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Short Communication

Scope for growth of juvenile Cortez oyster, *Crassostrea corteziensis* fed isocaloric diets prepared with microalgae and cereal flours

Alfonso N. Maeda-Martínez[1], Pedro E. Saucedo[2], José M. Mazón-Suástegui[2]
Héctor Acosta-Salmón[2] & Zoreyda Romero-Meléndez[2]
[1]Unidad Nayarit, Centro de Investigaciones Biológicas del Noroeste (CIBNOR), Nayarit, México
[2]Centro de Investigaciones Biológicas del Noroeste, Instituto Politécnico Nacional, La Paz, México
Corresponding author: Alfonso Maeda-Martínez (amaeda04@cibnor.mx)

ABSTRACT. We evaluated the effect of nine isocaloric diets prepared with a mixture of microalgae alone (*Tisochrysis lutea* + *Chaetoceros calcitrans* + *Ch. gracilis*; 1:1:1) and different combinations of the algal mixture and cornstarch or wheat flour, on the Scope for Growth (SFG) of Cortez oyster *Crassostrea corteziensis* juveniles (7.2 ± 1.1 mm shell length). A total 15,000 spat was acclimated to the experimental diets for 30 days at 21ºC, prior SFG determinations. Consumed energy, absorbed energy, and SFG decreased with a corresponding decrease in the proportion of live microalgae in the diet. Oysters fed the microalgal diet showed significantly increased physiological activity in all parameters except absorption efficiency. Oysters fed diets containing up to 50% dry feedstuff showed positive SFG values but these were significantly lower than SFG shown in oysters fed microalgae alone. Oysters fed diets containing ≥75% dry feedstuff showed negative SFG. These results suggest that cereals can be used as complementary food or *C. corteziensis* spat in hatchery operations, provided replacement of microalgae is kept below 50%.

Keywords: *Crassostrea corteziensis*, energy balance, oyster nutrition, microalgae, cornstarch, wheat flour.

Potencial de crecimiento de juveniles de ostión de placer, *Crassostrea corteziensis* alimentados con dietas isocalóricas preparadas con microalgas y harinas de cereales

RESUMEN. Se evaluó el efecto de nueve dietas isocalóricas preparadas con microalgas solamente (*Tisochrysis lutea* + *Chaetoceros calcitrans* + *Ch. gracilis*; 1:1:1), con diferentes proporciones de esa mezcla de microalgas con harinas de trigo y maíz, sobre el campo de crecimiento (SFG) de juveniles de ostión de placer *Crassostrea corteziensis* (7,2 ± 1,1 mm de longitud). Se aclimató un total de 15.000 juveniles durante 30 días a 21ºC, previamente a las determinaciones de SFG. Los resultados indicaron que la energía consumida, la energía absorbida y el SFG disminuyeron conforme disminuyó la proporción de microalgas en las dietas. Las ostras alimentadas solamente con microalgas, mostraron los mayores valores en todas las variables de la ecuación de SFG, excepto en eficiencia de absorción. En las dietas con 50% de reemplazo de microalgas por harinas de trigo y maíz, el SFG fue positivo pero significativamente menor que el obtenido con la dieta microalgal. Las dietas conteniendo ≥75% de harinas de trigo y de maíz, produjeron un SFG negativo. Con estos resultados se concluye que las harinas, podrían ser usadas como alimentos complementarios cuando exista escasez de microalgas en el laboratorio, siempre y cuando la proporción de reemplazo no exceda el 50%.

Palabras clave: *Crassostrea corteziensis*, balance energético, nutrición de ostras, microalgas, harina de maíz, harina de trigo.

Severe increases in die-offs of cultivated Pacific oyster, *Crassostrea gigas* during the last decade (Trabal *et al.*, 2012) in northwestern Mexico have motivated the development of culture technology of native species such as the Cortez oyster *C. corteziensis* (Chávez-Villalba *et al.*, 2005, 2008; Rivero-Rodríguez *et al.*, 2007). The study of inexpensive diets has been the subject of particular interest in this species (Mazón-Suástegui *et al.*, 2008, 2009). Partial replacement of microalgae by dry feedstuffs in the diet of juvenile oysters has been studied as a means to propose reduc-

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tions in the cost of laboratory nursery culture. A wide variety of ingredients have been investigated, including dried macroalgae and microalgae, bacteria and yeasts, microparticulated food and cereal flours (Knauer & Southgate, 1999). While most of these products have given satisfactory results in rearing marine bivalves, only a few have allowed replacing a large part of the algal proportion without affecting the nutritional balance of the diet or the condition of the animals. Cereals such as rice, oats, wheat, and corn are inexpensive, easy to prepare and assimilate, and energetically rich, hence, have emerged as promising diets for meeting the nutritional needs of bivalves (Mazón-Suástegui & Avilés-Quevedo, 1988; Fernández-Reiriz *et al.*, 1998; Pérez-Camacho *et al.*, 1998). Of these, corn-starch and wheat flour have been tried with success in the Cortez oyster (Mazón-Suástegui *et al.*, 2008, 2009). The physiological index Scope for Growth (SFG) determines the energy potentially available for growth and reproduction (Winberg, 1960) and is calculated as the difference between absorbed energy and respired and excreted energy. SFG has been used in marine bivalves to determine, for example, physiological plasticity of native and invasive species (Sará *et al.*, 2008; Nieves-Soto *et al.*, 2011), feeding range for aquaculture purposes (Ibarrola *et al.*, 1998; Yukihira *et al.*, 1998; Kesarcodi-Watson *et al.*, 2001a, 2001b; Velasco, 2007), identifying physical and chemical parameters for optimum growth (Yukihira *et al.*, 2000; Soria *et al.*, 2007), and population health (Din & Ahamad, 1995).

We investigated the consumed, absorbed, and respired energy, the absorption efficiency, and calculated SFG in juvenile Cortez oysters fed nine isocaloric diets prepared with a mixture of microalgae (*Tisochrysis lutea*, *Chaetoceros calcitrans* and *Ch. gracilis* at a 1:1:1 ratio by cell number; cell sizes = 3 to 4.5 µm), corn-starch and wheat flour (Table 1) to determine the degree at which microalgae can be replaced in the diet before affecting oyster growth. A total of 15,000 three-week old *C. corteziensis* juveniles with a mean (± SD) shell length (n = 30) of 7.2 ± 1.1 mm were separated into nine experimental groups and each was offered one experimental isocaloric diet.

The caloric content of the three microalgae and both cereal products (cornstarch and wheat flour) used to prepare the control diet is shown in Table 2. Caloric content was determined with a Parr 1261 calorimetric pump. Energy content of food particles was slightly higher for the two cereal products than for microalgae (Table 2), and diets containing higher proportion of cereals required a lower particle density to meet the energy required. With these data, the quantity of food particles constituting each experimental diet was calculated before starting the experiment to supply twice a day the energy equivalent to that in 8×10^6 cells to each oyster.

The density of food particles in each diet never exceeded 120,000 particles mL^{-1} to prevent pseudo-feces formation. Microalgae were cultured in 0.5 µm filtered seawater radiated with UV light and enriched with f/2 medium. Cultures were maintained at 22 ± 1ºC, salinity of 36, continuous light intensity of 44.6 $\mu E\ m^{-2}\ s^{-1}$, and vigorous aeration. Cereal products (particle size = 2.5 to 3.5 µm) were prepared by suspending commercial cornstarch (Maizena®, Unilever de Mexico, Mexico City) or ground wheat flour (Harinera Hasaya, Mexico City) in 0.5 L cold filtered freshwater. The resulting mixture was weighed and poured into 5 L boiling filtered freshwater and then gently stirred for 5 min to ensure a complete and homogeneous cooking. Cereal mixtures were diluted in filtered seawater (25ºC, salinity of 36) with vigorous aeration to be readily available to the oysters. Oysters were maintained for 30 days in 27 plastic containers (v = 16 L) with 1 µm filtered seawater (21ºC, salinity of 36 and constant aeration) and food particles of each experimental diet. Three containers holding *ca.* 550 oysters each were used as replicates for each of the nine experimental diets. As a routine procedure, 100% of the seawater within each container was renewed daily. After 30 days, Scope for Growth (SFG) was determined using a continuous flow-through system consisting of three 1.5 L independent chambers each containing the 550 juvenile oysters of each plastic container previously mentioned, and a fourth chamber corresponding to the blank. The experimental chambers received continuously 70 mL min^{-1} seawater with food of one experimental diet at a time. The chambers were maintained at 21ºC using temperature regulated water baths. SFG was measured (Winberg, 1960) by subtracting respiration and excretion energy from absorbed energy. A detailed description of the methods and formulae was described in a previous article (Nieves-Soto *et al.*, 2011). To detect significant differences, one-way ANOVA, followed by Tukey *post-hoc* analysis of means, was run between experimental diets. The significance level of this analysis was set at $P < 0.05$.

Significant differences were observed in most physiological parameters in oysters fed the experimental diets (Table 3). As a general pattern, consumed energy (C), absorbed energy (A), and SFG decreased with a corresponding decrease in the proportion of live microalgae in the diet. Oysters fed the control diet (live microalgae) showed significantly increased physiological activity in all parameters except absorption efficiency (*e*) (Table 3). Oysters fed diets 2, 3, 4, and 5 (containing up to 50% dry feedstuff) showed positive

Table 1. Composition (%) and energy content of diets given to juveniles of the Cortez oyster *Crassostrea corteziensis*. The microalgae constituting the basis of diet 1 (control) were: *Tisochrysis lutea*, *Chaetoceros calcitrans* and *C. gracilis* at a 1:1:1 ratio

Diet	Microalgae	Wheat flour	Cornstarch	Energy (J g^{-1})		
				Microalgae	Cereals	Total
1	100	0	0	985	0	985
2	75	25	0	737	247	984
3	75	0	25	737	247	984
4	50	50	0	492	492	984
5	50	0	50	492	492	984
6	25	75	0	247	737	984
7	25	0	75	247	737	984
8	0	100	0	0	985	985
9	0	0	100	0	985	985

Table 2. Dry weight and energy content of the food particles used to prepare the experimental diets fed to juveniles of the Cortez oyster *Crassostrea corteziensis*.

Particle	Dry weight (pg per particle)	Energy content (J g^{-1})	Reference
Tisochrysis lutea	28.5	16,383	Whyte (1987)
Chaetoceros calcitrans	44.7	12,612	Whyte (1987)
Chaetoceros gracilis	40.5	16,693	Lora-Vilchis *et al.* (2004)
1:1:1 diet	37.9	15,231	
Wheat flour	65	17,497	Present study
Cornstarch	44	16,512	Present study

SFG (27.4-43.3 J g^{-1} h^{-1}), but these were significantly lower than SFG shown in oysters fed microalgae (103.2 J g^{-1} h^{-1}). Oysters fed diets 6, 7, 8, and 9 (containing ≥ 75% dry feedstuff) showed the lowest C (11.6-47.6 J g^{-1} h^{-1}), and although *e* was the highest (97.4-99.9%) for these diets, C was not enough to provide more energy than that used for respiration.

Therefore, oysters fed these four diets showed negative SFG (Table 3). The lowest *e* was observed in oysters fed diet 1 (83.1%) and significantly increased with the corresponding increase in cereal content in the diets up to 99.6% and 99.9% for diets 8 and 9 (100% cereal meal), respectively. Respired energy did not show a clear relationship to the composition of the diets and ranged from 38.4 J g^{-1} h^{-1} in oysters fed diet 8 (100% wheat flour) to 100.5 J g^{-1} h^{-1} in oysters fed diet 6 (75% wheat flour). Excreted energy was negligible and was removed from the calculation of SFG. The Cortez oyster showed the highest SFG when fed the diet containing no cereal meals. However, oysters fed diets containing up to 50% of wheat flour or cornstarch showed positive SFG. These results are consistent with observations on other bivalve species including the Cortez oyster. For example, Cortez oyster spat fed diets containing 50% microalgae and 50% cornstarch or wheat flour showed growth comparable to that of spat fed 100% microalgae (Mazón-Suástegui *et al.*, 2008, 2009). Similarly, a diet of 50% microalgae and 50% cornstarch did not affect overall performance of the clam *Ruditapes decussatus* spat when compared to the diet composed by 100% microalgae (Pérez-Camacho *et al.*, 1998). On the other hand, negative SFG of spat fed ≥75% dry feedstuffs is consistent with poor growth of culture animals fed diets based mostly on these ingredients.

Despite significant research on artificial diets (Coutteau & Sorgeloos, 1992; Pérez-Camacho *et al.*, 1998; Knauer & Southgate, 1999), it has not been feasible to fully replace microalgae as the best source of nutrients for bivalves. The poor growth shown by bivalves fed drystuffs is likely due to the low consumed energy derived likely from reduced ingestion. Although *e* increased when microalgae were replaced with dry ingredients, the consumed energy was not enough to meet respiration energy. The high *e* recorded in oysters fed diets containing cornstarch or wheat flour may have been due to the easier digestibility of carbohydrates contained in these dry feedstuffs compared to the more complex structures and biochemical content of live microalgae, especially the two diatom species that have thick silicate walls. However, despite this increase in *e*, oysters did not consume enough energy (C) to satisfy

Table 3. Summary of mean (± SD) C: consumed energy, AE: absorption efficiency, A: absorbed energy, R: respired energy and SFG: scope for growth of Cortez oyster spat *Crassostrea corteziensis* fed nine isocaloric diets. Different superscripts indicate significant differences ($P < 0.05$).

Diet	C ($J g^{-1} h^{-1}$)	AE (%)	A ($J g^{-1} h^{-1}$)	R ($J g^{-1} h^{-1}$)	SFG ($J g^{-1} h^{-1}$)
1	199.3 ± 19.8^{a}	83.1^{e}	165.0 ± 16.5^{a}	61.8 ± 0.9^{b,c}	103.2 ± 15.6^{a}
2	130.8 ± 21.8^{b}	88.4^{d,e}	115.3 ± 19.2^{a,b}	71.9 ± 3.4^{a,b}	43.3 ± 21.9^{b}
3	124.1 ± 5.4^{b}	91.4^{c,d}	113.4 ± 4.9^{b,c,d}	54.7 ± 1.5^{c}	39.2 ± 34.0^{b}
4	105.1 ± 7.6^{b,c}	93.5^{b,c,d}	98.3 ± 7.1^{a,b,c}	68.3 ± 11.1^{a,b,c}	30.0 ± 14.4^{b}
5	76.2 ± 20.0^{c,d}	96.5^{a,b,c}	73.6 ± 19.3^{b,c,d}	46.1 ± 8.6^{b,c}	27.4 ±10.8^{b}
6	47.6 ± 4.1^{d,e}	97.4^{a,b}	46.4 ± 4.0^{b,c,d}	100.5 ± 6.2^{a}	-54.1 ± 3.7^{c}
7	28.9 ± 8.0^{e}	98.3^{a,b}	28.4 ± 7.8^{c,d}	55.5 ± 5.8^{b,c}	-27.1 ± 12.7^{c}
8	11.8 ± 1.6^{e}	99.6^{a}	11.8 ± 1.6^{d}	38.4 ± 5.5^{b,c}	-26.6 ± 6.0^{c}
9	11.6 ± 0.8^{e}	99.9^{a}	11.6 ± 0.8^{d}	62.6 ± 2.5^{b,c}	-51.0 ± 2.4^{c}

their needs. The nutritional value of a mixed diet depends on nutrient composition, digestibility, and palatability (Garr *et al.*, 2011). This means that oysters consumed a low number of food particles. This reduced ingestion may be related to the physical properties of the cereal particles, such as size, surface, and palatability. Absorption efficiency has been shown to vary widely depending on the species and habitat. Cockles for example, can modify the digestive process to maintain a fairly constant *e* (Ibarrola *et al.*, 1998; Nieves-Soto *et al.*, 2011), while pearl oysters showed increased *e* at increasing water temperature (Yukihira *et al.*, 2000). Absorption efficiency in the Cortez oyster was higher than that of *Anadara tuberculosa* (≤ 61%) used for bioremediation (Nieves-Soto *et al.*, 2011), and the pearl oysters *Pinctada maxima* and *Pinctada margaritifera* (≤58%) fed the microalgae *Tisochrysis lutea* (Yukihira *et al.*, 2000). The maximum SFG (103.2 J g^{-1} h^{-1}) of the Cortez oyster was also higher compared to the blood cockle *A. granosa*, that reached 60 J g^{-1} h^{-1} at similar temperatures and salinities (Din & Ahamad, 1995). The Atlantic surfclam *Spisula subtruncata*, showed similar SFG (100 J g^{-1} h^{-1}) on the Netherlands coast during an annual cycle (Rueda & Smaal, 2004) than oysters in our study.

In conclusion, the best diet for Cortez oyster spat production was the mixture of live microalgae. However positive SFG was found in those diets where microalgae were replaced with up to 50% cereal flours, constituting a useful finding in hatchery operations.

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