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Marine Diatom (*Chaetoceros calcitrans*) as a Monospecies Diet for Conditioning Oyster (*Crassostrea belcheri* Sowerby) Broodstock

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ABSTRACT

Chaetoceros calcitrans is a marine diatom widely used in aquaculture industries, as it is comprised of nutritional value suitable for most marine filter feeders. Large amounts of the diatom cells are routinely discharged from shrimp hatcheries as uneaten food. The effluent, rich in algal cells, can potentially be re-used to feed oyster broodstock, as the oysters are an effective filter feeder. This study attempted to investigate viability of *C. calcitrans* as a monospecies diet for conditioning broodstock of *Crassostrea belcheri*.

It was found that gametes of both sexes developed to spawning condition when rearing in captivity using *C. calcitrans*. Gametes were found in all four stages i.e. active, ripe, spawned and spent - at 15.4, 30.8, 30.8 and 23.1%, respectively when reared for 20 days. When the conditioning period was extended for 40 days, the development stages were found at 15.4, 15.4, 30.8 and 38.5%, respectively. The whole body weight decreased 2.01-2.65% after 20 days and 7.72% after 40 days. The condition indices were obtained between 6.31-7.78% and showed no relation to gametogenic stages of gonad. This study concluded that *C. calcitrans* can potentially be used as a monospecies diet for conditioning of oysters *C. belcheri* broodstock.

Key words: *Chaetoceros calcitrans* - *Crassostrea belcheri* - Broodstock

INTRODUCTION

Production of algal food *Chaetoceros calcitrans* used in marine shrimp hatcheries is labor intensive and costly. Unfortunately large amounts of uneaten algae are regularly lost from larval rearing systems through water exchange. Effluent rich in algal cells can potentially be re-used in a recirculating oyster-shrimp system as oysters are an effective filter feeder. Wang (1) states that an integrated shrimp-algae-oyster production system not only reduces water consumption but also turns waste into a profitable oyster production. For bivalve aquaculture, various species of microalgae have been used as an oyster life feed - either a monospecies or within a mixed species (2,3). Eight algal species including *Isochrysis* sp., *Chaetoceros gracilis*, *Chaetoceros calcitrans*, *Tetraselmis suecica*, *Thalassiosira pseudonana*, *Pavlova lutheri*, *Isochrysis*

galbana and *Skeletoema costatum* are widely used for oyster culture (2,3,4,5). For hatchery production selection of food algae for broodstock is essential as a control of reproduction involving the complex interaction of exogenous and endogenous factors. The exogenous factors include temperature, food, salinity and light, while the endogenous factors involve neuro-endocrine cycles and genotype (6). However, for many food species temperature is the major factor determining the timing of gametogenesis (6). Marunaka and Lannan (7) also stated that viable oyster broodstocks are only obtained when food nutrients reach requirement for their gametogenic development. While nutritional requirements of various species of oyster have been widely studied and well defined in western countries, information in this area especially for broodstock of *C. belcheri* in Thailand is relatively undetermined. This study attempted to determine the potential of *C. calcitrans* as a monospecies diet for conditioning of oyster, *Crassostrea belcheri*, broodstock when kept in captivity. The investigation was carried out to measure weight gain and gamete development of the oyster reared in a land-based holding facility.

MATERIALS AND METHODS

Experimental Organisms

Oysters, *Crassostrea belcheri*, were collected from an oyster farm in Ban Don Bay, Suratthani Province, southern Thailand. The oysters were cleaned to remove clay and epiphytes and kept for 5 days in fresh seawater in a flow through concrete tank. The oysters were then induced to spawn prior to use in experiments. Spawning was induced by thermal manipulation following the method of Ewart (8). A total of 100 oysters with a shell length of 90-120 mm were used and 36 oysters 11 males and 25 females spawned during induction. Only 36 individuals of the spawned oysters were further used as experimental organisms.

Experimental Protocol

A total of 36 spawned oysters were divided into 3 groups - of 10, 13 and 13 oysters. Group-1 was used for measuring gametogenic activity and condition index at the start (day-0). Group-2 was used for measuring weight gain and gametogenic activity at day-20. Group-3 was used for measuring gametogenic activity at day-40 and weight gain at day-20 and day-40. All groups were reared in a fiber glass vessel. Algal, *Chaetoceros calcitrans*, cells were added to the rearing tank at an initial concentration of 1.0×10^5 to 3.0×10^5 cells/ml as recommended by Ewart (8). Additional quantities of algae were continuously added to the rearing tank by gravity feed at a flow rate of 18-20 L/day/oyster following the method of Hickman and O'Meley (9). At this flow rate, the oysters ingested diatom at the amount of $2\text{-}10\times10^8$ cells/day/oyster as recommended by Pruder et al (1976 cited in 7). The rearing tank was emptied to clean and remove feces daily. Water qualities in the rearing tank were measured every 5 days, the results were as follows; salinity = 26±1 ppt, pH = 7.5-8.2, total ammonia <0.2 mgN/L and water temperature was 26-31°C.

Measuring of Weight Gain

Weight gain of oysters in this study is a measurement of percentage weight increments in a period of 20 days and 40 days. It assumes that shell growth is not detected in such a short experimental period. Thus, weight increment increases or loses in this experiment are presumed to be from the meat condition of the oysters.

Measuring of Condition Index and Gametogenic Cycle

Examination of germ cells development was done 3 times at day-0, day-20 and day-40 and groups of 10, 13 and 13 oysters were taken, respectively. Both male and female were taken at specified times as a proportional of the total sample sizes of 11 males and 25 females. The oysters were sacrificed for measurement. Whole body and meat weights were measured. Gonad and stomach were removed and cross-sectioned into 3 pieces. The tissue samples were preserved in 10% buffer formalin for 24 hours then rinsed with clean water and preserved in 70% ethyl alcohol for further analyses. Preparation of gonad tissue for histological examination was done following the method of Humason (10). Differentiation of gonad development was classified following the description of Dinamani (11) as spent, active, ripe and spawned stages. Somatic growth was investigated by measuring the condition index (CI) following the method of Hickman and Illingworth (12) using equation: CI = (Whole wet weight / meat wet weight) $\times 100$.

RESULTS

For histological examination, the results showed that gametes of both sexes developed to spawning condition when rearing in captivity using *C. calcitrans* as shown in **Table 1**. It was found that all gonads collected at day-0 were partially empty. A few eggs and sperm still remained in ovaries and testicles. When oysters were reared for 20 days oyster gametes were found in all four stages - active, ripe, spawned and spent - at 15.4, 30.8, 30.8 and 23.1%, respectively. When the conditioning period was extended for 40 days, the development stages of active, ripe, spawned and spent were found at 15.4, 15.4, 30.8 and 38.5%, respectively. This suggests that oysters are ready to spawn within a period of 20 days following previous spawning.

For weight gain, the results showed that body weight gradually decreased when the rearing period was prolonged **(Table 2)**. The whole body weight decreased 2.01-2.65% after 20 days and 7.72% after 40 days. This suggests that feeding of oysters with *C. calcitrans* at the amount of 2-10$\times 10^8$ cells/day/oyster was insufficient for growth of oysters.

For the condition index, it was found that condition indices were obtained between 6.31% to 7.78%. The condition index showed no relation to gametogenic stages of gonads **(Table 3)**. This suggests that the condition index is not applicable to indicate developmental stages of oyster gonads.

For water quality, the concentration of total ammonium in the rearing tank was at an acceptable level for molluscs (13). Water temperature, salinity and pH were compatible to the levels found in a natural oyster habitat.

Table 1. Gametogenic stages of *Crassostrea belcheri* broodstocks reared in a conditioning tank fed with *Chaetoceros calcitrans*, M = male and F = female.

Day(s)	Oysters of group	Gametogenic stages			
		Active Number (%)	Ripe Number (%)	Spawned Number (%)	Spent Number (%)
0	**1**, n=10	0	0	10 (100)	0
20	**2**, n=13	2M, (15.4)	1M-3F, (30.8)	1M-3F, (30.8)	3F, (23.1)
40	**3**, n=13	2F, (15.4)	2M, (15.4)	4F, (30.8)	1M-4F, (38.5)

Table 2. Weight gain of *Crassostrea belcheri* broodstocks reared in a conditioning tank fed with *Chaetoceros calcitrans*.

Day (s)	Weight gain (%)	
	Oysters of group-2 (n=13)	Oysters of group-3 (n=13)
20	- 2.65	-2.01
40		-7.72

Table 3. Condition index (CI) of *Crassostrea belcheri* broodstocks at various gametogenic stages after reared with *Chaetoceros calcitrans* for 20 or 40 days.

Developmental stages	CI (%)	Number of oyster used
Active	7.15±1.43	4
Ripe	6.31±1.01	6
Spawned	7.78±1.27	18
Spent	6.94±1.31	8

DISCUSSION

Though, many reports claim that a monospecies diet does not support well the development of oysters (2,3,4,5) Brown et al (14) states that algal species can vary significantly in their nutritional value. Coutteau (15) suggested that combinations of prymnessiophytes and diatoms were practically used as they provide a well balance diet that will accelerate development and metamorphosis of oysters in comparison with unialgal diets. PUFAs derived from microalgae, i.e. docosahexaenoic acid (22:6n-3, DHA), eicosapentaenoic acid (20:5n-3, EPA) and arachidonic acid (AA) are known to be essential for bivalves (16). While high concentrations of DHA are found in the prymnessiophytes such as *P. lutheri* and *Isochrysis* sp., diatoms such as *C. calcitrans* are rich in EPA (15). These three species practically comprise a mixed diet for feeding oysters at larval, juvenile and broodstock stages (3,15). However, this study found that gonads of *C. belcheri* actively developed when fed solely on *C. calcitrans*. Oysters also showed weight loss during the experiment. This suggests that even though the amount of food is not enough for somatic growth, the nutritional value of *C. calcitrans* is sufficient and meets the requirement for gametogenesis of *C. belcheri*.

Generally condition index (condition factor) is used to determine fatness of bivalves (12) and is a measurement of weight gain as a whole. This measurement may indicate growth of both somatic and germ cells, or one or the other. However, this study found no relation between condition index and gametogenic activity of germ cells. Condition indices of this oyster species also varied greatly according to their age, size, shape and thickness of shell. In natural water the condition indices of *C. belcheri* were reported to vary from 3.08% to 24.86% (17). Gosling (6) suggested that reproductive cycles of bivalves could be measured in terms of a gonad index (GI) that increases during gametogenesis. GI is a qualitative measurement calculated by multiplying the number of individuals at each development stage by the numerical ranking of that stage (developing = 1, ripe = 2, spawn = 3 and spent = 4), and dividing the result by the total number of individuals in the sample. Quantitative measurements such as gamete volume fraction (GVF) determined by stereological techniques is also used to measure reproductive cycles of bivalves (6). Kim et al (18) used various assessment methods for measuring gonadal development of the purplish Washing clam (*Saxidomus purpuratus*) including factor (fatness), gonad index (GI), ovarian egg diameter and biochemical composition (RNA, DNA content and their ratio RNA/DNA). They found that the RNA/DNA ratio most represented the processes of gonad development and spawning pattern of the clam.

CONCLUSIONS

This study concludes that *C. calcitrans* can potentially be used as a monospecies diet for conditioning of oysters *C. belcheri* broodstock. This finding is of particular benefit to integrated shrimp-oyster production where large amounts of *C. calcitrans* is produced. Further studies should focus on in improving weight gain of the broodstock. Investigation of optimal density of algae and stock density of broodstock in the holding tank is also essential for oyster hatchery. The methods used for assessment of the reproductive cycle suggested by Gosling (6) and Kim et al (18) should also be applied when a study on reproductive cycle of oysters is needed.

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บทคัดย่อ

ปิยะพงค์ โชติพันธุ์

การใช้ไดอะตอม *Chaetoceros calcitrans* เป็นอาหารสำหรับขุนพ่อแม่พันธุ์หอยตะโกรมกรามขาว (*Crassostrea belcheri* Sowerby)

ไดอะตอมชนิด *Chaetoceros calcitrans* เป็นสาหร่ายที่มีคุณค่าทางอาหารสูง นิยมใช้เลี้ยงสัตว์ทะเลวัยอ่อนที่กินอาหารโดยวิธีกรอง สาหร่ายชนิดนี้จะถูกผลิตขึ้นจำนวนมากเพื่อใช้สำหรับเลี้ยงลูกกุ้งวัยอ่อน และมีจำนวนหนึ่งถูกถ่ายทิ้งขณะที่มีการเปลี่ยนถ่ายน้ำในบ่ออนุบาลลูกกุ้ง น้ำทิ้งจากบ่ออนุบาลที่มีเซลล์สาหร่ายอาจนำไปใช้เลี้ยงหอยนางรมได้อีกเนื่องจากหอยนางรมเป็นสัตว์ที่กรองสาหร่ายได้อย่างมีประสิทธิภาพ การทดลองครั้งนี้เป็นการศึกษาความเป็นไปได้ในการใช้สาหร่าย *C. calcitrans* สำหรับขุนหอยตะโกรมกรามขาว (*Crassostrea belcheri* Sowerby) ให้สามารถพัฒนาเซลล์สืบพันธุ์จนถึงขั้นผสมพันธุ์ได้ในที่กักขัง

ผลการศึกษาพบว่าเซลล์สืบพันธุ์ของหอยทั้งสองเพศพัฒนาจนถึงขั้นที่สามารถผสมพันธุ์ได้เมื่อเลี้ยงในที่กักขัง หลังจากเลี้ยงด้วยสาหร่ายที่ใช้ทดลองเป็นเวลา 20 วัน และ 40 วัน ตรวจพบเซลล์สืบพันธุ์ได้ในทุกระยะของการพัฒนาคือ active, ripe, spawned และ spent จำนวน 15.4, 30.8, 30.8 และ 23.1% ตามลำดับ และ 15.4, 15.4, 30.8 และ 38.5% ตามลำดับ แต่น้ำหนักของหอยลดลง 2.01 - 2.65% และ 7.72% หลังจากเลี้ยงเป็นเวลา 20 วัน และ 40 วัน ตามลำดับ วัดค่าดัชนีความสมบูรณ์ได้ตั้งแต่ 6.31% ถึง 7.78% ซึ่งค่าที่วัดได้ไม่มีความสัมพันธ์กับระยะพัฒนาของเซลล์สืบพันธุ์แต่อย่างใด การศึกษาครั้งนี้สรุปว่าการขุนพ่อแม่พันธุ์หอยตะโกรมกรามขาวโดยใช้สาหร่าย *C. calcitrans* เพียงชนิดเดียวสามารถทำให้เซลล์สืบพันธุ์พัฒนาจนถึงระยะผสมพันธุ์ได้ในที่กักขัง

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