salinity: 39.7±0.8 PSU; pH: 8.2±0.3 and dissolved oxygen: 6.4±0.5 mg L^{-1}). Adults were held on PVC rods with net bags so as not to retain faeces and detritus.

Feeding. Four strains of microalgae (*Isochrysis galbana*, *Pavlova lutheri*, *Tetraselmis chui* and *Chaetoceros calcitrans*) were used in furtherance to reveal the influence of their combination on spawning efficiency, fertilization rates and larvae rearing. Two bispecific diets, R1 (75% *I. galbana*; 25% *P. lutheri*) and R2 (75% *C. calcitrans*; 25% *T. chui*), and one tetraspecific diet R3 (75% *I. galbana* and *C. calcitrans*; 25% *P. lutheri* and *T. chui*) were mainly tested in this study. Moreover, three different rations (triplicate) were tested (2%, 4% and 6% of clam dry meat weight in dry weight of microalgae) within each diet. The required food ration estimation followed herein has been already cited (Utting & Millican 1997). Adults were receiving the daily ration halved twice per day in an open flow system.

Spawning and larvae rearing. After 48 days of conditioning, each broodstock batch was cleaned externally and left to dry for one hour. Clams were induced to spawn by thermal stimulation, through placing them in cool filtered water (8±2°C) over 20 minutes. Through the rapid increase of temperature, the water was drained and replaced by warm water (28°C) for one hour. During this step a small amount of microalgae and male sperms were added to stimulate the opening of adult shells and start pumping activity. After two repetitions, the clams began spawning and the individuals were separated by gender and put in beakers containing filtered seawater. The spawned individuals per diet and per ration were counted. The oocytes were fertilized by addition of 5 mL mixture of sperms from different males. After two hours of fertilization, the fertilized oocytes of each treatment (diets and ration) were sieved to eliminate the remaining sperms and unfertilized eggs.

All fertilized eggs were grouped per diets and larval rearing was handled in cylindro-conical tanks (500 L) with known initial density. Water was renewed each 48 h under natural conditions of seawater (temperature: 21.5±0.6°C; salinity 36-39 PSU; and pH: 8.2±0.3). During the larvae process, the filtred seawater (1 µm) has been also treated by Ultraviolet lights to avoid microbial contamination.

The influence of food quality on larvae performance, survival and deformation until day 18 was performed by feeding larvae with three different diets based on 100 cell μL^{-1} per day of food supply. Two bispecific diets R1 (75% *I. galbana*; 25% *P. lutheri*) and R2 (75% *C. calcitrans*; 25% *T. chui*), and one mixture diet R3 (75% *I. galbana* and *C. calcitrans*; 25% *P. lutheri* and *T. chui*) were daily administered. Each two days, larvae performance, survival and deformation were recorded based on 0.1 mL aliquots measurements (triplicate).

Statistical analysis. Induced spawning and fertilization success were evaluated through visual observation of the presence or absence of gametes in each container. If spawning occurs, the number of oocytes and the amount of fertilized eggs are calculated. Counts were submitted to Levene test to verify homogeneity of variance. In order to estimate the effect of each treatment (diet and ration) on both spawning and fertilization rates, a one-way analysis of variance (ANOVA) was performed at a 95% level of confidence (α = 0.05). When ANOVAs were significant, the post hoc Tukey multiple comparison test was used to determine which treatments differed. All statistical tests were analyzed using Rcmdr interface (Fox & Bouchet-Valat 2016) implemented in R package version 3.1.2 (R Development Core Team 2008). Reared larvae performance, survival and deformation under different treatments were visually examined using the inverted microscopy.

Results

Spawning and fertilization. Generally, the number of spawned females was always higher than male's for all treatments (56% and 44% respectively). For diets R1 and R2, the release of oocytes increases as much as the microalgae availability increases (Figure 1A). As regards to diets efficiency, the broodstock reared under R2 showed a slight increase of oocytes release comparing to broodstock reared under R1 for 2% (2.12×10^6 and 1.8×10^6 oocyte ind^{-1} respectively). But, for other rations, the oocytes release in R2 has approximatively twice as much R1 for 4% (5×10^6 and 2.45×10^6 oocyte ind^{-1} respectively) and for 6% (5.67×10^6 and 2.68×10^6 oocyte ind^{-1} respectively). Whereas, the broodstock reared under diet mixture (R3) has release the highest number of oocytes as regards other regimes especially under the ration 4% (7.65×10^6 oocyte ind^{-1}) followed by 6% and 2% respectively (6.20×10^6 and 4.60×10^6 oocyte ind^{-1}).

In terms of diet influence (among the 39 spawned females regardless the food ration), the mean total number of oocytes released was higher for broodstock reared under R3 ($(6.15\pm1.36) \times 10^6$ oocyte ind^{-1}) than under R2 and R1 respectively ($(4.26\pm1.67) \times 10^6$ and $(2.31\pm0.39) \times 10^6$ oocyte ind^{-1}) (Table 1). On the other hand, with regard only to food ration (regardless the dietary), clams have exhibited high oocytes release under 4% ($(5.03\pm2.28) \times 10^6$ oocyte ind^{-1}) followed by broodstock reared under 6% ($(4.85\pm1.65) \times 10^6$ oocyte ind^{-1} and 2% ($(2.84\pm1.34) \times 10^6$ oocyte ind^{-1}). The one-way analysis of variance indicates that the main factor that significantly influences spawning was quality of diets ($F = 20.7$, $p < 0.001$) followed by food availability (fed ration) ($F = 4.092$, $p < 0.05$) (Table 1).

The fertilization rate (FR) was likewise influenced by both diet and feeding ration during broodstock conditioning. Within R2 and R3 regimes, we observed that FR decreases consistently once the availability of microalgae is increased (Figure 1B). The percentage of fertilization success ranges from 67 to 79% and from 50 to 80% for R2 and R3 respectively depending on food ration. Although, clams reared under R1 diet have released oocytes with the highest FR across this work with 95% and 91% of fertilization success respectively for feeding ration 2% and 6% while ration 4% has shown only 70%. In terms of diet influence (among 38 million oocytes), R1 has shown the highest mean frequency of fertilized eggs (0.859 ± 0.119) followed respectively by diets R2 and R3 (0.739 ± 0.065 and 0.664 ± 0.131) (Table 1). A significant influence among nutritional regimes was observed in FR of released oocytes ($F = 7.347$, df. = 2, $p < 0.01$). On the other hand, in terms of ration influence, ration 2% revealed the highest FR (0.851 ± 0.087) followed by 4% and 6% (0.713 ± 0.041 and 0.698 ± 0.179 respectively). However, the food availability of microalgae during clams conditioning had a significant influence on fertilization rate ($F = 4.645$, df. = 2, $p < 0.05$) (Table 1).

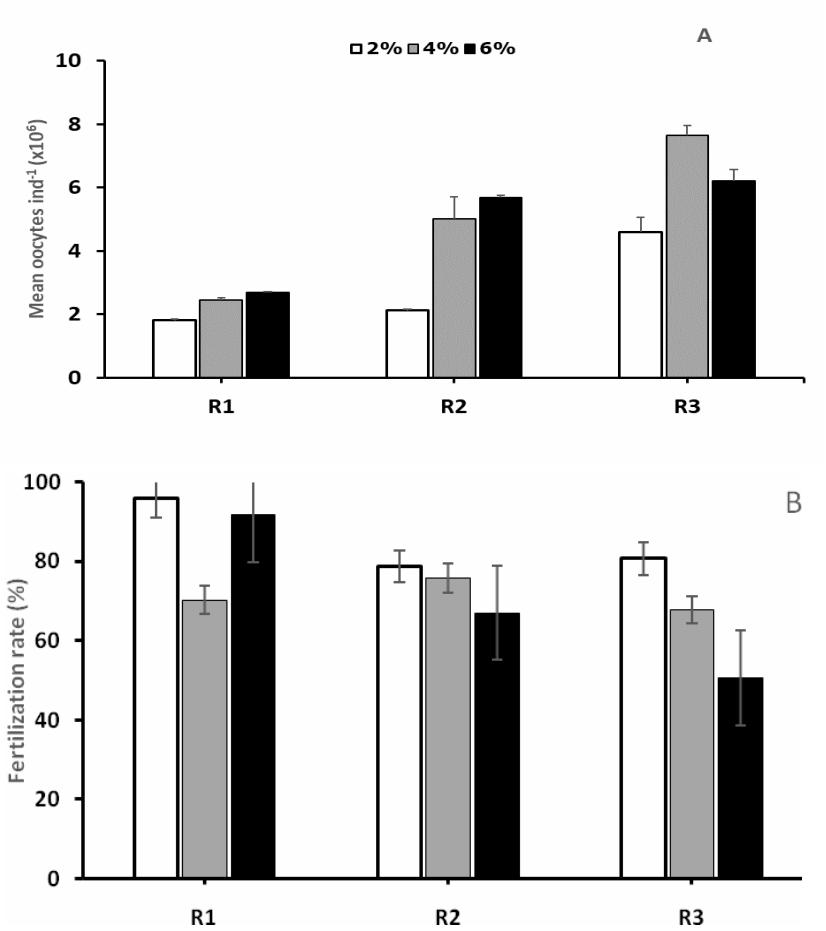

Figure 1. Influence of diet and ration of nutrition during broodstock conditioning on spawning (A) and fertilization rate (B) of carpet shell clam *R. decussatus.*

Table 1

Oocytes release and fertilization rate of *R. decussatus* as regards food quality and availability

Treatments		*Nb. SI*	*Nb. SF*	*Mean oocyte ind^{-1} (±SE)*	*df*	*F value*	*Mean fertilization rate (±SE)*	*df*	*F value*
Ration	2%	22	11	2.84 (±1.34) x 10^6	2	4.092*	0.851 (±0.087)	2	4.645*
	4%	24	15	5.03 (±2.28) x 10^6			0.713 (±0.041)		
	6%	23	13	4.85 (±1.65) x 10^6			0.698 (±0.179)		
Diet	R1	24	14	2.31 (±0.39) x 10^6	2	20.7***	0.859 (±0.119)	2	7.347**
	R2	23	13	4.26 (±1.67) x 10^6			0.739 (±0.065)		
	R3	22	12	6.15 (±1.36) x 10^6			0.664 (±0.131)		

Nb = number; SI = spawned individuals; SF = spawned female; SE = standard error; df = degree of liberty. One-way ANOVA was used to compare mean differences across rations and diets treatment (significance level: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Larvae rearing. The total number of larvae has been decreased during the larvae rearing process under all treatments (R1, R2 and R3). After 24 h of fertilization, the survival of trochophore larvae was very important for R2 (44%) followed by R1 and R3 respectively (22% and 18%). During the larvae development from trochophore into D shape, the highest survival was observed for larvae reared under R1 diet (30%) followed by larvae reared under R3 and R2 (14% and 10% respectively). Throughout development from D shape to veliger phase, the most important survival was attained for larvae reared under R3 diet (57%) followed by R2 and R1 respectively (33% and 23%). While regarding larvae development from veliger to pediveliger phase, the highest survival was reached for larvae reared under R3 diet (48%) then R1 (38%) where no pediveliger larvae was attained under R2 regime.

Generally, among 12.7 x 10^7 fertilized eggs, only 8 x 10^5 larvae have reached the pediveliger phase with an approximate survival 0.61% by the end of larval experiment for R1 diet (Table 2). For R3 regime, only 0.74% has reached the pediveliger phase among 7.94 x 10^7 fertilized eggs. Whereas, for R2 diet, among 5.19 x 10^7 fertilized eggs, an absolute mortality was obtained during veliger phase where no pediveliger larvae have been attained.

Table 2

Larvae performance of *R. decussatus* under different diets (R1, R2 and R3)

		R1	*R2*	*R3*
Trochophore	Mean (x 10^7)	2.90	2.30	1.47
	SD (x 10^7)	±0.165	±0.043	±0.061
	Survival (%)	22.7	44.3	18.5
D-shape larvae	Mean (x 10^7)	0.88	0.25	0.22
	SD (x 10^7)	±0.018	±0.023	±0.018
	Survival (%)	30.3	10.8	14.7
	Cum. Surv (%)	6.88	4.82	2.72
Veliger	Mean (x 10^7)	0.21	0.08	0.12
	SD (x 10^7)	±0.021	±0.008	±0.012
	Survival (%)	23.5	33.3	56.9
	Cum. Surv (%)	1.62	1.61	1.55
Pediveliger	Mean (x 10^7)	0.08	0.00	0.06
	SD (x 10^7)	±0.003	±0.00	±0.002
	Survival (%)	37.7	0	47.9
	Cum. Surv (%)	0.61	-	0.74
Spat	Fixation (%)	0	0	3.1

SD = standard deviation; Cum. Surv = cumulative survival.

Discussion

Effect of feeding on spawning. Conditioning is a crucial process that aims preparing *R. decussaus* broodstock for spawning. Maturation is regularly controlled by several factors, such as nutrition, temperature and photoperiod (Matias et al 2016). Mainly, temperature and food quality and availability were considered the most fundamental factors that affect the gonad development (Matias et al 2016).

The influence of feeding regime during broodstock conditioning has previously been investigated on many bivalve species, such as *Ostrea edulis* (Millican & Helm 1994), *Pecten maximus* (Utting & Millican 1998) and *Mytilus* (Pronker et al 2008; Aghzar et al 2013). As regards clam (*Ruditapes*), some studies were often carried out on manila clam *R. philippinarum* (Leal 1994; Laing & Loppez-Alvarado 1994), while several were established on *R. decussatus* (Ojea et al 2008; Matias et al 2014; Abbas et al 2018). Referring to this study, the results obtained demonstrate the effect of both type and availability of food during conditioning, as evidenced by the differences in spawning efficiency.

In terms of the effect of diet quality, clams that received *T. chui* + *C. calcitrans* have approximatively twice the average of oocytes released with 56.5% of female spawning success than clams fed with *I. galbana* + *P. lutheri* with 58% of female spawning success. This, indicates that the combination of diatom strain *C. calcitrans* with flagellate strain especially *T. chui* is most suitable for providing energy during maturation process. Previous study was handled on *R. decussatus* where clams reared with *T-iso* + *C. calcitrans* showed the highest oocytes release (0.87 x 10^6 per female) (Matias et al 2016). Whereas, the average attained herein is approximatively five times higher when we replaced *I. galbana* (clone *T-iso*) with *T. chui* strain. This actually do not systematically indicate the over-suitableness of *T. chui*, but many factors such as temperature (e.i. Ojea et al 2008), photoperiod (Pazos et al 2003), origin (Matias et al 2009) and conditioning period (Helm & Bourne 2004; Delgado & Pérez-Camacho 2005)

can contribute to explain the observed differences in spawning efficiency of local clams. Abbas et al (2018), has concluded that the combination of *C. calcitrans* and *T. suecica* has revealed the highest performance which is congruent with our results when using *T. chui* combined with *C. calcitrans*. Furthermore, clams reared with the mixture of four strains *I. galbana* + *P. lutheri* + *C. calcitrans* + *T. chui* have spawned the highest average number of oocytes even only with 54.5% of spawning success.

As regards the quantity of available food within the studied ration, our results have shown spawning was significantly increased with food quantity, whereas, the emission of gametes appeared to be accelerated by more favorable food rations. In fact, this can be clearly explained by the influence of available food on gonadal maturation (Buchanan et al 1998; Kent et al 1998). Accordingly, Delgado & Pérez-Camacho (2005) have described that high food availability has accelerated the characteristics of gametogenesis and then increased the quantity of gonads comparing to restricted food ration. Similar study has shown higher condition index and speed up maturation for *C. chione* broodstock when reared under 6% rather than 3% dry weight (Martinez-Pita et al 2016). Furthermore, the period of spawning appeared to be accelerated by more microalgae availability in natural population of *R. decussatus* (Rodriguez-Moscoso 2000). Moreover, the period of conditioning process (50 days herein) could also affect the spawning yield of reared clams (Delgado & Pérez-Camacho 2005).

Effect of feeding on egg fertilization. The quality of released oocytes has been clearly confirmed by fertilization rate. In fact, the food abundance does systematically influence the spawning success but not necessary the fecundity rate. Some previous studies were carried out on the effect of diet quality on *R. decussatus* fertilization rate (Matias et al 2016), but none have dealt with the influence of food availability. Consequently, many hypothesizes could be upheld to explain the negative response in terms of fertilization process toward food abundance during conditioning. Nonetheless, the most logical hypothesis assuming that abundance of nutrition speeds up gonadal development of local clams and enhances the release of maximum oocytes but with low fertilization efficiency. This means that further histological analysis should be carried out during gonadal maturation under different level of nutrition availability to reject or confirm our assumption. Whereas, many studies have demonstrated that the size of gonads and the fecundity are primarily determined by the amount of food available during bivalves broodstock conditioning (Heasman et al 1996). In contrast to our study, the fecundity and eggs quality of *Pecten maximus* were increased when food supplements were administered (Devauchelle & Mingant 1991). However, in *Mytilus edulis* a daily low ration (1.6%) was revealed enough to obtain high fecundity and egg quality (Pronker et al 2008).

Effect of diet on larvae performance. Generally, during the early larvae stage, the egg uses its endogenous energetic reserves and dissolved organic matter to insure the larvae development process. However, several experimental studies in many species, *R. decussatus* (Matias et al 2014), *Meritrix meritrix* (Tang et al 2006) and *Crassostrea gigas* (Rico-Villa et al 2006) have demonstrated the effect of nutrition on the larvae performance even since early larvae. Several authors have studied the effect of biochemical composition of different diets on bivalve larvae performance (Matias et al 2014; Rico-Villa et al 2006). The current results reveal also the prominence of providing *R. decussatus* larvae with appropriate regime during each development stage.

During the embryonic stage, regimes mainly composed of *I. galbana* and *P. lutheri* were more appropriate for growth and survival of early larvae. This could be explained by the relatively small size and bio-volume of this strains comparing to larvae shape and size (Fernandez-Pardo et al 2016; da Costa et al 2020). Furthermore, the high lipid content of microalgae in diet R1 could also explain the high larvae mortality (Aranda-Burgos et al 2014). Nonetheless, none of the pediveliger larvae have been settled which may be clearly explained by crucial requirement of diatoms especially for larvae metamorphosis (Pettersen et al 2010; Ragg et al 2010). The diatoms availability is most

beneficial during late larvae development for many bivalve species (Pettersen et al 2010; Ragg et al 2010; Aranda-Burgos et al 2014; Fernandez-Pardo et al 2016).

Whereas, in a previous work, Matias et al (2014) demonstrated that *R. decussatus* early larvae did not efficiently assimilate *C. calcitrans*. On the other hand, the same authors have proposed that optimal larvae performance consisted on a mixture of *T. lutea* and *C. calcitrans*. Nevertheless, the observed absolute mortality for R2 diet could be explained by restricted assimilation of available microalgae (*C. calcitrans* and *T. chui*) during early larvae development and starvation after exhausting the endogenous reserves of fertilized eggs. Actually, reduced survival due to high *C. calcitrans* treatment during early larvae has been also observed in other bivalve species such as *Perna canaliculus* (Ragg et al 2010).

Although, diet based on the admixture of four strains (R3) has shown better larvae performance especially since D-shape where survival was around 50%. Hence, the quality of microalgae mosaic used since the beginning has greatly influenced the quantity of beneficial strains during the appropriate phase. For instance, the concentration used was 100 cell μL^{-1}, which means only 25 cell μL^{-1} of each strain per day. Consequently, the amount of appropriate strain needed for larvae growth during each phase was very critical for better performance, whilst other strains are not consumed. Whereas, during late larvae, diatoms and large size microalgae are quite beneficial and increased the survival of larvae to achieve metamorphosis. The first interpretation explaining high mortality and low metamorphosis is the availability of appropriate food during each development phase. The appropriateness of each species may be due to its morphology (Robert et al 2004) and biochemical composition especially fatty acids EPA and DHA (da Costa et al 2016). Diatoms such as *C. calcitrans* are rich of EPA and DHA and the ratio (DHA+EPA)/ARA is considered the main key factor for larvae performance and settlement success (Pettersen et al 2010).

Generally, many other factors could be associated to explain this issue including temperature (Beiras et al 1994), rearing density (Perez-Camacho et al 1977) and microbacterial aspect (Mechri et al 2015; Dubert et al 2017). However, more insight should be given into the nutrition effect on eggs quality in terms of their biochemical inherent and physiological variability.

Conclusions. This work is the first preliminary study devoted to understand the response of *R. decussatus* to production success in hatcheries in Morocco. In the meanwhile, many aspects (biochemical, physiological and genetic) are already undertaken to optimize zoo-technical performance of local clam and to define its taxonomic status.

Acknowledgements. This study is a product of partnership between National Institute of Fisheries Research (INRH) and AZURA hatchery (Dakhla, Morocco). We would like to covey special thanks to all participants for their contribution, ongoing support and valuable advices throughout this work.

References

Abbas A. S., El-Wazzan E., Khafage A. R., El-Sayed A. F. M., Razek F. A. A., 2018 Influence of different microalgal diets on gonadal development of the carpet shell clam *Ruditapes decussatus* broodstock. Aquaculture International 26:1297-1309.

Aghzar A., Miñambres M., Alvarez P., Presa P., 2013 A cost-benefit assessment of two multi-species algae diets for juveniles of *Mytilus galloprovincialis*. Thalassas 29(1):9-16.

Amane Z., Tazi L., Idhalla M., Chlaida M., 2019 Morphometric analysis of European clam *Ruditapes decussatus* in Morocco. AACL Bioflux 12(5):1623-1634.

Aranda-Burgos J. A., da Costa F., Nóvoa S., Ojea J., Martínez-Patiño D., 2014 Effects of microalgal diet on growth, survival, biochemical and fatty acid composition of *Ruditapes decussatus* larvae. Aquaculture 420-421:38-48.

Bax N., Carlton J. T., Mathews-Amos A., Haedrich R. L., Howarth F. G., Purcell J. E., Rieser A., Gray A., 2001 The control of biological invasions in the world's oceans. Conservation Biology 15(5):1234-1246.
Beiras R., Perez-Camacho A., Albentosa M., 1994 Influence of temperature on the physiology of growth in *Ruditapes decussatus* (L.) larvae. Journal of Shellfish Research 13(1):77-83.
Bidegain G., Barcena J. F., García A., Juanes J. A., 2015 Predicting coexistence and predominance patterns between the introduced Manila clam (*Ruditapes philippinarum*) and the European native clam (*Ruditapes decussatus*). Estuarine Coastal and Shelf Science 152:162-172.
Buchanan J. T., Roppolo G. S., Supan J. E., Tiersch T. E., 1998 Conditioning of eastern oysters in a closed, recirculating system. Journal of Shellfish Research 17(4):1183-1189.
Cohen A. N., Carlton J. T., 1998 Accelerating invasion rate in a highly invaded estuary. Science 279(5350):555-558.
da Costa F., Petton B., Mingant C., Bougaran G., Rouxel C., Quéré C., Wikfors G. H., Soudant P., Robert R., 2016 Influence of one selected *Tisochrysis lutea* strain rich in lipids on *Crassostrea gigas* larval development and biochemical composition. Aquaculture Nutrition 22(4):813-836.
da Costa F., Cerviño-Otero A., Iglesias Ó., Cruz A., Guévélou E., 2020 Hatchery culture of European clam species (family Veneridae). Aquaculture International 28:1675-1708.
Delgado M., Pérez-Camacho A., 2005 Histological study of the gonadal development of *Ruditapes decussatus* (L.) (Mollusca: Bivalvia) and its relationship with available food. Scientia Marina 69(1):87-97.
Devauchelle N., Mingant C., 1991 Review of the reproductive physiology of the scallop, *Pecten maximus* L., applicable to intensive aquaculture. Aquatic Living Resource 4(1):41-51.
Dubert J., Aranda-Burgos J. A., Ojea J., Barja J. L., Prado S., 2017 Mortality event involving larvae of the carpet shell clam *Ruditapes decussatus* in a hatchery: isolation of the pathogen *Vibrio tubiashii* subsp. europaeus. Journal of Fish Diseases 40(9):1185-1193.
Fernandez-Pardo A., da Costa F., Rial D., Nóvoa S., Martínez-Patiño D., Vázquez J. A., 2016 Use of response surface methodology to determine optimum diets for *Venerupis corrugata* larvae: effects of ration and microalgal assemblages. Aquaculture 452:283-290.
Fox J., Bouchet-Valat M., 2016 Rcmdr: R Commander. R package version 2.3-1.
Gosling E., 2002 Bivalve molluscs: biology, ecology and culture. Fishing News Books, (UK) Blackwell Publishing, 455 pp.
Heasman M. P., O'Connor W. A., Frazer A. W., 1996 Temperature and nutrition as factors in conditioning broodstock of the commercial scallop *Pecten fumatus* Reeve. Aquaculture 143(1):75-90.
Helm M. M., Bourne N., 2004 Hatchery operation: broodstock conditioning, spawning and fertilization. In: Hatchery culture of bivalves: a practical manual. Helm M., Bourne N., Lovatelli A. (eds), FAO, Rome, Fisheries Technical Paper No. 471, pp. 59-81.
Kamara A., Rharbi N., Berraho A., Ramdani M., 2005 [Comparative study of the sexual cycle of the *Ruditapes decussatus* clam from three parallel environments of the Moroccan coasts]. Mar Life 15(1-2):43-50. [in French]
Kent G. N., Maguire G. B., John M., Cropp M., Frankish K., 1998 Broodstock conditioning, spawning induction, and larval rearing of the stepped venerid, *Katelysia scalarina* (Lamarck 1818). Journal of Shellfish Research 17(4):1065-1070.
Laing I., Lopez Alvarado J., 1994 Effect of dried algae diets on conditioning' and fecundity of Manila clam, *Tapes philppinarum* (Adams and Reeve). Aquaculture Research 25(2):157-166.
Leal A. M., 1994 Conditioning of Manila clam broodstock on natural and artificial diets. PhD Thesis. UK: Bangor University, United Kingdom, 343 pp.

Martínez-Pita I., Sánchez-Lazo C., García F. J., 2016 Influence of microalga lipid composition on the sexual maturation of *Mytilus galloprovincialis*: a hatchery study. Aquaculture Nutrition 22(1):202-216.
Matias D., Joaquim S., Leitão A., Massapina C., 2009 Effect of geographic origin, temperature and timing of broodstock collection on conditioning, spawning success and larval viability of *Ruditapes decussatus* (Linné, 1758). Aquaculture International 17(3):257-271.
Matias D., Ben-Hamadou R., Joaquim S., Matias A., Sobral P., Leitao A., 2014 The influence of different microalgal diets on European clam (*Ruditapes decussatus*, Linnaeus, 1758) larvae culture performances. Aquaculture Research 46(10):2527-2543.
Matias D., Joaquim S., Matias A. M., Leitão A., 2016 Reproductive effort of the European clam *Ruditapes decussatus* (Linnaeus, 1758): influence of different diets and temperatures. Invertebrate Reproduction & Development 60(1):49-58.
Mechri B., Salem I. B., Medhioub A., Medhioub A., Medhioub M. N., Aouni M., 2015 Isolation and genotyping of potentially pathogenic *Vibrio alginolyticus* associated with *Ruditapes decussatus* larva and juvenile mass mortalities. Aquaculture International 23(4):1033-1047.
Millican P. F., Helm M. M., 1994 Effects of nutrition on larvae production in the European flat oyster, *Ostrea edulis*. Aquaculture 123(1-2):83-94.
Ojea J., Pazos A. J., Martínez D., Novoa S., García-Martínez P., Sánchez J. L., Abad M., 2008 Effects of temperature regime on broodstock conditioning of *Ruditapes decussatus*. Journal of Shellfish Research 27(5):1093-1100.
Pazos A. J., Silva A., Sordo A., Abad M., Sánchez J. L., 2003 [Influence of the photoperiod on the conditioning of the fine clam (*Ruditapes decussatus*), Linné 1787]. Proceedings of the IX Congreso Nacional de Acuicultura, Cádiz, Spain, pp. 293-295. [in Spanish].
Pérez-Camacho A., Román G., Torre M., 1977 [Experiences in larval cultures of three species of bivalve molluscs: *Venerupis pullastra* (Montagu), *Venerupis decussata* (Linnaeus), and *Ostrea edulis* (Linnaeus)]. Boletim do Instituto Oceanográfico n° 235, pp. 10-61. [in Spanish]
Pettersen A. K., Turchini G. M., Jahangard S., Ingram B. A., Sherman C. D., 2010 Effects of different dietary microalgae on survival, growth, settlement and fatty acid composition of blue mussel (*Mytilus galloprovincialis*) larvae. Aquaculture 309(1-4):115-124.
Pronker A. E., Nevejan N. M., Peene F., Geijsen P., Sorgeloos P., 2008 Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus 1758): part I. Impact of different micro-algae mixtures on broodstock performance. Aquaculture International 16:297-307.
Puigcerver M., 1996 Analysis on individual growth of juvenile carpet shell clams, *Tapes decussatus* (L.), in three different rearing conditions employed in the Ebro's Delta, Spain. Aquaculture Research 27(6):399-404.
Ragg N. L. C., King N., Watts E., Morrish J., 2010 Optimising the delivery of the key dietary diatom *Chaetoceros calcitrans* to intensively cultured Greenshell™ mussel larvae, *Perna canaliculus*. Aquaculture 306(1-4):270-280.
R Development Core Team, R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria 2008, ISBN 3-900051-07-0, URL: http://www.R-project.org.
Rico-Villa B., Le Coz J. R., Mingant C., Robert R., 2006 Influence of phytoplankton diet mixture on microalgae consumption, larval development and settlement of the Pacific oyter *Crassostrea gigas* (Thunberg). Aquaculture 256(1-4):377-388.
Robert R., Chrétiennot-Dinet M. J., Kaas R., Martin-Jézéquel V., Moal J., Le Coz J. R., Nicolas J. L., Bernard E., Connan J. P., Le Dean L., Gourrierec G., Leroy B., Quéré C., 2004 [Improvement of phytoplankton productions in mollusc hatchery: characterization of forage microalgae]. RI DRV/RA-2004-05, 149 pp. [in French]

Rodriguez-Moscoso E., 2000 [Histophysiology of the reproduction of the fine clam *Ruditapes decussates* (Linné, 1758) in the Ría de Arosa (natural population and culture population)]. PhD thesis, Santiago de Compostela University, Spain. [in Spanish]
Roméo M., Gnassia-Barelli M. M., 1995 Metal distribution in different tissues and in subcellular fractions of the Mediterranean clam *Ruditapes decussatus* treated with cadmium, copper, or zinc. Comparative Biochemistry and Physiology Part C 111(3):457-463.
Shafee M. S., Daoudi M., 1991 Gametogenesis and spawning in the carpet-shell clam, *Ruditapes decussatus* (L.) (Mollusca: Bivalvia), from the Atlantic coast of Morocco. Aquaculture Research 22:203-216.
Tang B., Liu B., Wang G., Zhang T., Xianga J., 2006 Effects of various algal diets and starvation on larval growth and survival of *Meretrix meretrix*. Aquaculture 254(1-4):526-533.
Utting S. D., Millican P. F., 1997 Techniques for the hatchery conditioning of bivalve broodstocks and the subsequent effect on egg quality and larval viability. Aquaculture 155(1-4):45-54.
Utting S. D., Millican P. F., 1998 The role of diet in hatchery conditioning of *Pecten maximus* L.: a review. Aquaculture 165(3-4):167-178.

Received: 06 November 2020. Accepted: 30 November 2020. Published online: 17 December 2020.
Authors:
Yassine Ouagajjou, National Institute of Fisheries Research, INRH, BP 31 M'diq, Morocco, e-mail: ouagajjou@inrh.ma; yassinewag200@gmail.com
Touryia El Aloua, Faculty of Science, Chouaib Doukkali University, El Jadida 24000, Morocco, e-mail: touryanaim@gmail.com
Mohamed El Moussaoui, Department of Biology, Faculty of Science, Abdelmalek Essaadi University, Tétouan 93002, Morocco, e-mail: mohamedalielmoussaoui@gmail.com
El Mustafa Ait Chattou, National Institute of Fisheries Research, INRH, BP 127 (bis) Dakhla, Morocco, e-mail: mustachattou@gmail.com
Adil Aghzar, Department of Environmental Engineering and Agrobiotechnology, Higher School of Technology, Sultan Moulay Slimane University, Khénifra, Morocco, B.P. 170, 54000 Khénifra, Morocco, e-mail: aaghzar@gmail.com/a.aghzar@usms.ma
Younes Saoud, Department of Biology, Faculty of Science, Abdelmalek Essaadi University, Tétouan 93002, Morocco, e-mail: syounes60@yahoo.fr

How to cite this article:
Ouagajjou Y., El Aloua T., El Moussaoui M., Ait Chattou E. M., Aghzar A., Saoud Y., 2020 First insight into nutrition effect on spawning and larvae rearing of the clam *Ruditapes decussatus* L. from Dakhla Bay. AACL Bioflux 13(6):3664-3673.