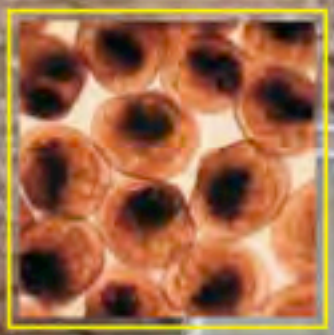
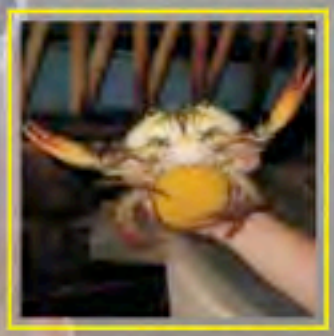
larvi 2009

september 7 - 10, 2009
ghent university, belgium


5th fish & shellfish larviculture symposium

european aquaculture society

special publication N° 38

LARVI '09 – FISH & SHELLFISH LARVICULTURE SYMPOSIUM
C.I. Hendry, G. Van Stappen, M. Wille and P. Sorgeloos (Eds)
European Aquaculture Society, Special Publication No. 38, Oostende, Belgium, 2009

POTENTIAL VALUE OF *NAVICULA INCERTA, PROSCHKINIA* SP., *NITZSCHIA* SP., AND *AMPHORA* SP. AS FEED FOR *HALIOTIS TUBERCULATA COCCINEA* POST-LARVAE: EFFECT OF INOCULUMS DENSITY ON ALGAL GROWTH RATES

G. Courtois de Vicose*, A. Porta, M.P. Viera, A. Bilbao, H. Fernández-Palacios, and M.S. Izquierdo

Grupo de Investigación en Acuicultura (GIA) ICCM & IUSA P.O. Box 56. 35200 Telde, Las Palmas, Islas Canarias, España. (*Corresponding author. Tel: +34 928132900, Fax: +34 928132908. Email address: gtricor@hotmail.com)

Introduction

Benthic diatoms are the principal food source for postlarval abalone (Kawamura, 1996,). Various studies found a relation between benthic diatom density and postlarval abalone settlement, growth, and survival (Searcy-Bernal et al., 2003, Daume et al., 2004, and Roberts et al., 2007). However, only few studies have focused on their nutritional quality and the influence of algal biochemical composition on newly settled abalone (Daume et al., 2003; Gordon et al., 2006; Uriate et al., 2006; Viana et al., 2007). The present study examines the characteristics of four benthic diatoms species and the effect of density on their growth response and nutritional quality for abalone, in terms of their proximate and fatty acid composition.

Material and methods

Diatom cultures
Four species of diatoms *Navicula incerta*, *Proschkinia* sp., *Nitzschia* sp., and *Amphora* sp. were selected and grown in f/2 medium (Guillard, 1975) plus silicate under continuous light of 62±8µmol photon.m^{-2}.s and 28.5±1.4ºC. Growth curve and cell attachment capacity were determined at initial inoculums of 0.05×10^6 cells.ml^{-1}, 0.10×10^6 cells.ml^{-1}, and 0.25×10^6 cells.ml^{-1} for each species, and growth was monitored during 7 days. Each species, inoculated at 0.10×10^6 cell.ml^{-1}, were harvested in logarithmic and stationary phases of growth for biochemical analysis.

Cell counts and growth
Average growth rates were estimated using the following formulae:

$\mu = \ln (N_1/N_0)/ t_1-t_0$ (Guillard, 1973).

Ten randomly chosen fields of view were photographed at 400× and attached cells were then counted. The number of cells.mm^{-2} was calculated.

Analytical methods

Triplicate samples of freezed cells from each of the experimental cultures were analyzed for total lipids (Folch et al., 1957), protein (AOAC 1995), carbohydrate, ash, and total fatty acids (Izquierdo et al., 1989).

Data analysis

An analysis of variance (one-way ANOVA) was performed with growth rate, cell number, lipid, protein and fatty acids as the source of variance. Data showing significant differences ($P<0.05$) were analyzed by paired comparisons using Tukey's HSP test. Equality of variance was assessed with Bartlett's test.

Results

Biometric parameters and growth rates

GR were significantly higher ($P<0.05$) for all species when inoculated at 50×10^3 cells.ml^{-1}, the highest ($P<0.05$) being for *Proschkinia* sp. in all treatments (0.47-0.65µ.day^{-1}), while cell production at stationary phase harvest was higher for all species when inoculated at 100×10^3 cells.ml^{-1} (Table I) The GR found in the present study suggest a nutrient limitation and/or a shadowing effect when original inoculums density was increased as well as the effect of cell size on nutrient assimilation (Richmond, 1986). Attachment capacity was the highest at low inoculums' density for all species except *Proschkinia* sp. and *Amphora* sp. had the highest cell attachment capacity.

Table I. Growth rate and cell count at harvest of four species of diatoms grown at three different densities, (mean±SD, n=9)

Species	**Densities (10^3 cells.ml^{-1})**		
	50	**100**	**250**
Growth rate µ (day^{-1})			
Amphora sp.	0.47±0.01^a	0.43±0.00^b	0.29±0.01^c
Nitzschia sp.	0.47±0.00^a	0.44±0.00^b	0.29±0.00^c
Proschkinia sp.	0.65±0.01^a	0.58±0.01^b	0.47±0.01^c
Navicula incerta	0.35±0.01^a	0.30±0.00^b	0.16±0.02^c
	Cell count at stationary phase harvest ×10^6 (ml^{-1})		
Amphora sp.	1.37±0.09^b	2.02±0.05^a	1.92±0.16^a
Nitzschia sp.	1.31±0.05^c	2.22±0.05^a	1.92±0.04^b
Proschkinia sp.	4.61±0.37^b	5.81±0.43^a	6.57±0.42^a
Navicula incerta	0.60±0.04^b	0.83±0.06^a	0.80±0.10^a

Different superscripts across a row indicate difference between growth rates and densities at 95% level (ANOVA Tukey's, test; $P<0.05$)

Production of nutrients

All diatoms except *N. incerta* showed a significantly ($P<0.05$) higher lipid content during the exponential phase (Table II). Protein content followed a similar trend. Carbohydrate content followed a trend inverse to that of lipid content. No significant differences ($P>0.05$) were found in the ash content among all diatoms cultures. Brown et al. (1996, 1997) reported these facts as typical of cultures becoming nutrient limited. *N. incerta* increased its storage products as lipid instead of carbohydrate that could be an indication of silicon deficiency. *Amphora* sp. and *Proschkinia* sp. presented the highest protein, lipid, and carbohydrate content in their logarithmic growth, as well as good energy contents. These values being within the range needed for juvenile abalone they can be considered suited to cover abalone postlarvae nutritional requirements.

Table II. Proximate chemical analysis (% dry weight) and gross energy (GE) at an initial inoculum of 1x 10^5 cells.ml^{-1} and harvested in logarithmic (Exp) and stationary (Sta) phase of growth, (mean±SD, n=9)

Species	Growth Phase	Lipid (%)	Protein (%)	Ash (%)	Carbohydrates (%)	GE (kJ.g^{-1})
Amphora sp.	Exp	9.74±1.70^{a}	19.70±0.95^{a}	57.10±3.40	13.50±5.01^{cd}	10.82
	Sta	7.31±0.68^{cd}	13.07±0.71^{bc}	60.50±0.90	19.15±1.77^{bc}	9.24
Navicula incerta	Exp	6.11±0.17^{de}	13.00±0.60^{bc}	53.70±3.80	27.23±3.77^{a}	10.16
	Sta	8.88±0.63^{ab}	13.00±1.07^{bc}	58.00±0.43	20.20±0.70^{abc}	10.00
Nitzschia sp.	Exp	4.90±0.55^{ef}	14.50±0.40^{b}	62.00±1.50	18.70±1.70^{bc}	8.56
	Sta	3.11±0.24^{g}	14.20±1.22^{b}	61.00±0.00	21.80±1.21^{ab}	8.33
Proschkinia sp.	Exp	7.82±0.72^{bc}	20.72±1.40^{a}	61.40±8.02	10.05±6.70^{d}	9.68
	Sta	4.00±0.56^{fg}	11.60±1.93^{c}	60.80±2.80	23.73±5.03^{ab}	8.39

Different superscripts down a column indicate means which differ significantly at 95% level (ANOVA tukey's test; $P<0.05$)

Fatty acid composition

Polyunsaturated fatty acids (PUFA) constituted the largest fraction of the total fatty acids (TFA). The proportion of the various PUFAs varied among the diatom species and between growth phases. All of the analyzed diatoms had significant quantities of 20:5n-3 (eicosapentaenoic acid - EPA) *Proschkinia* sp. had the highest quantity of 20:4n-6 (arachidonic acid - ARA). *Proschkinia* sp., *Nitzschia* sp. and *Navicula incerta* showed a decrease in EPA and ARA between the logarithmic and the stationary phase. Levels of 22:6n-3 (docosahexaenoic acid - DHA) (0.19%-1.90% TFA) increased between logarithmic and stationary phase and were generally low among the diatoms tested and are also reported to be low in abalone tissues. The fatty acid profiles of the diatoms tested were characteristic of most diatoms. Based on previous abalone nutritional studies (Mai et al., 1995, Daume et al., 2003, Gordon et al., 2006, Viana et al., 2007), the levels of PUFA, n-3 PUFA and more specifically EPA and DHA found in this study suggest the diatoms tested could be suited for *H. tuberculata coccinea* postlarvae and fulfill their nutritional requirements.

Acknowledgements

This study has been financed by the National Development Plan of Marine Cultures (JACUMAR, TR 2003/2008) and the Scholarship INNOVA.

References

Brown M.R., G.A. Dunstan, S.J. Norwood and K.A. Miller. 1996. Effects of harvest stage and light on the biochemical composition of the diatom *Thalassiosira pseudonana*. Journal of Phycology 32:64–73.

Daume S., B.M. Long, and O. Crouch. 2003. Changes in amino acid content of algal feed species (Navicula sp.) and the effect on growth and survival of juvenile abalone (*Haliotis rubra*). Journal of Applied Phycology 15:201–207.

Gordon N., Neori, A., Shpigel, M., Lee, J., Harpaz, S., 2006. Effect of diatom diets on growth and survival of the abalone *Haliotis discus*. Aquaculture 252: 225-233.

Kawamura T., 1996. The role of benthic diatoms in the early life stages of the Japanese abalone (*Haliotis discus hannai*). p.355–367 In: Survival Strategies in Early Life Stages of Marine Resources. Watanabe Y., Yamashita Y., Oozeki Y. (Eds.), A.A. Balkema, Brookfield.