ISSN Versión Impresa 1816-0719 | ISSN Versión en linea 1994-9073 | ISSN Versión CD ROM 1994-9081

The Biologist (Lima), 2017, 15(1), jan-jun: 109-117.

The Biologist (Lima)

The Biologist (Lima)

ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

USE OF MICROALGAE FOR BROODSTOCK CONDITIONING OF *MESODESMA DONACIUM* (MESODESMATIDAE)

USO DE MICROALGAS PARA EL ACONDICIONAMIENTO DE REPRODUCTORES DE *MESODESMA DONACIUM* (MESODESMATIDAE)

Sheyla Zevallos[1] & Pedro Toledo[2]

[1] *Laboratorio de Investigación Acuícola (LIA) del Instituto del Mar del Perú (IMARPE), sede Ilo, Jr. Mirave 101 Ilo, Moquegua 18601, Perú*
[2] *Departamento Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo 1780000, Chile*
Corresponding author. E-mail: szevallos@imarpe.gob.pe (Sheyla Zevallos)

ABSTRACT

Adult *Mesodesma donacium* were conditioned with the following microalgae diets: D1) *Isochrysis galbana* (local) + *Chaetoceros* sp. (local) + *Phaeodactylum tricornutum* (local); D2) *I. galbana* (local) + *Ch. gracilis* (commercial) + *Pavlova lutheri* (commercial); D3) *Chaetoceros* sp. (local) + *I. galbana* (commercial) + *P. lutheri* (commercial); and a control (C) with commercial *I. galbana*, *Ch. gracilis*, and *P. lutheri*. *M. donacium* were fed with a continuous feeding system (2.0 x 10^5 cells/mL) and maintained at 16 °C, with constant aeration and daily full water replacement. Chemical and histological analyses determined gonadal development and total lipid content. Gonadal development significantly differed as a result of the different diets, with D2 individuals showing the best response at day 44 of conditioning (56% mature). Total lipid content did not significantly differ between groups, although levels were higher than ocean-sampled *M. donacium*. Moreover, temperature did not influence gonadal development or total lipid content, with no correlation between this factor and dependent variables. To optimize broodstock conditioning of *M. donacium*, a continuously administered microalgae diet is recommended under constant temperatures and with consideration to total lipid content.

Keywords: bivalves – gonadal development – local microalgae – total lipids

RESUMEN

Ejemplares adultos de *Mesodesma donacium* fueron acondicionados bajo condiciones controladas aplicando tres diferentes mezclas de microalgas: D1) *Isochrysis galbana* (local) + *Chaetoceros sp* (local) + *Phaeodactylum tricornutum* (local); D2) *I. galbana* (local) +*Ch. gracilis* (comercial) + *Pavlova lutheri* (comercial); D3) *Chaetoceros sp* (local) +I. galbana (comercial) + *P. lutheri* (comercial) y un control (C) con las microalgas comerciales *I. galbana*, *Ch. gracilis* y *P. lutheri*. Las microalgas fueron suministradas a los adultos de *M. donacium* mediante un sistema de alimentación continuo en una concentración de 2,0 x 10^5cel/mL, a una temperatura promedio de 16°C, aireación constante y recambio diario de agua. Los análisis químicos e histológicos determinaron el desarrollo gonadal y el contenido total de lípidos. El desarrollo gonadal mostró diferencias significativas como resultado de las dietas suministradas, siendo D2 la que presenta la mejor respuesta a los 44 días de acondicionamiento (56% maduros). El contenido total de lípidos no presentó diferencia significativa entre los tratamientos, pero fueron superiores a los evidenciados en medio natural. Además, la temperatura no influyó en el desarrollo gonadal ni en el contenido total de lípidos, descartando la correlación entre este factor y las variables dependientes. Para optimizar el acondicionamiento de reproductores de *M. donacium*, se recomienda utilizar un sistema de alimentación continuo, manteniendo la temperatura constante y considerando los lípidos totales como una estrategia de manejo en medio controlado.

Palabras clave: bivalvos – maduración gonadal – microalgas locales – lípidos totales

INTRODUCTION

Mesodesma donacium (Lamarck, 1818) is a Lamellibranchia bivalve of commercial interest that inhabits the mid- and infralittoral zones of sandy beaches exposed to waves (Segura *et al.,* 1998), extending from the intertidal zone to a depth of 15 m (Carré, 2007). This mollusk is distributed from the Bay of Sechura, Peru (5°S) to the Inio river mouth located at the extreme southern end of Chiloé, Chile (43°S) (Alamo & Valdivieso, 1997; Vargas, 2003; Zaro, 2004; Santelices, 2006). The commercial extraction of *M. donacium* and increasing ocean surface temperatures (Quiroz & Barriga, 1997) are factors that have contributed to the decrease and collapse of natural beds of this bivalve (Segura *et al.*, 1998). In fact, the capture of *M. donacium* is banned in Peru.

A potential alternative for recovering populations of this species is through the incorporation of broodstock-obtained juveniles into the natural environment. For this to occur, considerable consideration should be given to nutritional aspects (Farías & Uriarte, 2002) and the survival of bivalve larvae and juveniles (Contreras, 2006). In the last 40 years, hundreds of microalgae have been assessed for nutritional applications, but only 20 species are currently used in the aquaculture industry – (Priyadarshani *et al.*, 2012). The nutritional quality of an alga is a determinant of its final utilization –(Volkman *et al.*, 1989), with local microalgae acting as natural food sources (Borowitzka, 1997).

Microalgae are an important food source in the commercial breeding of aquatic animals, especially in larval and juvenile bivalve molluscs, rotifers use to feed larvae of crustaceans and marine fish (Borowitzka, 1997); source depends on the content and nature of its biochemical constituents, representing the basis for the formation of new tissue, restoration of those who have been affected and for normal metabolism of organisms breeding. A combination of algae as diet provides more nutritional value because they contain an assortment of most nutritional requirements for growth of the larvae, are these shrimps, molluscs and fish (Álvarez, 1994).

They have reported numerous studies aimed at analyzing the effect of different diets and nutritional components on both the gonadal maturation and larval development in bivalve; as reporting on the microalgal most commonly use for

the production of molluscs cultured controlled were T - Iso and *Chaetoceros neogracile* (S.L.VanLandingham, 1968), associated with elevated levels of total lipid and docosahexaenoic acid (DHA) in T - Iso and high levels of carbohydrates, eicosapentaenoic acid (EPA) and riboflavin in *C. neogracile* (Farías & Uriarte, 2002).

Farias (2008) reports that within the requirements lipid are the main energy source and in particular triglycerides of fatty acids, saturated short chain are predominantly used for energy purposes, β oxidation way (Borowitzka, 1997). On the other hand, the main function of essential fatty acids in bivalves, as part of membrane phospholipids, is the regulation of the flow of cellular and subcellular membranes, and acclimation to different temperature ranges.

Highlighting reproductive studies *Argopecten purpuratus* (Lamarck, 1819) maintained at 16 ° C and fed with a mixture of microalgaes *Isochrysis galbana* (Parke, 1949) and *Chaetoceros gracilis* (Pantocsek, 1892), obtaining lipid and gonadal recoveries achieved with higher percentages of 42.64 % ± 4.68 maturity after the first 10 days in a period of 80 days reproductive conditioning (Martínez *et al*., 2000).

In the case of *M. donacium*, there is a lack of information Salgado & Ishiyama (1979) showed that immature and less mature stages occur June through August; intermediate stages and full maturity from August to November; stages of partial or total evacuation from December to March, and are gradually descending until July; stages of gonadal reversal observed from February to July.

In this context, the aim of the present study was to evaluate the effect of different mixed local and commercial microalgae diets on the gonadal development of broodstock *M. donacium.*

MATERIALS AND METHODS

Collection area and samples
Adult *M. donacium* were collected from La Punta, Arequipa, Peru (17º11'S-71º47'W) and transported to the Laboratory of Mollusk Research at the Instituto del Mar, Ilo, Peru.

Random samples of 21 specimens were made before the start of laboratory tests and 29 specimens during the fortnight of December for the morphometric measurement, determination of gonadal status and biochemical profile.

Broodstock conditioning
Adult *M. donacium* (56-84 mm length) were conditioned in a tank with four 20 L trays (30 x 20 cm) that contained sifted and washed sandy substrate (0.5 mm diameter, phi 2-1) (Wentworth, 1922). Twenty *M. donacium* individuals were placed in each tray. The seawater in the tank was maintained at an average of 16±1°C and was fully replaced every 36 h by an air lift pump. The dissolved oxygen content was kept at 3.61 mL/L, while salinity was maintained at 34.824 psu.

Dietary trials
Strains of local microalgae were collected from the surface of the water column at sandy ocean floor beaches in Vila Vila (Tacna, Peru; 18°06'S-70°43'W) and La Punta (Arequipa, Peru; 17º11'S–71º47'W) using a 20 µm mesh net. The microalgae were isolated using either successive dilutions or dish isolation with a Pasteur pipette (Almaguer *et al*., 2004). The microalgae were then acclimated in dishes with a liquid medium enriched with F/2 Guillard (1975) for growth and maintenance for a month. Samples of each microalgae were sent to the Germplasm Bank for Aquatic Organisms of the Instituto del Mar, Peru and were identified as *Isochrysis galbana* (Parke, 1949), *Chaetoceros* sp, and *Phaeodactylum trycornutum* (Bohlin, 1898).

The commercial microalgae used in the dietary assays were *Ch. gracilis*, *Pavlova lutheri* (J.C.Green, 1975), and *I. galbana.* These microalgae were obtained from the Central Laboratory for Marine Cultures of the Faculty of Marine Sciences at the Universidad Católica del Norte, Coquimbo, Chile.

Three diets were formulated using mixes of local and commercial microalgae. The diets respectively used each microalgae in a 35-30-35% proportion: D1) *I. galbana* (local) + *Chaetoceros* sp (local) +

Ph. tricornutum (local); D2) *I. galbana* (local) + *Ch. gracilis* (commercial) + *P. lutheri* (commercial); D3) *Ch.* sp (local) + *I. galbana* (commercial) + *P. lutheri* (commercial); and C) a control using commercial *I. galbana*, + *Ch. gracilis,* + *P. lutheri*. These microalgae diets were administered daily using a continuous feeding system at a concentration of 2.0×10^5 cells/mL.

Analysis of gonadal development

Of the collected *M. donacium*, eight were measured and examined under an optic microscope to determine sex and gonad development. Every 15 days during the dietary trials, all *M. donacium* individuals were measured (total length, TL; ± 0.01 mm) and weighed (total weight, TW; and soft tissue weight, WST; ± 0.001 g). Additionally, the sex and gonadal development of eight individuals were determined every 15 days during the dietary trials through smear analysis under a microscope using the scale proposed by Buitrón & Perea (1996). In addition to these determinations, four individuals were sampled every 15 days and preserved in 96° ethanol for posterior histological analyses. The remaining samples were frozen at -13 °C until determinations of fat content.

Chemical assays

The soft tissues of *M. donacium* from the different diets were separated by sex and homogenized. The moisture content (AOAC, 2000) was determined for the soft tissue samples of *M. donacium* by drying them at 95 °C in a forced air drying oven (H.W. Kessel) until reaching a constant weight. Total lipid content was determined with the Soxhlet Soxtec Fat Extraction System 2045, using 25-50 mL of hexane as a solvent (Foss, 1992).

Statistical analysis

The Chi-squared test was used for categorical data to compare the proportion of each sex and gonadal development in function diets of *M. donacium* individuals. A one-way analysis of variance (ANOVA) was used to compare total lipid results, while a multivariate ANOVA ($p = 0.05$) was used to determine the influence of temperature on gonad development and the percent of total lipids. Data normality and homocedasity were verified before analyses, which were performed in the SPSS v.21.0 statistical software. If significant differences were found, an *a posteriori* Tukey test was applied.

RESULTS

Effect of diet on gonadal development in M. donacium

The Chi-squared test ($X^2 = 18.46$) demonstrated significant differences in gonad maturity between the *M. donacium* samples ($p < 0.05$) in relation to the distinct diets during the reproductive conditioning. In the D2 group, 60% were classified in stage 3 (mature) of the reproductive cycle, followed by the C group with 55%; the D1 group with 50%; and the D3 group with 10%. Likewise, the D1 group presented the lower percentage of *M. donacium* in stage 4 (20%), followed by D2 and C (35%), and D3, with 70% of individuals in the spawning stage (Table 1).

Regarding gonadal development at the beginning of November, the majority of the broodstock were spawning (stage 4; 44%), while 25% were in recovery (stage 5). Over the trial period, gonadal development was progressive, and by the first and second halves of December, 56 and 50% of the conditioned individuals respectively reached maturity (stage 3) (Table 2).

In November, 38% of the *M. donacium* sampled from natural ocean conditions were in stage 3, while 63% were in stage 4. In December, 100% of the individuals collected from the ocean were spawning (stage 4).

Gonadal development of broodstock conditioned M. donacium

The Chi squared test ($X^2 = 89.54$) demonstrated significant differences ($p < 0.05$) in sexual maturity over the period of broodstock conditioning. At the beginning of November, 44% of the *M. donacium* individuals were spawning (stage 4) and 25% were in recovery (stage 5). Sampling at day 23 evidenced progressive gonadal development towards maturity (stage 3), with 56 and 50% of the broodstock respectively reaching this stage by the first and second halves of December (Table 2).

The D2 diet favored rapid gonad development, with 50% of this group reaching maturity (stage 3) during the first 44 days of broodstock conditioning (16 ±1°C, 3.61 mL/L dissolved oxygen, 34.82 psu). This treatment also resulted in the release of gametes, with 50% of the broodstock spawning

(stage 4), with decreased biomass found in the individuals sampled during this period. A similar situation was observed with the D1 group fed a diet comprised of local microalgae.

3.3 Effect of microalgae diets on the lipid content of M. donacium

One-way ANOVA indicated that there were no significant differences between the different dietary groups in regards to total lipids ($p > 0.05$). However, a higher amount of lipids was found in mollusks of the D3 group during the second half of December, which coincided with 50% of the female reaching maturity (stage 3) (Table 3). The total lipid content of broodstock conditioned *M. donacium* decreased over the first 13 days before evidencing a slight increase at day 40 of the trial. Due to this, the November total lipid content of laboratory individuals was very similar to that of *M. donacium* sampled from the ocean, the majority of which were in stage 4 or 5 of sexual development. In December, the total lipid content of the broodstock conditioned *M. donacium* increased, albeit insignificantly, as compared to November. However, these values were significantly higher than the December values of *M. donacium* sampled from the ocean. These observations indicate a correlation between lipid content and gonadal development (until stage 3) as a result of the local and commercial microalgae diets consumed during sexual maturation.

Table 1. Effect of microalgae diets on gonadal development in *Mesodesma donacium.*

			Reproductive Cycle Stage			Total
			3	4	5	
Diets	D1	Number of individuals	10*	4	6	20
		% of diet	50%	20%	30%	100%
	D2	Number of individuals	12*	7	1	20
		% of diet	60%	35%	5%	100%
	D3	Number of individuals	2	14	4	20
		% of diet	10%	70%	20%	100%
	C	Number of individuals	11*	7	2	20
		% of diet	55%	35%	10%	100%
Total		Number of individuals	35*	32	13	80
		% of diet	44%	40%	16%	100%

$X^2 = 18.46$ $p < 0.05$*

* indicates significant differences ($p < 0.05$)

Table 2. Stage of gonadal development in *Mesodesma donacium* over time under controlled conditions (three treatments and a control).

Reproductive Cycle Stage	Period of Broodstock Conditioning									
	15 Nov		28 Nov		13 Dec		25 Dec		08 Jan	
	Nº	%	Nº	%	Nº	%	Nº	%	Nº	%
2	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
3	5	31.3*	6	37.5*	9	56.3*	8	50.0*	7	43.8
4	7	43.8	7	43.8	7	43.8	8	50.0	3	18.8
5	4	25.0	3	18.8	0	0.0	0	0.0	6	37.5
Total	16	100	16	100	16	100	16	100	16	100

$X^2 = 89.54$ $p < 0.05$*

* indicates significant differences ($p < 0.05$).

Table 3. Effect of microalgae diets on total lipid content in *Mesodesma donacium.*

Diet	Period of Broodstock Conditioning				
	15 Nov	28 Nov	13 Dec	25 Dec	08 Jan
D1	2.86±1.92	1.34±0.47	1.11±0.81	0.46±0.25	0.77±0.54
D2	3.16±1.59	1.18±0.71	0.88±0.40	0.50±0.37	1.44±0.22
D3	0.96±0.68	1.32±0.82	0.64±0.45	0.75±0.64	0.66±0.96
C	1.95±0.76	0.70±0.15	1.89±0.88	1.64±0.71	0.40±0.47
Statistics					
Fo	0.89	1.01	2.64	0.95	2.10
Ft	3.49	3.49	3.49	3.49	3.49
*Sig.	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

* indicates significant differences ($p < 0.05$).
Fo: test statistic

DISCUSSION

Broodstock conditioning was achieved in adult *M. donacium* at 44 days through a continuous feeding regimen that used mixed microalgae local and commercial diets, whereas *M. donacium* in the natural environment evidenced only 38% in stage 3 and the remaining individuals in stage 4. During December, 100% of the clams sampled from the ocean were spawning. These observations corroborate that reported by Salgado & Ishiyama (1979) about stage partial or total evacuation evidenced from December to March, and the results obtained by FIP (2009) where females evaluated during November in Tacna presented more than 80% on high maturity and the onset of spawning occurred in December 2007; and Tejada concluded that females collected in Tacna during December 2010 found mostly spawned and 39% in gonadal recovery period (Tejada, 2009; 2010). Considering all of this, the mixed diets used in the treatments provided positive benefits, more quickly inducing sexual maturity in laboratory-maintained clams through microalgae commercial and local obtained from the littoral region of Peru, as compared to a purely commercial diet. Considering all of this, the mixed diets used in the treatments provided positive benefits, inducing sexual maturity in laboratory-maintained clams through microalgae obtained from the littoral region of Peru. This is in line with Borowitzka (1997), who indicated that better bivalve production under controlled conditions would require better microalga species as a dietary foundation.

The microalgae diets given to broodstock *M. donacium* influenced gonad development. The D2 diet, composed of the local *I. galbana* and commercial *Ch. gracilis* and *P. lutheri*, was the most apt for obtaining the greatest percentage of mature (stage 3) individuals by day 44 of broodstock conditioning. Following this point, gametes were released, resulting in 50% of the broodstock moving into stage 4 (recovery) and a consequent decrease in the biomass of *M. donacium* individuals. A similar situation was observed in the D1 group fed a diet composed only of local microalgae. These results contrast with those obtained by Martínez *et al.* (2008), who demonstrated that adult specimens of *A. purpuratus* maintained at 16 °C and fed a mix of microalgae (*I. galbana* and *Ch. gracilis*) and fats resulted in 42,64 ± 4,68% gonad recovery during the first 10 days of an 80-day broodstock conditioning period. Martínez (2000) later indicated that a diet of mixed microalgae enriched with unsaturated fats led to better results of sexual maturity and the number of spawning *A. purpuratus.*

Lipid content decreased in the gonads of broodstock conditioning *M. donacium* during the first month, but increased slightly during the second month. Due to this, the lipid content values

were initially similar to those of clams sampled from the ocean, where the majority of adults were in stage 4 or 5 of the reproductive cycle. However, the lipid content of captive adults was significantly greater than that of ocean samples in December, although these values were not significantly higher than those of laboratory individuals in November. This occurrence was related to the progressive development of the gonad until reaching stage 3, which in turn was associated with the distinct diets consumed during the reproductive cycle. The assessed diets were primarily composed of local *I. galbana* (local) and commercial *P. lutheri* and *Ch. gracilis*. According to Ching & Liang (2001), *I. glabana* and *P. lutheri* are dominated by the fatty acids 18:1n-9 and 18:4n-3 and 20:5n-3, respectively. This would indicate that these lipids are a primary energy source, as also supported by Farías & Uriarte (2002), Hendricks *et al.* (2003), and Farías (2002). Specifically, these authors found lipids to be a necessary element of the adult bivalve diet during spawning, as well as during gamete development since energy reserves are absorbed during gametogenesis (Martínez, 2000). Fatty acids also play a role in forming part of the phospholipids present in cell membranes, aiding in the regulation of fluidity and acclimatization to different temperature ranges (Farias, 2008).

The temperature of seawater did not influence the progress of gonadal development in individuals fed distinct diets. Likewise, the percentage of total lipids in *M. donacium* individuals subject to the experimental trials was not influenced by temperature. The recorded temperatures fluctuated within a range of ± 0.6 °C, values that did not change the process of broodstock conditioning. Due to this, the progressive variation in gonadal development is likely attributable to the various mixed diets given over the experimental period.

ACKNOWLEDGMENT

This research was carried out thanks to the logistical support of the Institute of the Sea of Peru and its own contribution. The authors are grateful to all the people who provided their support in the field and experimental phase of this research.

BIBLIOGRAPHIC REFERENCES

Álamo, V. & Valdivieso, V. 1997. *Lista sistemática de moluscos marinos del Perú, Instituto del Mar del Perú, Callao, Perú,* accessed January 27, 2016, http://biblioimarpe.imarpe.gob.pe:8080///handle/123456789/1436.

Almaguer, Y.; Alfonso, E. & Leal, S. 2004. Aislamiento y cultivo de dos especies de diatomeas bentónicas, Revista de Investigación Marina, 25: 57–64.

Álvarez. H. 1994. *Introducción al método ficológico*. Folleto de Algas. Escuela Superior Politécnica del Litoral. pp.19-27.

AOAC. 2000. *Official Methods of analysis of association of official analytic chemist international.* Vol I, II. 17th Ed AOAC Gaithersburg.

Borowitzka, M. 1997. Microalgae for aquaculture: Opportunities and constraints, Journal of Applied Phycology, 9: 393–401.

Buitrón, B. & Perea, A. 1996. *Informe Interno sobre el estado reproductivo de "macha" Mesodesma donacium de la zona de Ilo durante febrero y marzo de 1996,* Instituto del Mar del Perú, Ilo, Perú. 1996.

Carré, M. 2007. El mes de recolección de la macha (*Mesodesma donacium*) determinado por sus líneas de crecimiento: aplicaciones arqueológicas, Bulletin de l'Institut français d'études andines. XX: 299–304.

Ching, P.L. & Liang, P.L. 2001. Ultrastructural study and lipid formation of *Isochrysis sp*., Botanical Bulletin of Academia Sinica. Shanghai, 42: 207–214.

Contreras, J. 2006. *Aplicación de diferentes dietas microalgas durante el desarrollo gametogénico de Mesodesma donacium (Lamarck, 1818): Efectos sobre la maduración sexual, viabilidad de gametos y desarrollo larval temprano*, Universidad Católica del Norte, Coquimbo, Chile.

Farías, A. & Uriarte, I. 2002. *Nutrición en larvicultura de pectínidos: relevancia de proteínas y lípidos.* In: L.E. Cruz-Suárez, D. RicqueMarie, M. Tapia-Salazar, M.G. Gaxiola-Cortés, N. Simoes (Eds.), Av. En *Nutr. Acuícola VI Mem. VI Simp. Int. Nutr. Acuícola* 3 Al 6 Septiembre 2002 Cancún Q Roo México, Universidad Autónoma de

Nuevo León, Monterrey, N.L., México, p. 5, accessed January 27, 2016, http://www.uanl.mx/utilerias/nutricion_acuicola/VI/archivos/A21.pdf.

Farías, A. 2008. *Nutrición y alimentación en moluscos bivalvos. Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina.* Taller Técnico Regional de la FAO. Puerto Montt, Chile. FAO Actas de Pesca y Acuicultura. No. 12. Roma, FAO. 297–308, accessed January 27, 2016, ftp://ftp.fao.org/docrep/fao/011/i0444s/i0444s.pdf.

Foss, S. 1992. *SoxtecTM 2045 Fat Extraction System*, User Manual. Rev. 1.0, Part no 1001 1992.

Guillard, R. 1975. Culture of phytoplankton for feeding marine invertebrates. in "Culture of Marine Invertebrate Animals." (eds: Smith W.L. & M.H. Chanley) Plenum Press, New York, USA. 26-60.

Hendriks, I.E.; Van Duren, I.E. & Herman, P.M.J. 2003. Effect of dietary polyunsaturated fatty acids on reproductive output and larval growth of bivalves, Journal of Experimental Marine Biology and Ecology, 296: 199–213.

Martínez, G.; Aguilera, C. & Mettifogo, L. 2000. Interactive effects of diet and temperature on reproductive conditioning of *Argopecten purpuratus* broodstock, Aquaculture, 183:149–159.

Martínez, G. 2008. *Control de la reproducción y producción de semillas de bivalvos en sistemas controlados.* En Lovatelli, A.; Farias A. & Uriarte I., (eds) Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: Factores que afectan su sustentabilidad en América Latina. Taller Técnico Regional de la FAO. 20–24 de agosto de 2007, Puerto Montt, Chile. FAO Actas de Pesca y Acuicultura. No. 12. Roma, FAO. pp. 267–275, accessed January 27, 2016, ftp://ftp.fao.org/docrep/fao/011/i0444s/i0444s.pdf.

Priyadarshani, I.; Sahu, D. & Rath, B. 2012. Algae in Aquaculture. International Journal of Health Sciences and Research, 2: 108–114.

Quiroz, M. & Barriga, E. 1997. *Evaluación del recurso macha Mesodesma donacium en el litoral sur del Perú, 1996.* Instituto del Mar del Perú, Callao, Perú, accessed January 27, 2016, http://biblio imarpe.imarpe.gob.pe:8080///handle/123456789/1436.

Salgado, I. & Ishiyama, V. 1979. Ciclo de madurez sexual y desove de la macha *Mesodesma donacium*, Revista de Ciencias Universidad Nacional Mayor de San Marcos, 71: 20–28.

Santelices, A. 2006. *Evaluación del crecimiento y supervivencia de juveniles de Mesodesma donacium (Lamarck, 1818) mantenidos en diferentes densidades de cultivo y tipos de sustrato.* Aquaculture Engineer, Universidad Católica del Norte, Coquimbo, Chile.

Segura, M.; Galindo, Ó. & Flores D. 1998. *Evaluación del recurso macha (Mesodesma donacium) en el litoral de Ica y Arequipa. Marzo-abril 1998.* Instituto del Mar del Perú, Ilo, Peru, accessed January 27, 2016, http://biblioimarpe.imarpe.gob.pe:8080/handle/123456789/1025.

Tejada, A. 2009. Monitoreo de las actividades de pesca experimental del recurso macha *Mesodesma donacium* en el litoral de la Región Tacna RM No035-2009-PRODUCE. Instituto del Mar del Perú, Ilo, Perú, accessed January 27, 2016, http://biblioimarpe.imarpe.gob.pe:8080/handle/123456789/1025.

Tejada, A. 2010. Monitoreo de las actividades de pesca experimental del recurso macha *Mesodesma donacium* en el litoral de la Región Tacna RM No035-2010-PRODUCE., Instituto del Mar del Perú, Ilo, Perú, accessed January 27, 2016, http://biblioimarpe.imarpe.gob.pe:8080/handle/123456789/1025.

Vargas, L. 2003. *Asentamiento larval de la macha, Mesodesma donacium (Lamarck, 1818) sobre tres sustratos de diferente granulometría.* Universidad Católica del Norte, Coquimbo, Chile.

Volkman, J.K.; Jeffrey, S.W.; Nichols, P.D.; Rogers, G.I. & Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture, Journal of Experimental Marine Biology and Ecology, 128: 219–240.

Wentworth, C.K. 1922. A scale of Grade and Class

Terms for Clastic Sediments. The Journal of Geology. 30: 377–392.

Zaro, M. 2004. *Desarrollo embrionario y larval de Mesodesma donacium (Lamarck, 1818), a tres tratamientos de temperatura*. Universidad Católica del Norte, Coquimbo, Chile.

Received January 28, 2017.
Accepted March 2, 2017.