ผลของการเสริม DHA จากแหล่งต่างๆ ในสาหร่ายเซลล์เดียวต่อการอนุบาลลูกหอยนางรมพันธุ์ตะโกรมกรามขาว *Crassostrea belcheri* (Sowerby, 1871) ระยะวัยรุ่น

The Effects of Using DHA-rich Supplements from Different Sources in Microalgal Diets on the Nursery Culture of the Juvenile Oyster, *Crassostrea belcheri* (Sowerby, 1871)

สุวัจน์ ธัญรส [1*] จรัญ บุญรงค์ [2] และ วรพร ธารางกูร [1]
Suwat Tanyaros [1*], Jaran Boonrong [2] and Woraporn Tarangkoon [1]

บทคัดย่อ

การอนุบาลลูกหอยนางรมพันธุ์ตะโกรมกรามขาว (*Crassostrea belcheri*) ระยะวัยรุ่นในระบบน้ำหมุนเวียนกึ่งปิดเป็นระยะเวลา 4 สัปดาห์ โดยใช้สาหร่ายเซลล์เดียวเสริมด้วย DHA จากสาหร่ายขาว (*Schizochytrium limacinum*) และน้ำมันปลาทูน่าในรูปอิมัลชั่นในระดับความเข้มข้นต่างๆ กัน ผลการศึกษาพบว่าการเสริม DHA จากทั้ง 2 แหล่งมีผลกระทบต่อการเจริญเติบโตของลูกหอย โดยการเสริม DHA จากสาหร่ายขาวในอัตรา 0.5 เปอร์เซ็นต์ต่อกรัมน้ำหนักเปียกของลูกหอยมีค่าอัตราการเพิ่มขึ้นของความกว้างและความยาวเปลือกสมบูรณ์ไม่แตกต่างจากการใช้สาหร่ายเซลล์เดียว 100 เปอร์เซ็นต์ ขณะที่การเสริม DHA จากสาหร่ายขาวในอัตรา 1 เปอร์เซ็นต์ต่อกรัมน้ำหนักเปียกของลูกหอย และการเสริมน้ำมันปลาทูน่าในอัตรา 0.1 และ 0.2 เปอร์เซ็นต์ต่อปริมาตรน้ำทั้งหมดที่ใช้อนุบาลลูกหอยมีผลทำให้ค่าอัตราการเพิ่มขึ้นของความกว้างและความยาวเปลือกสมบูรณ์ลดลงเมื่อเปรียบเทียบกับการใช้สาหร่ายเซลล์เดียว 100 เปอร์เซ็นต์เป็นอาหารและการอนุบาลลูกหอยในน้ำทะเลจากธรรมชาติ ลูกหอยนางรมที่อนุบาลโดยใช้สาหร่ายเซลล์เดียวผสมโดยไม่เสริม DHA พบมีค่าผลผลิตรายวันสูงกว่าลูกหอยนางรมที่อนุบาลโดยการเสริม DHA จากแหล่งและความเข้มข้นที่ต่างๆ กัน ในช่วงสิ้นสุดการทดลองพบว่าลูกหอยนางรมที่เสริม DHA จากสาหร่ายขาวในอัตรา 1 เปอร์เซ็นต์ และน้ำมันปลาทูน่า 0.2

[1] คณะวิทยาศาสตร์และเทคโนโลยีการประมง มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย อำเภอสิเกา จังหวัดตรัง 92150
[1] Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Sikao, Trang 92150, Thailand.
[2] สถาบันทรัพยากรธรรมชาติและสิ่งแวดล้อม มหาวิทยาลัยเทคโนโลยีราชมงคลศรีวิชัย อำเภอสิเกา จังหวัดตรัง 92150
[2] Natural Resources and Environment Institute, Rajamangala University of Technology Srivijaya, Sikao, Trang 92150, Thailand.
[*] Corresponding author, e-mail: stanyaros@gmail.com

เปอร์เซ็นต์ มีอัตราการรอดต่ำกว่าลูกหอยนางรมที่ใช้สาหร่ายเซลล์เดียว 100 เปอร์เซ็นต์ การเสริม DHA จากสาหร่ายขาวในอัตรา 0.5 เปอร์เซ็นต์ และน้ำมันปลาทูน่า 0.1 เปอร์เซ็นต์ และลูกหอยที่อนุบาลในน้ำทะเลธรรมชาติการเสริม DHA จากทั้ง 2 แหล่งมีผลกระทบต่อสัดส่วนขนาดของลูกหอยภายหลังสิ้นสุดการทดลอง

คำสำคัญ: กรดไขมันชนิดไม่อิ่มตัวสูง, สาหร่ายขาว, น้ำมันปลาทูน่า, การเจริญเติบโตรายวัน, หอยนางรมพันธุ์ตะโกรมกรามขาว

ABSTRACT

Juvenile oysters, *Crassostrea belcheri* were nursed in a semi-closed recirculation system for four weeks. Supplementation with DHA-rich *Schizochytrium limacinum* and tuna oil emulsion in microalgal diets were tested in the nursery culture of juvenile oysters. The results reveal that using DHA-rich supplements from both sources affected the growth performance of juvenile oysters. Supplementation with 0.5% DHA from *S. limacinum*/g wet weight was found to cause non-significant differences in absolute shell growth compared with 100% microalgal, while 1.0% DHA from *S. limacinum*/g wet weight, 0.1% and 0.2% of tuna oil/water volume supplemented in feed were decreased in absolute shell growth compared with a 100% microalgal diet and natural sea water. Juvenile oysters fed on non-supplemented mixture microalgal diets were found to have a higher daily yield than those fed on a diet using DHA-rich supplements from different sources and in different ratios. At the end of the experiment, a lower survival rate was found for juvenile oysters fed on a mixed microalgal diet supplemented with *S. limacinum* (1%) and tuna oil (0.2%) than for oysters fed on 100% microalgal diets, 0.5% DHA from *S. limacinum*/g wet weight, 0.1% of tuna oil/water volume and natural sea water. Using DHA-rich supplements from different sources and in different ratios affected on the size fraction of juvenile oysters.

Key words: HUFAs, *Schizochytrium limacinum*, tuna oil, daily growth rate, *Crassostrea belcheri*

INTRODUCTION

Oyster culture in Thailand has been practiced for several decades along the country's coasts. *Crassostrea belcheri* is one of the most commercially important bivalves, and many studies have been done on its biology and culture over many years (Department of Fisheries, 1994). The majority of spats for grow-out farms are collected from natural sources, but the amount of oyster seed produced from those sources is

limited and insufficient. Oyster seed production from hatcheries has continued to develop and is a subject of great interest in Thailand. A spat larger than 0.5 cm is suitable for moving to a nursing pond or the sea, having a high specific growth rate and low mortality (Tanyaros and Tarangkoon, 2014). Highly unsaturated fatty acids (HUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported to be essential for optimal growth of juvenile oysters (Langdon and Waldock, 1981; Knauer and Southgate, 1999). Generally, the ability to synthesize EPA and DHA in bivalves is limited (Waldock and Holland, 1984; Soudant *et al.*, 2000). Lipid emulsions offer a practical solution to avoid HUFA-deficiency in hatchery-reared molluscs since they have been described as effective carriers for essential fatty acids in bivalves such as juvenile *Placopecten magellanicus* (Coutteau *et al.*, 1996), *Tapes philippinarum* and *Crassostrea gigas* spat (Caers *et al.*, 1998, 2000). *Schizochytrium limacinum*, a heterotrophic marine microorganism, was first discovered in a mangrove area of the Yap Islands, Micronesia (Honda *et al.*, 1998). It has attracted recent attention as it is made up of more than 50% in the dry cell weight; and about 30% of the fatty acids in this strain are DHA (Yokochi *et al.*, 1998). Past study has shown that *S. limacinum* can support a greater growth rate for both *T. semidecussata* and *C. gigas* at 40% and 80% replacement, respectively, in comparison with the control group (Boeing, 2005). The present investigation aimed to assess the use of DHA-rich supplements from *S. limacinum* and tuna oil emulsion in the nursery culture of the juvenile oyster, *C. belcheri*.

MATERIALS AND METHODS

Experimental oysters

The oyster spats *C. belcheri* used in this experiment were produced from the hatchery at the Marine Shellfish Breeding Research Unit, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang campus, Trang, Thailand. Oyster spats were graded by size to prevent possible growth retardation, and had a mean (±SD) shell width (dorso-ventral measurement) and shell length (antero-posterior measurement) of 1.86±0.18 cm and 1.73±0.23 cm, respectively. The animals were kept for two days in a semi-closed recirculation system for acclimatization. The water was totally renewed every day, and food was added twice a day (morning and evening) at a rate of 50 cells μl^{-1} of *Chaetoceros calcitran* and *Tetraselmis suecica*.

Experimental design

A mixed microalgal diet consisting of 50/50% *C. calcitrans* and *T. suecica* was used as feed during the experiment. The daily use of microalgae concentrates of both species was calculated by the method of Helm *et al.*, (2004). Six treatments were compared: (i) a 100% mixed microalgal diet; (ii) a 100% mixed microalgal diet supplemented with 0.5% DHA from *S. limacinum* (35% DHA)/g wet weight;(iii) a 100%

mixed microalgal diet supplemented with 1.0% from *S. limacinum* /g wet weight; (iv) a 100% mixed microalgal diet supplemented with 0.1% tuna oil emulsion (A1 DHA SELCO®INVE, HUFAs 200 mg/g dwt)/water volume; (v) a 100% mixed microalgal diet supplemented with 0.2% tuna oil emulsion /water volume; and (vi) natural sea water. The experiment was set up using a completely randomized design (CRD), and each treatment was conducted in triplicate.

Experimental system and procedures

A semi-closed recirculation system was designed for nursing hatchery-reared juvenile oysters (See Figure 1). Four sets were used, each consisting of a submersible pump, a rectangular 105 L (50 x 70 x 30 cm) fiber glass tank for placement of the nursing units, a cylindrical plastic 30 L container (60 cm diameter and 25 cm depth) for food storage, and three sets of cylindrical PVC pipe (15.2 cm diameter x 12 cm depth) used as nursing units. Each cylindrical PVC pipe was drilled 10 cm from the bottom for a 1.86 cm diameter overflow pipe. A screen using 600 µm mesh was fixed by a PVC clamp to the bottom of each cylindrical PVC section. The volume of water in each nursing unit was 1.82 L during system operation. Each nursing unit had a capacity of 754 juvenile oysters, equal to a stocking density of 4 juveniles per cm^{-2} (Tanyaros *et al.*, 2012). The water flow in each nursing unit was controlled by an adjustable valve at the overflow pipe to maintain a flow rate of 4 L min^{-1} (Tanyaros *et al.*, 2012).

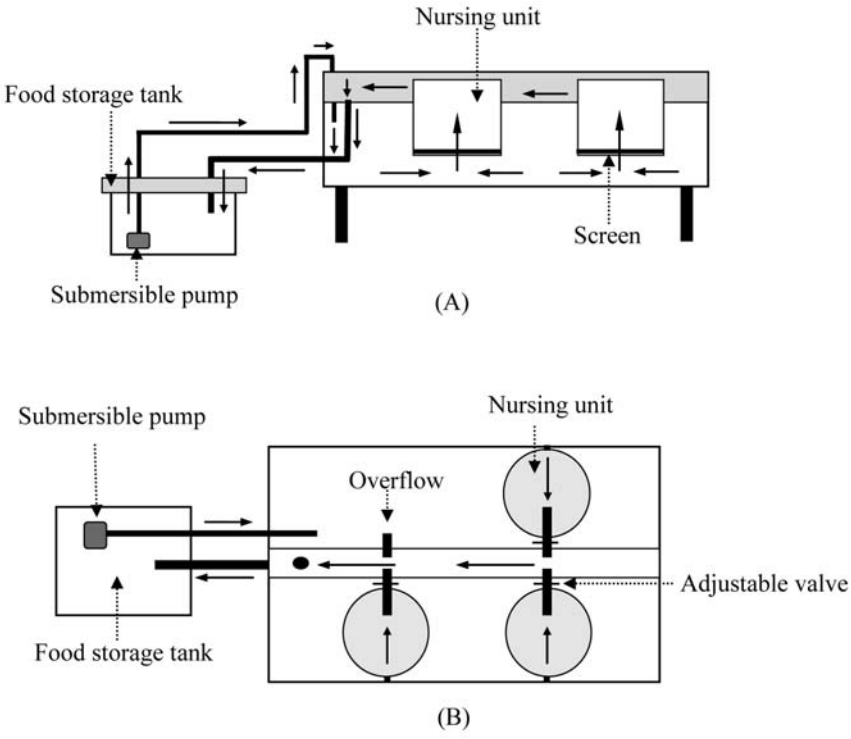

Figure 1 Diagram showing the experimental system: (A) side view and (B) top view.

Sample collection and analysis

Every fifteen days during the experiment, the oysters were removed from the plastic mesh net for weighing. Twenty oysters from each net were randomly selected for measurement of shell width and length. Growth was expressed as absolute growth rates for shell width and length (AGRW, AGRL). The daily yield was calculated using the method of Dégremont *et al.* (2007).

At the end of the experiment, all juvenile oysters from each experimental unit were graded using sieves with mesh sizes of 0.42 and 0.72 cm in diameter, and then counted. The survival rates and size fractions were calculated and expressed as percentages.

Statistical analysis

Data from the experiments were analyzed statistically to test for differences among sieving groups using an analysis of variance (one-way ANOVA) using SPSS 17.0 for Windows. If significant effects were found, a further analysis using Tukey's test was used to determine the pairwise comparison of their means.

RESULTS

The juvenile oysters were nursed in a semi-closed recirculation system for four weeks. There were significant differences ($P< 0.05$) in their mean absolute shell width and lengthfor the different treatments. Using a 0.5% DHA supplement from *S. limacinum*/g wet weight in feed showed non-significant differences in absolute shell growth compared with a 100% microalgal diet. Using a 1.0% DHA supplement from *S. limacinum*/g wet weight, 0.1% and 0.2% tuna oil emulsion/water volume in feed caused a decrease in absolute shell growth compared with a 100% microalgal diet. On the other hand, pumping sea water and directly to feed juvenile oysters caused a non-significant difference in absolute shell growth compared with a 100% microalgal diet (Figures 2A and B). Mean absolute shell widths were 0.30±0.06, 0.30±0.03, 0.18±0.03, 0.15±0.03, 0.13±0.02 and 0.23±0.001 mm d^{-1} and mean absolute shell lengths were 0.24±0.01, 0.23±0.001, 0.20±0.01, 0.20±0.01, 0.16±0.01 and 0.20±0.03 mm $d^{-1}$for a 100% mixture microalgal diet using 0.5% and 1.0% DHA supplements from *S. limacinum* and ones supplemented with 0.1% and 0.2% tuna oil emulsion and natural sea water, respectively.

Mean daily yield differed significantly among the treatments over the study period ($P< 0.05$). A 100% mixture of microalgae without supplementation showed the highest daily yield in comparison with of the use of DHA-rich supplements from different sources and ratios (Figure 3). Mean daily yields were 243.3±7.1,169.4±7.2, 121.8±10.2, 101.2±13.2, 55.9±9.1 and 116.1±10.4 %/day for a 100% mixed microalgal diet and those using 0.5% and 1.0% DHA supplements from *S. limacinum*, 0.1% and 0.2% tuna oil emulsion and natural sea water, respectively.

Differences among the mean percentages for survival were found at the end of the experiment ($P< 0.05$). Mean survival rates

were 90.43±8.1, 85.3±8.1, 71.2±8.1, 73.8±16.1, 53.5±19.3and 63.9±4.3 % for 100% mixed microalgal diets supplemented with 0.5% and 1.0% DHA from *S. limacinum*, and those using 0.1% and 0.2% supplements of tuna oil emulsion and natural sea water, respectively (Figure 4).

Use of DHA-rich supplements from *S. limacinum* and tuna oil emulsion at different ratios in microalgae affected the growth of juvenile oysters. The fraction of oysters retained on a sieve 0.72 cm in diameter increased when DHA-rich supplements from different sources and ratios were used in comparison with a 100% mixture of microalgae and natural sea water, while the fraction of medium size (0.42-0.72 cm) was significantly lower than for those fed on a 100% mixture of microalgae and natural sea water. Use of a DHA-rich supplement from

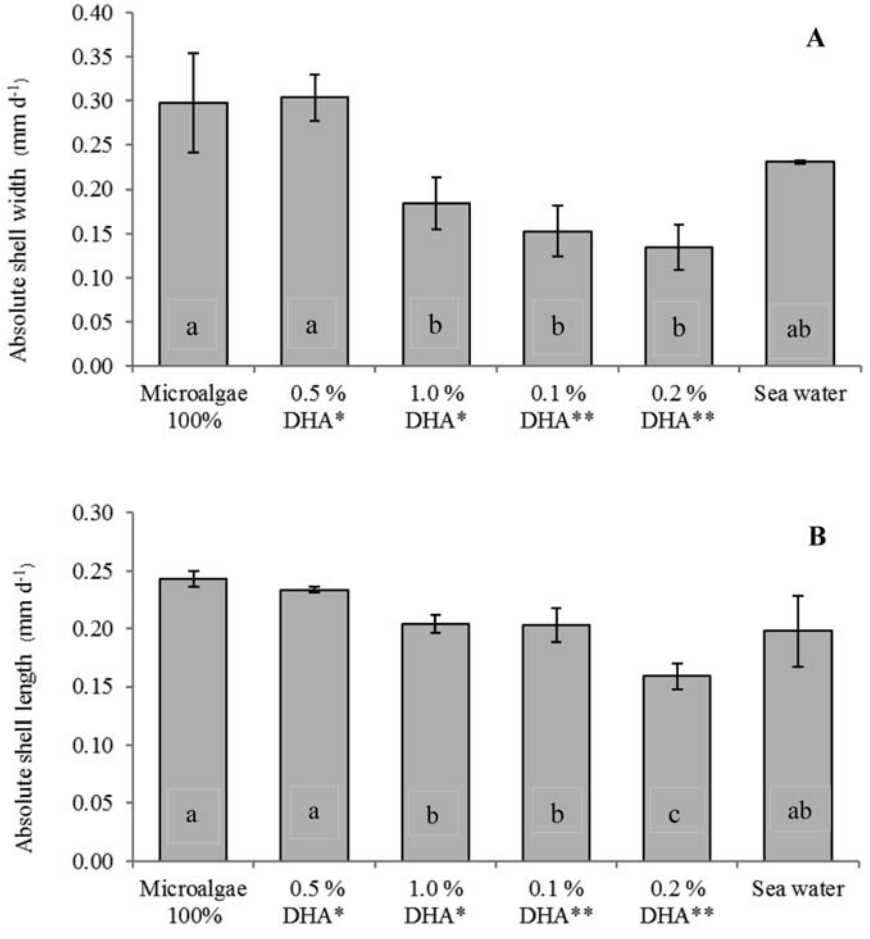

Figure 2 Mean (±SD) absolute shell width (A) and length (B) of hatchery-reared juvenile oysters fed on microalgae with DHA-rich supplements from different sources and in different ratios. Bars labelled with different letters are significantly different (P< 0.05). (* *S. limacinum* (35% DHA)/g wet weight; ** tuna oil emulsion/water volume).

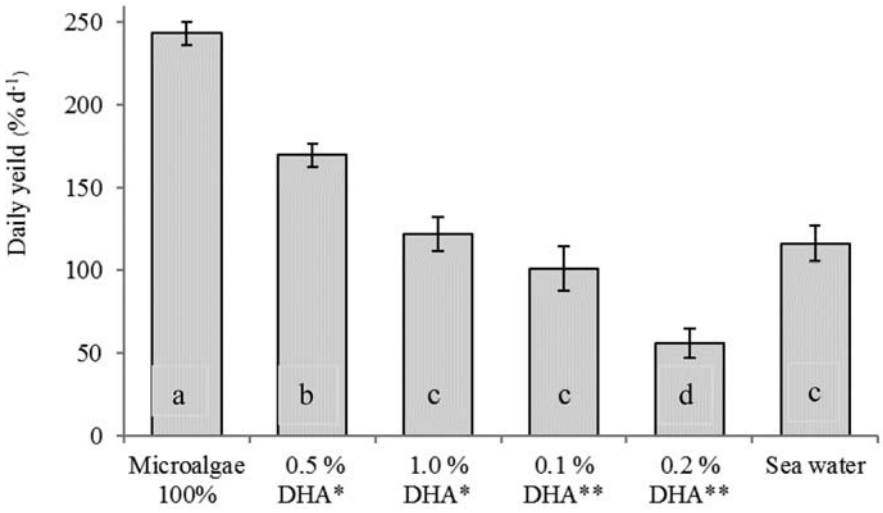

Figure 3 Mean (±SD) daily yield of hatchery-reared juvenile oysters fed on microalgae with DHA-rich supplements from different sources and in different ratios. Bars labelled with different letters are significantly different (P< 0.05). (* *S. limacinum* (35% DHA)/g wet weight; ** tuna oil emulsion/water volume).

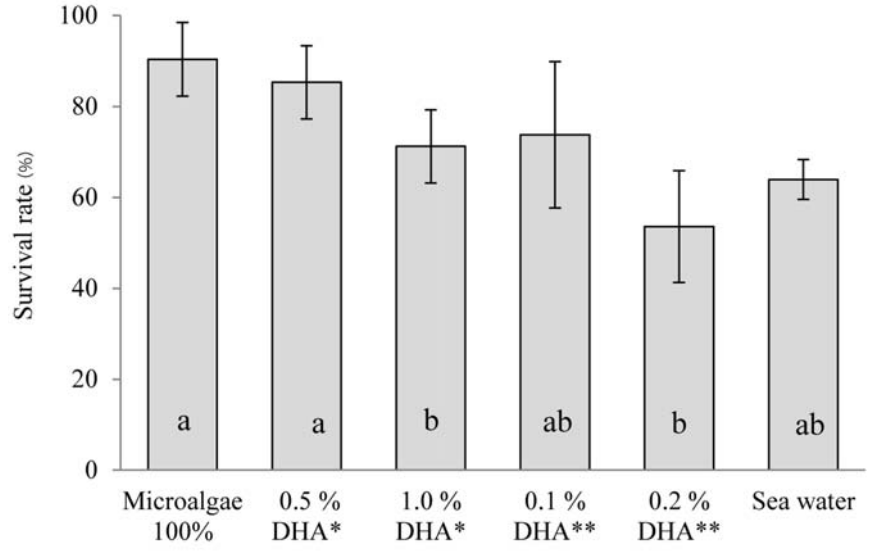

Figure 4 Mean (±SD) survival rate of hatchery-reared juvenile oysters fed on microalgae with DHA-rich supplements from different sources and in different ratios. Bars labelled with different letters are significantly different (P< 0.05). (* *S. limacinum* (35% DHA)/g wet weight; ** tuna oil emulsion/water volume).

S. limacinum led to a higher fraction of small size juvenile oysters (<0.42 cm) than a diet of a 100% mixture of microalgae and natural sea water (Table 1).

DISCUSSION

Lipids in general, and specifically n-3 highly unsaturated fatty acids (HUFAs), have an essential role in bivalve mollusc nutrition. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been reported to be essential for optimal growth of Pacific oyster, *C. gigas* oyster (Langdon and Waldock, 1981; Knauer and Southgate, 1999), scallop juveniles (Parrish *et al.*, 1995). In our findings, use of the DHA-rich supplements, *S. limacinum* and tuna oil emulsion effected the growth performance of juvenile *C. becheri*. Increasing the ratio of *S. limacinum* effected the growth and survival rate in juvenile oysters.The opposite result was found in the previous report by Boeing (2005). He found that *S. limacinum* supports a greater growth rate in both *Tapes semidecussata* and *C. gigas* at 40% and 80% replacement, respectively. Particle cells of *S. limacinum* used in this study may be of an unsuitable size for feeding. Particle size is known to be an important criterion for the selection process, with juvenile oysters exhibiting varying feeding rates for different sizes of food particles (Langdon and Newell, 1996). Mature cells of *S. limacinum* are globular and measure 10.0-14.0 µm in diameter and this is larger than the *Isochrysis galbana* (6 µm) or *Chaetoceros calcitrans* (8 µm). It was observed that more *S. limacinum* cells were ejected as pseudofeces as the supplementation rate increased. High pseudofeces production effects the scope for growth, as has been reported in several bivalves,

Table 1 Mean fraction of juvenile oysters in different size ranges fed on microalgae with DHA-rich supplements from different sources and in different ratios after nursing for four weeks. (* *S. limacinum* (35% DHA)/g wet weight; ** tuna oil emulsion/ water volume). Data labelled with different letters (vertical only) are significantly different (p <0.05).

Treatments	Size fraction (%)		
	>0.72 cm	0.42-0.72 cm	<0.42 cm
Microalgae 100%	28.9 ± 2.6^{c}	62.3 ± 6.2^{b}	8.7 ± 3.6^{bc}
0.5 % DHA*	32.3 ± 1.3^{bc}	46.1 ± 1.9^{c}	21.5 ± 2.5^{a}
1.0 % DHA*	30.6 ± 3.7^{bc}	53.9 ± 2.6^{bc}	15.4 ± 1.6^{a}
0.1 % DHA**	37.1 ± 2.8^{b}	52.5 ± 1.5^{bc}	10.3 ± 1.5^{b}
0.2 % DHA**	44.8 ± 2.3^{a}	50.2 ± 4.9^{c}	4.7 ± 3.5^{c}
Sea water	1.3 ± 1.3^{d}	88.9 ± 6.2^{a}	9.6 ± 5.6^{bc}

such as the cockle, *Cerastoderma edule* (Navarro *et al.*, 1992) and *Mytilus galloprovincialis* (Albentosa *et al.*, 2012). This is the main cause effecting the growth of oysters in the present study.

The non-supplemented mixture microalgal diet showed better growth performance in comparison with the diet supplementated with tuna oil emulsion. The growth performance of *C. belcheri* juvenile decreased with increasing concentrations of tuna oil emulsion. High pseudofeces production at high supplementation ratesof tuna oil emulsion were observed during the experiment. Tuna oil emulsion may effect the filtration mechanism in oysters. Lipid spheres might not have been available for the juveniles as food particles because particles that are too large for consumption or for suspension in seawater were formed. In addition, ingestion and handling of this unprofitable food may have caused the use of excess of energy, leading to a lower growth rate. A similar result was found by Hendriks *et al.* (2003), who reported that supplementation with lipid emulsions in *C. gigas* at the larval stage was not effective. However, our findings contradict the results of previous studies in which lipid emulsions offered a practical solution to avoiding HUFA-deficiencies in hatchery-reared juvenile *Placopecten magellanicus* (Coutteau *et al.*, 1996), *T. philippinarum* and *C. gigas* (Caers *et al.*, 1998, 2000). The DHA-rich *S. limacinum* and tuna oil emulsion used as a supplement to a microalgal diet did not improve the growth rate in juvenile *C. belcheri*, but finding a suitable form and increasing their lipid reserves, especially their triacylglycerol content, are important factors for future investigation.

ACKNOWLEDGMENT

The authors thank Supatcha Chuseingjaw and Tamrong Pattanatong for help collecting the samples. We also wish to thank William Martin for assistance in editing the manuscript. This study was funded by the Rajamangala University of Technology Srivijaya, annual budget year 2014.

REFERENCES

Albentosa, M., Sánchez-Hernández, M., Campillo, J.A. and Moyano, F.J. 2012. Relationship between physiological measurements (SFG -scope for growth-) and the functionality of the digestive gland in *Mytilus galloprovincialis*. **Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology** 163: 286–295.

Boeing, P. 2005. **Use of Spray-dried *Schizochytrium* sp. as a Partial Algal Replacement for Juvenile Bivalves**. Bio-Marine, INC. Aquafauna. Available source: http:// www.aquafauna.com/Use%20of%20Spraydried%20Schyz%20for%20Bivalves.pdf, July 11, 2015

Caers, M., Coutteau, P., Lombeida, P., and Sorgeloos, P. 1998. The effect of lipid supplementation on the growth and fatty acid composition of *Tapes philippinarum*

(L.) spat. **Aquaculture** 162: 287–299.

Caers, M., Coutteau, P. and Sorgeloos, P. 2000. Incorporation of different fatty acids, supplied as emulsions orliposomes, in the polar and neutral lipids of *Crassostrea gigas* spat. **Aquaculture** 186: 157–171.

Coutteau, P., Castell, J.D., Ackman, R.G. and Sorgeloos, P. 1996. The use of lipid emulsions as carriers for essential fatty acids in bivalves: a test case with juvenile *Placopecten magellanicus*. **Journal of Shellfish Research** 15: 259–264.

Dégremont, L., Ernande, B., Bédier, E. and Boudry, P. 2007. Summer mortality of hatchery produced Pacific oyster spat (*Crassostrea gigas*). I. Estimation of geneticparameters for survival and growth. **Aquaculture** 262: 41-53.

Department of Fisheries. 1994. **Oyster culture**. Ministry of Agriculture and Cooperative. Bangkok, Thailand.

Helm, M.M., Bourne, N. and Lovatelli, A. 2004. **Hatchery culture of bivalves. A practical manual.** FAO Fisheries Technical Paper No. 471. Food and Agriculture Organization of the United Nations Publishing, Rome, Italy.

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

Honda, D., Yokochi, T., Nakahara, T., Erata, M. and Higashihara, T. 1998. *Schizochytrium Limacinum* sp. nov., a new thraustochytrid from a mangrove area in the west Pacific Ocean. **Mycological Research** 102: 439–448.

Knauer, J. and Southgate, P.C. 1999. A review of the nutritional requirements of bivalves and the development of alternative and artificial diets for bivalve aquaculture. **Review in Fisheries Science** 7: 241–280.

Langdon, C.J. and Newell, R.I.E. 1996. Digestion and nutrition in larvae and adults, pp. 231–269. *In* V.S. Kennedy, R.I.E. Newell and A.F. Able, eds. **The Eastern Oyster.** Maryland Sea Grant College, Maryland.

Langdon, C.J. and Waldock, M.J. 1981. The effect of algal and artificial diets on the growth and fatty acid composition of *Crassostrea gigas* spat. **Journal Marine Biology Association of United Kingdom** 61: 43–448.

Navarro, E., Iglesias, J.I.P. and Ortega, M.M. 1992. Natural sediment as a food source for the cockle *Cerastoderma edule* (L.): effect of variable particle concentration on feeding, digestion and the scope for growth. **Journal of Experimental Marine Biology and Ecology** 156: 69–87.

Parrish, C.C., Mckenzie, C.H., MacDonald, B.A. and Hatfield, E.A. 1995. Seasonal studies of seston lipids in relation to microplankton species composition and scallop growth in South Broad Cove,

Newfoundland. **Marine Ecology Progress Series** 129: 153–164.

Soudant, P., Chu, F.L.E. and Samain, J.F. 2000. Lipids requirements in some economically important marine bivalves. **Journal of Shellfish Research** 19: 605.

Tanyaros, S., Pattanatong, T. and Tarangkoon, W. 2012. Effect of water flow rate and density on nursing hatchery-reared juvenile oysters, *Crassostrea belcheri* (Sowerby, 1871) in a semi-closed recirculation system. **Journal of Applied Aquaculture** 24: 364–373.

Tanyaros, S. and Tarangkoon, W. 2014. Water quality, growth and mortality of tropical Oyster *Crassostrea belcheri* spat in the conserved natural oyster beds of Trang province, southern Thailand, pp. 34-39. *In* **2nd International Conference on Fisheries Science.** Colombo, Sri Lanka.

Waldock, M. and Holland, D.L. 1984. Fatty acid metabolism in young oysters, *Crassostrea virginica*: polyunsaturated fatty acids. **Lipids** 19: 332–336.

Yokochi, T., Honda, D., Higashihara, T. and Nakahara, T. 1998. Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. **Applied Microbiol Biotechnology** 49: 72–76.