

かき研究所 ニュース

Oyster Research Institute News No.24 2009.10

Special Edition of the Proceedings of
the 1st & 2nd International Oyster Symposiums



The Foundation of Oyster Research Institute

まえがき

「かき研究所ニュース 特別号」は、第2回国際かきシンポジウム^{*1} (IOS2)及び第1回国際かきシンポジウム^{*2} (IOS1)における発表論文の一部を掲載しています。

世界かき学会^{*3} (WOS) では発表論文を世界に広く発信するために、国際的に評価の高い米国発行の国際誌“Journal of Shellfish Research” (JSR) に投稿することになり、私のほか WOS 運営委員の Maguire 博士 (オーストラリア)、Wu 教授 (中国) で構成するプロシーディング編集委員会を設置し、2008年4月より審査・選考作業を行いました。

論文募集の概要は以下の通りです。

- ・ WOS 編集委員会と JSR 編集委員会の双方が認めた論文について、JSR への掲載料の一部を財団法人かき研究所が助成する。
- ・ 執筆者が希望すれば、財団法人かき研究所が発行するかき研究所ニュースに掲載することができる。
- ・ 学生が IOS2 で発表した論文のうち、彼らが第一執筆者の場合に限り、優秀な論文3編について JSR 掲載料を財団法人かき研究所が全額助成する。

2008年3月末日の締切までに計17編の論文が提出されました。プロシーディング編集委員会では、Dr. Greg Maguire が中心となって応募論文の中から JSR 投稿候補論文の選考と校閲を行いました。当初の予想を超える労力と時間を要し、JSR へ論文をまとめて提出することが難しい状況となりました。そこで、2009年8月、執筆者に JSR へ論文を直接提出するよう要請し、了承されました。また、JSR 投稿論文以外の論文については、執筆者より許可を得たものを本号に掲載することになりました。

上記のような事情により、プロシーディングの発行が遅れたことをお詫び申し上げますとともに、本号の発行に際し、論文の掲載にご協力いただきました執筆者の皆様には心より御礼申し上げます。

*1) The 2nd International Oyster Symposium (IOS2)。2007年11月、中国浙江省杭州市にて浙江大学との共催で行われた。

*2) The 1st International Oyster Symposium (IOS1)。2005年7月、東京ビッグサイトにて財団法人かき研究所の主催で行われた。

*3) The World Oyster Society (WOS)。IOS1 総括討議にて設立された。

2009年10月

世界かき学会 会長

財団法人かき研究所 理事長

森 勝 義

Preface

The “Oyster Research Institute News Special Edition” carries some of the research papers presented at the 2nd International Oyster Symposium^{*1} (IOS2) and the 1st International Oyster Symposium (IOS1)^{*2}.

The World Oyster Society^{*3} (WOS) began the process of submitting its research papers to the “Journal of Shellfish Research” (JSR)—an internationally renowned research journal published in the United States—to disseminate our research efforts to the world. The Society established a *Proceedings* Editorial Board comprising three Steering Committee members of WOS: President Katsuyoshi Mori, Dr. Greg Maguire and Prof. Xinzhong Wu. The Board began its selection process in April 2008.

The Outline of the Call for Papers is the following.

- For papers accepted by the editorial boards of both WOS and JSR, the Foundation of Oyster Research Institute provides partial funding necessary for the JSR publication.
- The paper can be published in the Oyster Research Institute News if requested by the author.
- For three outstanding papers presented at IOS2, each of which must have a student as the first author, the Foundation of Oyster Research Institute provides full funding necessary for JSR publication.

Seventeen manuscripts were submitted by the deadline, the end of March, 2008. The *Proceedings* Editorial Board began the selection and editorial process headed by Dr. Greg Maguire, which required much more time and energy than we expected. Consequently, it became difficult for us to submit papers to JSR all together. In August 2009, I suggested that each author submits a paper directly to JSR. That suggestion was accepted. Papers other than those submitted to JSR with the authors’ permission are included in this special edition.

We apologize for the delayed publication of the *Proceedings* under these circumstances. We would like to express our gratitude to the authors of the papers on the occasion of this publication.

^{*1)} Held in November 2007 in Hangzhou City, Zhejiang Province, People's Republic of China jointly with Zhejiang University

^{*2)} Held in July 2005 at Tokyo Big Sight hosted by the Foundation of Oyster Research Institute

^{*3)} Established at General Discussion in IOS1

October, 2009

Katsuyoshi MORI, Ph.D.

President of the World Oyster Society
&
Board Chairman of the Foundation of
Oyster Research Institute

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- History, Status and Future of Oyster Culture in France
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- The Changing Face of Oyster Culture in New South Wales, Australia
Wayne A. O'CONNOR & Michael C. DOVE
- Nitric Oxide and Superoxide Anion Generation in *Crassostrea Ariakensis* Hemocytes Stimulated with Rickettsiales-Like Organisms and Zymosan
Jingfeng SUN & Xinzhong WU

The Taxonomic Status and Origin of the Portuguese Oyster *Crassostrea angulata* (Lamarck, 1819)

F. M. BATISTA^{1,2}, A. LEITÃO^{1,3}, A. HUVET⁴, S. LAPEGUE⁵,
S. HEURTEBISE⁵ & P. BOUDRY^{4,5}

¹ Instituto Nacional de Recursos Biológicos (INRB, I. P.) / L-IPIMAR, Av. 5 de Outubro, 8700-305 Olhão, Portugal; ² Instituto de Ciências Biomédicas Abel Salazar (ICBAS), Universidade do Porto, Largo Prof. Abel Salazar, 2, 4099-003 Porto, Portugal; ³ Departamento de Genética e Biotecnologia, Centro de Genética e Biotecnologia da Universidade de Trás-os-Montes e Alto Douro, CGB/UTAD, P-5000-911 Vila Real, Portugal; ⁴ Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer), Unité Mixte de Recherche Physiologie et Ecophysiologie des Mollusques Marins, Centre de Brest, B. P. 70, 29280 Plouzané, France; ⁵ Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer), Laboratoire de Génétique et Pathologie (LGP), 17390 La Tremblade, France.

ABSTRACT: The taxonomic status of the Portuguese oyster *Crassostrea angulata* (Lamarck, 1819) and the Pacific oyster *C. gigas* (Thunberg, 1793) has often been a matter of controversy. Based on larval shell morphology, experimental hybridization and electrophoretic studies of enzyme polymorphism, several authors have considered these two species to be synonymous. Recently, several genetic studies based on mitochondrial DNA and microsatellite data indicated that the two taxa are genetically distinct although closely related. Karyotype analysis has also supported the close genetic similarity of these taxa in comparison with other cupped oysters. However, a recent comparative analysis of chromosomes using restriction enzyme banding patterns highlighted differences between all chromosome pairs of *C. angulata* and *C. gigas* except chromosomal pair 10. In addition, significant phenotypic differences between the two taxa were observed in terms of aquaculture production, ecophysiological characteristics and aneuploidy levels. Moreover, differences in shell shape and muscle scar pigmentation were observed between adults of the two taxa. *C. angulata* and *C. gigas* were long assumed to be native to the north-eastern Atlantic and Asia, respectively. However, phylogenetic analysis firmly places both Portuguese and Pacific oysters within an Asian *Crassostrea* clade, supporting the hypothesis of the introduction of *C. angulata* from Asia to Europe. Pure populations of *C. angulata* were observed in Taiwan as well as presumed mixed populations of *C. angulata* and *C. gigas* in northern China. These findings suggest that (1) *C. angulata* and *C. gigas* are very closely related, but they cannot be considered synonymous and (2) the Portuguese oyster has an Asian origin.

Short running title: TAXONOMIC STATUS AND ORIGIN OF *C. ANGULATA*

KEY WORDS: *Crassostrea angulata*, *C. gigas*, taxonomy, origin, phylogenetic analysis

INTRODUCTION

Oysters belonging to the genus *Crassostrea*, known as cupped oysters, are among the most important commercial aquatic species in the world. These species have been harvested from the wild and cultivated since ancient times. They include the Portuguese oyster *Crassostrea angulata* (Lamarck, 1819) and the Pacific oyster *C. gigas* (Thunberg, 1793) which were long assumed to be natives of the north-eastern Atlantic and Asia, respectively.

The Portuguese oyster was a species of major economic importance in Europe up to the early 1970's and sustained European oyster production for almost one century, before major mortalities between 1967 and 1973 almost wiped it out from Europe (Comps 1988). Now there is only a small production of *C. angulata* in Europe but it is presumed to be the main oyster species produced in Taiwan and possibly one of the main oyster species produced in China (Lapègue et al. 2004, Guo et al. 2006). The Pacific oyster was widely introduced and is

presently farmed throughout the Americas, Africa, Australasia, Europe, and Asia: it is one of the main marine organisms produced in the world (FAO 2006).

The taxonomic status of the Portuguese and the Pacific oysters has been a matter of controversy (Carriker & Gaffney 1996). The main justification of their distinction into two different species is their geographical separation, whereas some morphological, experimental hybridization and molecular genetic data suggest synonymy. In the last decade several studies have been published that shed more light on the taxonomic status and origin of *C. angulata* and *C. gigas*.

The objective of the present work is reviewing the published studies about *C. angulata* and *C. gigas* that can help to clarify the taxonomic status and origin of the Portuguese oyster. The potential role for *C. angulata* in the future development of the oyster industry is also discussed.

MORPHOLOGICAL ANALYSES

Classification of oysters based only on morphological analysis can be problematic, especially in the genus *Crassostrea*, due to high variation and plasticity that are strongly influenced by environmental conditions (Galtsoff 1964). Although some adult shell features can be used in the classification of *Crassostrea* species, their utility is limited especially in closely related taxa. Other morphological characteristics, such as the morphology of late larval shells (Hu et al. 1993) and anatomical features (Wang et al. 2004), are considered more useful as taxonomic attributes. Ranson (1960) reported no differences between the morphology of larval shells of *C. angulata* and *C. gigas*, whereas he could separate other species using the same approach. Consequently, he considered them to be the same species. According to Menzel (1974) and Biocca & Matta (1982), the adult shells of *C. angulata* and *C. gigas* are indistinguishable, but no morphometric analyses were performed and the criteria used to differentiate them were not specified. A recent study by Batista et al. (2008) revealed differences in shell shape between adults of *C. angulata* and *C. gigas* reared under common conditions. *C. angulata* had deeper shells with shorter adductor muscle scars as well as smaller ligamental areas than *C. gigas* (Batista et al. 2008). Moreover, *C. angulata* had highly pigmented adductor muscle scars whereas in *C. gigas* the pig-

mentation was lighter (Galtsoff 1964, Batista et al. 2008). Furthermore, Evseev et al. (1996) found anatomical differences in the arrangements of the interlamellar septa of *C. gigas* when compared with observations of *C. angulata* by Nelson (1960, in Evseev et al. 1996). While a direct comparison of the anatomy of the two taxa using animals from different populations is still lacking, both taxa are morphologically very similar which suggests that they are very closely related.

GROWTH AND ECOPHYSIOLOGICAL COMPARISONS

Significant phenotypic differences between Portuguese and Pacific oysters have been reported in recent decades (Héral & Deslous-Paoli 1991). Production yield is an important economic trait in shellfish farming that takes into account survival and growth. A higher production yield in the natural environment has been observed for *C. gigas* than *C. angulata* (Parache 1989, Soletchnik et al. 2002). Héral et al. (1986) observed that production was always higher for *C. gigas* than for *C. angulata* at various stocking levels in Marennes-Oléron Bay, France. These differences were mainly attributed to the faster growth of *C. gigas*, but are also related in some cases to higher mortality of *C. angulata* (His 1972, Bougrier et al. 1986, Soletchnik et al. 2002, Batista et al. 2007).

Some ecophysiological comparisons have been made between the two taxa to better understand these growth rate differences. His (1971) observed that the Pacific oyster had a higher clearance rate and that below 10°C the difference in valve activity between the taxa increased such that only *C. gigas* was active at 2°C. Juvenile Pacific oysters had higher oxygen consumption rates than juvenile Portuguese oysters (Gouletquer et al. 1999). Haure et al. (2003) found differences in feeding time activity but not in clearance and oxygen consumption rates. These ecophysiological results suggest that *C. angulata* and *C. gigas* have different strategies for the allocation of the available energy, which could explain their dissimilar growth. A negative correlation between the degree of somatic aneuploidy (i.e., the proportion of somatic cells with one or more chromosomes missing) and growth rate was observed in *C. gigas* (Thiriou-Quévieux et al. 1988, Leitão et al. 2001). Recently, Batista et al. (2007) recorded higher values for the proportion of aneuploid cells and numbers of missing chromosomes

in *C. angulata* than in *C. gigas*. These authors also observed a negative correlation between somatic aneuploidy and growth rate for both species.

SUSCEPTIBILITY TO DISEASE AND PARASITES

Major mortalities between 1967 and 1973, commencing in France in 1966, almost led to the disappearance of the Portuguese oyster from Europe (Renault 1996). The “gill disease”, identified as the main cause of these mortalities, was characterized by the appearance of gill lesions (Arvy & Franc 1968, Alderman & Gras 1969, Marteil 1969, Comps 1970). A second period of mass mortalities followed, but this time without distinctive clinical signs (Comps 1988). Different agents were implicated in the mortalities such as fungi, protozoans and viruses (Arvy & Franc 1968, Besse 1968, Gras 1969, Comps & Duthoit 1976). Irido-like viruses are regarded as one of the most probable causes of the mortalities and they were designated as gill necrosis virus (Comps & Duthoit 1976) and haemocyte infection virus (Comps 1988). However, these viruses were not isolated and neither were experimental transmission studies performed to demonstrate their pathogenicity (Comps 1988). Large-scale introduction of a replacement species in France, the Pacific oyster *C. gigas*, was initiated in 1970 in order to overcome the crisis (Grizel & Héral 1991). However, smaller scale introductions beginning in 1966 may have led to the introduction of the putative agent that caused the mortalities (Renault 1996). The irido-like viruses associated with the mortalities were also observed in the Pacific oyster (Comps & Duthoit 1976, Comps & Bonami 1977), but no losses were detected in this species during a period when the Portuguese oyster suffered mass mortalities (Comps 1988). Hence, these results suggest that *C. angulata* and *C. gigas* have different susceptibility to the putative disease that threatened the Portuguese oyster with extinction in Europe.

A significant higher prevalence of the parasitic copepod *Myicola ostreae* was observed in *C. angulata* in comparison with *C. gigas* (Batista et al. 2009). Moreover, the infestation was significantly less intense in affected *C. gigas* than in *C. angulata*. Histopathological analyses indicate that the higher susceptibility of *C. angulata* to *M. ostreae*, in comparison with *C. gigas*, may have been due to differences in the host's response to the parasite

(Batista et al. 2009).

GENETIC DIFFERENTIATION AND VARIABILITY

The high genetic similarity between *C. angulata* and *C. gigas* observed by several authors using allozyme markers supported the hypothesis that the Portuguese and Pacific oysters should be classified as the same species (Mathers et al. 1974, Buroker et al. 1979, Biocca & Matta 1982, Mattiucci & Villani 1983). However, studies on the mitochondrial cytochrome oxidase subunit I (COI) gene have shown clear genetic differences between the two taxa (Boudry et al. 1998, O'Foighil et al. 1998). An average of 2.3% difference in this nucleotide sequence suggests that populations of *C. angulata* and *C. gigas* may have diverged several hundred thousand years ago (Hedgecock et al. 2004). Huvet et al. (2000) revealed low but significant genetic differences between Portuguese and Pacific oyster populations using microsatellite markers (mean Wright's fixation index; $F_{st} = 0.022$). These authors reported that the genetic differentiation observed in pairs of populations of the two different taxa were twice as large as in pairs of populations of the same taxon. Studies based on satellite DNA (López-Flores et al. 2004) and the 5S rRNA gene (Cross & Rebordinos 2006) highlighted the genetic similarity between *C. angulata* and *C. gigas*. All these genetic studies clearly indicate close phylogenetic ties between the Portuguese and Pacific oysters. Nevertheless, studies using mitochondrial and microsatellites markers have shown that there are small but clear genetic differences between them.

The level of mitochondrial genetic variation of *C. gigas* (samples collected in different parts of the world) appears to be lower than that of *C. angulata* (samples collected in Portugal), based on PCR-RFLP haplotypes of a COI fragment (Boudry et al. 1998, Lapègue et al. 2004). Using sequence data for the same mitochondrial gene, O'Foighil et al. (1998) observed no polymorphism in individuals of *C. gigas* ($n=20$) with different origins whereas high polymorphism was observed in *C. angulata* ($n=5$) from the Sado estuary, Portugal. In addition, the analysis of the sequence of a 584 bp region of the same gene revealed higher genetic variability in a *C. angulata* population ($n = 47$) from the Sado River (Portugal) than a *C. gigas* population ($n = 44$) from the Seudre River (France) (Batista, 2007).

Nevertheless, Huvet et al. (2000) using microsatellite markers observed similar levels of genetic variation in both taxa. The low mitochondrial variability of *C. gigas*, when compared with *C. angulata*, could be related to the dissemination of *C. gigas* from Miyagi Prefecture to different regions in Japan (O'Foighil et al. 1998) and other places in the world where the Pacific oyster was introduced.

CYTOTAXONOMY

Oysters of the genus *Crassostrea* have a diploid chromosome number ($2n=20$) which is a common feature in the *Ostreidae* family (Thiriou-Quévieux 2002). Comparative standard karyological analysis of *C. angulata* and *C. gigas* supported the close genetic similarity of these two taxa when compared with other cupped oyster species (Leitão et al. 1999a). A study in which the G-banding technique was applied to chromosomes of *C. angulata* and *C. gigas* confirmed the high similarity between karyotypes of both taxa, but showed differences supporting their taxonomic separation (Leitão et al. 1999b). Leitão et al. (2004) confirmed the previous findings, through the application of a restriction endonuclease banding technique that revealed differences between the banding patterns in all chromosomes pairs of *C. angulata* and *C. gigas* except for chromosome pair 10. As already mentioned, these two taxa have different levels of aneuploidy (negatively correlated with growth), but the chromosomal pairs absent in aneuploid situations (mainly pairs 1, 9 and 10) were the same for the two taxa (A. Leitão, unpublished data). From a cytogenetic perspective, these taxa are indeed very similar, but nevertheless can be distinguished.

ORIGIN OF THE PORTUGUESE OYSTER

Their disjunct geographical distribution, *C. angulata* being present in the north-eastern Atlantic and *C. gigas* in Asia, before the voluntary introduction of *C. gigas* in Europe (Grizel & Héral 1991) in the early 1970s, led to the question of the origin of these two closely related taxa. Three hypothesis have been proposed to explain their disjunct geographic distributions: (i) *C. angulata* (Iberian Peninsula and Morocco), *C. gigas* (China, Japan, to Sakhalin Island) and *C. cattuckensis* (India) were derived from a fossil ancestor toward the end of the Miocene but became isolated as three populations, and eventually three species, as later, tectonic

events produced land barriers (Stenzel 1971); (ii) *C. angulata* was native to Europe but transported to Asia some centuries ago by European merchant ships (Menzel 1974); (iii) *C. angulata* was introduced to Europe by undocumented anthropogenic transfer during the earliest days of circumglobal navigation from the Far East (Ranson 1960).

Based on mitochondrial DNA sequence data, O'Foighil et al. (1998) estimated a divergence time of 1 to 2 million years for the Portuguese and Pacific oysters, long after closure of the Tethyan Seaway, estimated at approximately 7 million years ago. Although this study may have overestimated divergence times, it clearly undermines Stenzel's hypothesis, suggesting that the closure of the Tethyan Seaway occurred before the appearance of the last common ancestor of the Portuguese and Pacific oysters. According to Ranson (1948, in Edwards 1976), there is no evidence of the presence of *C. angulata* in the Miocene, Pliocene and Quaternary beds of Portugal, which supports the hypothesis of a recent introduction of *C. angulata* into Europe from another region. Conversely, Lawrence (1995) argues that the fossil record does not promote the notion that the Portuguese and Pacific oysters may have been imported into the eastern Atlantic by humans. In fact, *C. angulata* has been reported to exist for at least 2200 years in Spain (Ruiz et al. 2004). It is possible that other *Crassostrea* species, native to the eastern Atlantic, were derived from a fossil ancestor, but are no longer present in Europe: these could have been incorrectly identified in the late Holocene sediments as *C. angulata*. Another possibility is that in some palaeontological studies other species of oysters were mistakenly classified as *C. angulata* because of the high morphological plasticity of oysters. The close genetic relationship between *C. angulata*, *C. gigas*, and other *Crassostrea* species from Asia (Fig. 1) such as the Kumamoto oyster *C. sikamea* and the Suminoe oyster *C. ariakensis* suggests that the Portuguese oyster has an Asian origin (Buroker et al. 1979, O'Foighil et al. 1998, Boudry et al. 2003). Moreover, studies using mitochondrial markers reported the presence of populations of *C. angulata* in Taiwan (Boudry et al. 1998) as well as presumed mixed populations of *C. angulata* and *C. gigas* in Northern China (Yu et al. 2003, Lapègue et al. 2004). These findings suggest that the Portuguese oyster has an Asian origin and is probably a case of recent undocumented anthropogenic introduction.

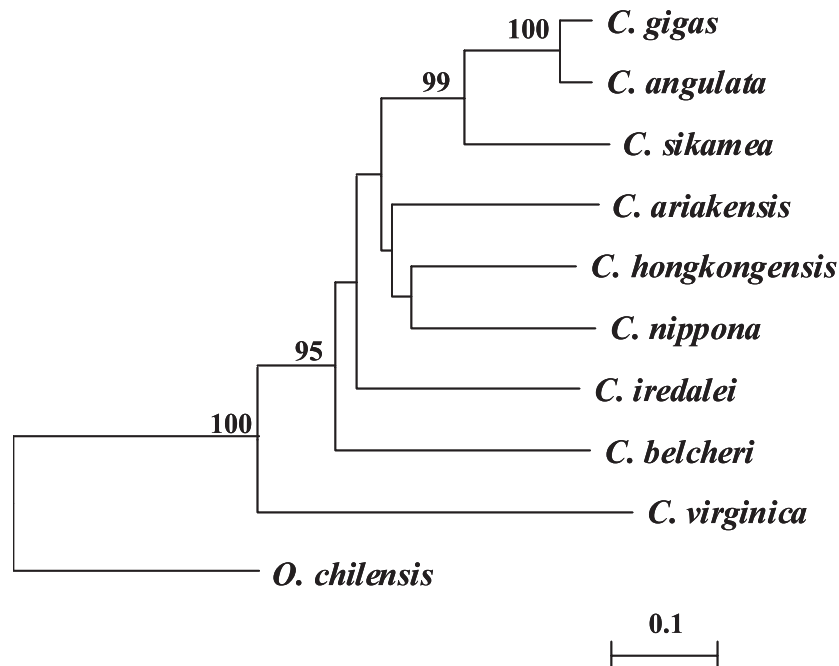


Figure 1. Phylogenetic tree based on neighbor-joining analyses of *Crassostrea* species of a partial cytochrome oxidase subunit 1 gene sequence using Kimura two-parameter distances with 1000 bootstrap. *Ostrea chilensis* was used as an outgroup. Numbers at specific nodes represent bootstrap values greater than 70%.

THE BIOLOGICAL SPECIES CONCEPT

Several species concepts can be found in the literature which arises from the difficulty to define species which is known as the “species problem”. Hence, it is important to define what species concept is being employed and to bear in mind that the different concepts may have flaws. The biological species concept defines a species as a group of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups (Mayr 1942). Several studies have been done to determine if there are pre- and/or post-zygotic mechanism that can contribute to the reproductive isolation of *C. angulata* and *C. gigas*. High fertilization rates in crosses of *C. angulata* with *C. gigas* have been reported by different authors (Imai & Sakai 1961, Menzel 1974, Huvet et al. 2001). Molecular analysis of six-hour old embryos revealed no evidence of preferential fertilization between gametes from the same taxon when spermatic competition was allowed between taxa (Huvet et al. 2001). However, the results of Huvet (2000) and Soletchnik et al. (2002), based on the analysis of sexual maturation of *C. angulata* and

C. gigas, suggest that asynchronous spawning may occur. In addition, differences between these two taxa in the minimum temperature at which eggs or sperm are released were reported by Lubet (1994) based on the work of Le Dantec (1968). Normal viability of the F1 hybrids between the Portuguese and Pacific oysters has been observed by several authors (Imai & Sakai 1961, Menzel 1974, Bougrier et al. 1986, Huvet et al. 2002). According to Menzel (1974) meiosis in the F1 hybrids of the Portuguese and Pacific oysters as well as mitosis in the F2’s embryos appeared normal. However, Numachi (1966, in Gaffney & Allen 1993) have previously reported that F1 hybrids of both taxa display normal viability and fertility, but the F2 progeny did not survive to settlement. These results were not corroborated by Huvet et al. (2002) who produced F2 hybrids that showed normal fertilization rates, developmental yields, and settlement rates. Huvet et al. (2004) provided evidence for the existence of hybridization between the Portuguese and Pacific oysters in the natural environment where the two taxa were recently put in contact. Moreover, Batista (2007) reported that the recruitment of *C. angulata*, *C. gigas* and there hybrids

overlapped during most of the setting season in Ria Formosa (Portugal). However, differences in haplotypes and allelic frequencies were observed for almost all setting periods which suggest that pre- and/or post-zygotic mechanism may lead to partial reproductive isolation between *C. angulata* and *C. gigas*. It is worth noting that the two previous studies were done in Portugal where both *C. angulata* and *C. gigas* were introduced and hence further studies should be done in areas where the two taxa are thought to be native. Nevertheless, although little is known about the distribution of the two taxa in Asia they remain apparently genetically distinct in areas of allopatry (*C. angulata* in Taiwan and *C. gigas* in Japan; Boudry et al. 1998) despite the prolonged planktonic larval stage that should facilitate dispersal and consequently gene flow.

FINAL CONSIDERATIONS

The current knowledge on the Portuguese and Pacific oysters shows that they are very closely related, but also that there are clear genetic and phenotypic differences between them. Consequently, these two taxa cannot be considered synonymous. More data about putative barriers to gene flow between the two taxa would be useful for defining their taxonomic status. Different lines of evidence suggest that the Portuguese oyster is of Asian origin. A better knowledge on the distribution of *C. angulata* in Asia can provide new insights regarding the origin of the Portuguese oyster, namely in regions where the most common commercial routes between Europe and Asia were established during the earliest days of circumglobal navigation.

Other cases of closely related bivalves that are genetically and phenotypically distinct, but interbreed to produce sexually viable offspring, have been reported. One of the best studied cases are mussels of the *Mytilus* complex comprising the closely related but genetically distinct taxa *M. edulis*, *M. galloprovincialis* and *M. trossulus* (McDonald et al. 1991). Two of these species, *M. edulis* and *M. galloprovincialis*, co-occur in Western Europe and readily interbreed and produce hybrid zones with parental genotypes, high frequencies of F1 and F2 hybrids, and mussels of mixed genetic ancestry (Hilbish et al. 2002, Bierne et al. 2003). Another case of closely related taxa that hybridize in the natural environment involves the hard clams *Mercenaria mercenaria* and *M. campechiensis* on

the east coast of North America (Bert & Arnold 1995). Despite the taxonomic difficulties posed by these taxa, hybridization between them and the existence of hybrid zones offer excellent opportunities to study speciation and processes, which contribute to reproductive isolation (Gardner 1997). Evidence of natural hybridization between *C. angulata* and *C. gigas* in the south of Portugal, where the two taxa are in contact due to recent anthropogenic transfer, opened new perspectives for studying the evolutionary history of the *Crassostrea* genus (Huvet et al. 2004). The development of nuclear markers will be of crucial importance for investigating natural hybridization between these two cupped oysters.

The differences observed between the closely related taxa *C. angulata* and *C. gigas* in growth, aneuploidy levels, adult shell shape, ecophysiological characteristics, and parasite-disease susceptibility also offer new opportunities for examining the genetic basis of growth, shell shape and disease resistance. *C. angulata* can also be seen as a valuable genetic resource for the development of selective breeding programs as well as for production diversification and biodiversity preservation. Hence, the conservation of the remaining pure populations of *C. angulata* in Europe (Lapègue et al. 2004) is of importance since these genetic resources can be used for the development of the European oyster industry without the zoosanitary risks associated with the introduction of exotic oysters (Berthe & Boudry 1999).

ACKNOWLEDGMENTS

The authors thank Florence Rivet and Teresa Drago for helping compile some of the papers cited. We would like to acknowledge Greg Maquire and Bob Ward for comments that helped to improve this article.

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Oyster Culture in North America: History, Present and Future

René E. LAVOIE

Bedford Institute of Oceanography, Canada

ABSTRACT: There are several species of oysters in North America. On the Atlantic Coast, the American oyster *Crassostrea virginica* (Gmelin, 1791) is the most important, being cultivated in Mexico, the United States and Canada. The European oyster *Ostrea edulis* (Linnaeus, 1758) was introduced from Europe and is found in Canada and the north eastern part of the United States. On the Pacific Coast of Canada, there are three species of oysters: the native Olympia oyster *Ostrea lurida* (Carpenter, 1864), the American oyster *Crassostrea virginica*, and the Pacific oyster *Crassostrea gigas* (Thunberg, 1793). The Pacific (Miyagi) oyster *Crassostrea gigas* is the most important. On the market, it has replaced the native oyster which had been depleted by overfishing and water pollution. The Pacific oyster was first introduced into British Columbia waters about 1912 or 1913 from Miyagi Prefecture in Japan and is now established along the Pacific Coast of Mexico, the United States (including Alaska) and Canada. In its new North American home, the Miyagi oyster has adapted to a diversity of natural habitats and culture methods. After suffering heavy mortalities in conditions of high water temperatures and low water circulation, it seems to have adapted. In addition, there are signs that its presence is improving the environment in which it lives and that these improvements are beneficial to the Native oyster. Given the public interest in clean environments, and in healthy and sustainable food from the sea, there may be a greater role to play for the Miyagi oyster in its new North American home.

KEY WORDS: Pacific oyster, introductions, adaptability, disease, environment, leases

*Corresponding author: E-mail: rene.lavoie@ns.sympatico.ca

INTRODUCTION

Oysters have been exploited for a long time in North America. In 1608, Samuel de Champlain tasted succulent oysters on Isle Saint-Jean, in the Gulf of St-Lawrence. This discovery led to a long relationship between francophone Canadian consumers and oyster producers from the Gulf of St-Lawrence, a relationship which still endures today (Lavoie 1978).

Following the gradual colonisation of North America by the French, the British and the Spaniards, and the subsequent creation of the United States of America and Canada, natural oysters stocks on both coasts became depleted first by overexploitation, then by habitat degradation and pollution. Towards the middle of the 19th century, oyster culture started to be seen as a solution to save what was left of natural stocks and to fill the ever increasing market demand.

On the Atlantic coast of North America, the native American Oyster (*Crassostrea virginica*) responded well to aquaculture. On the Pacific

Coast, the native Olympia oyster *Ostrea lurida* did not show similar success. The Pacific Oyster *Crassostrea gigas*, imported from the Miyagi Prefecture in Japan, gradually established itself along a vast portion of the Pacific Coast, and became the dominant oyster species.

This paper first presents a very brief overview of oyster culture on the Pacific Coast of North America to set the context. It then briefly describes the history and present status of the Pacific oyster *Crassostrea gigas* in Canada, the United States and Mexico. A number of factors related to its successful implantation are discussed. The paper then describes current markets and trends, and concludes with ideas about the future. It does not cover the Canadian, U.S. and Mexico Atlantic Coast oyster industries in detail.

HISTORY

Canada

There are three species of oysters on the West

Coast of Canada: the native Olympia oyster *Ostrea lurida*, the Atlantic oyster *Crassostrea virginica*, and the Pacific oyster *Crassostrea gigas* (Quayle 1988). Records show that the native oyster was marketed in 1884. Efforts to cultivate it took place in the 1930's but over time, its populations dwindled possibly because of deleterious environmental factors. The Atlantic oyster was introduced to several areas in 1903, but it failed to establish itself in any significant quantity except in estuaries of rivers flowing into Boundary Bay, British Columbia (Carlton & Mann 1996). By 1936, production of both native and Atlantic oysters had virtually ceased (Quayle 1969).

The Pacific oyster *Crassostrea gigas* was first introduced in Ladysmith Harbour and Fanny Bay, Vancouver Island in 1912-13 and natural oyster sets were found in Ladysmith Harbour in 1925. These discoveries led to the first large scale transplants in 1926 when 2,000 individual oysters of 2-3 years of age were transplanted from Samish Bay, Washington State, USA. The same year, 20 cases of seed (approximately 400,000 seed) were imported from Japan. Between 1929 and 1932, four million seed were imported. Seed importation from Japan had virtually ceased by 1961.

United States of America

There are five species of oysters on the West Coast of the United States: the native Olympia oyster *Ostrea lurida*, the Atlantic oyster *Crassostrea virginica*, the Kumamoto oyster *Crassostrea sikamea* (Amemiya, 1928), the European flat oyster *Ostrea edulis*, and the Pacific oyster *Crassostrea gigas*. The Pacific oyster is by far the dominant species (Chew 1991, FAO 2005).

In 1899, the possible importation of Japanese oysters was raised by the United States Fish Commissioners with the Imperial University of Tokyo (Galtsoff 1929). The University responded that oyster from the beds located at Akkeshi Bay, Hokkaido would be best adapted for transplanting in North America. From 1902 to 1920, several transplants of oysters from various locations in Japan were made to Samish Bay near Bellingham, and other areas of Puget Sound, Washington State (Steele 1964). From 1924 to 1960, the Pacific Oyster Growers Association imported 1,012,638 cases of seed for distribution in Washington State, Oregon, California, Alaska, and British Columbia. In addition, 214,456 cases of seed were bought by

non-members of the Association. There were no seed purchases in the years 1942-1946.

Mexico

The Pacific Coast of Mexico has a number of native oyster species, namely *Crassostrea corteziensis* (Hertlein, 1951), *Crassostrea rhizophorae* (Lamarck, 1819), *Crassostrea iridescens*, *Ostrea lurida* and *Ostrea megadon* (Hanley, 1846). In the early 1980s, these oysters were located mainly in the States of Baja California, Sonora, and Nayarit. *Crassostrea corteziensis* was the most important cultivated species (Haro et al. 1982). The Pacific oyster *Crassostrea gigas* was introduced in 1973 using seed purchased from a hatchery owned by the Lumi Indians in Marietta, Washington State. Its culture gradually spread to the northern states of Baja California, Baja California Sur, and Sonora.

ADAPTATIONS OF THE PACIFIC OYSTER TO THE WEST COAST OF NORTH AMERICA

Environment

The Pacific oyster had to overcome many challenges in order to occupy new territory on the North American Continent. First was the hardship of being harvested, packed and shipped across the Pacific Ocean. Several accounts of the gradual evolution of the shipping process are given by Steele (1964). Sometimes the seed was out of the water for more than one month from the time it was harvested and packed in Japan to the time it was replanted on seeding grounds in North America. Upon arrival, the seed had to adapt to new temperature, salinity and circulation regimes.

Predators and pests

The young oysters also had to cope with native predators and pests of their new habitat (Chew 1991) including the Dungeness crab *Cancer magister* (Dana, 1852), the Red Rock crab *Cancer productus* (J.W. Randall, 1840), the Graceful crab *Cancer gracilis* (Dana, 1852), four species of sea stars, two species of ducks, and even one species of stingray.

In addition, some of its natural predators in Japan came with the seed in early shipments, before Canadian and American authorities developed and

implemented control measures in cooperation with Japanese authorities and seed growers (Quayle 1988). These included the flatworm *Pseudostylochus ostreophagus* (Hyman, 1955), the parasitic copepod *Mytilicola orientalis* (Mori, 1935), and the Japanese oyster drill *Ocenebra japonica* (Dunker, 1860).

Reproduction

The Pacific oyster is a very fecund bivalve with reproductive organs that can form at least 50% of the body volume (Quayle 1988). It also seems to have an ability to respond to its environmental conditions by having more males when food is scarce and more females when food is abundant (Chew 1991). For aquaculture purposes, it has shown an ability to respond quickly and positively to selection for yield (Langdon et al. 2003).

Diseases and mortalities

Diseases were another challenge. In 1960, a disease occurred in an area of Baynes Sound ranging from Henry Bay to a point approximately 3 miles south of Denman Island, British Columbia. This disease, subsequently named the Denman Island disease, killed 30% of the oysters in its initial outbreak (Quayle 1988) and is now considered to be caused by the protist *Mikrocytos mackini* (Bower 2005).

Beginning in the mid 1960's and through the early 1970's, during the Summer months (Chew 1991), mortalities as high as 60-80% were observed in certain areas. Several studies found no associated disease organisms, but determined that these mortalities were associated with warm water temperatures and low water circulation. Perdue et al. (1981) found that oysters were usually fully ripe when mortalities occurred, and that their other tissues had virtually no glycogen reserves. It was speculated at the time that that lack of glycogen was a consequence of the high fecundity of the Pacific oyster. Since such Summer mortalities greatly diminished by mid to late 1970's, it would appear that the oyster, through natural selection, had adapted its own physiology to the conditions of its new habitat.

More recently, oyster seed losses have been associated with an ostreid herpesvirus in Tomales Bay, California (Burge et al. 2006).

An unintended consequence of the introduction

of *Crassostrea gigas* to the Pacific Coast of North America was the introduction of oyster disease MSX (Bower 2006). MSX is caused by the protozoa *Haplosporidium nelsoni* (Haskin et al. 1966) which occurs within the native range of *C. gigas* in Japan. *H. nelsoni* eventually found its way to the Atlantic Coast where it became a deadly disease to *Crassostrea virginica*, first in the United States (Burreson & Ford 2004), and, more recently, in the Bras d'Or Lake, Nova Scotia, Canada (Stephenson et al. 2003).

HUMAN COMPETITION

Human competition to oyster culture takes several forms (Bourne & Chew 1994). Recreational users of the near shore waters for sports such as sailing and water skiing resent floating long lines and culture rafts. Recreational shellfish harvesters may wander on leases at low tide and, willingly or by ignorance, take oysters from private leases. Wealthy recreational property owners tend to dislike the sight of oyster culture rafts, the boat traffic, and noise generated by culture operations. Many believe that aquaculture operations nearby have a negative effect on shore property value. Diesel engines running continuously to operate seed growth accelerators (flupsies) attract the anger of shore residents who profoundly resent the never-ending noise pollution.

Industrial competition can take the form of physical habitat deterioration, water pollution or both. The most often cited source of damage is sulphite waste, from pulp and paper mills, which may cause mortalities or depressed growth and meat quality. One example is from Samish Bay where seed oysters that used to grow to market size in two years took five years to reach market size after their growing area was affected by pollution. Meat yields went from 80-120 oysters per gallon (3.78 l) to 130-200 oysters per gallon (Steele 1964). Although considerable progress had been made to control industrial pollution in recent years, much remains to be done.

Fecal contamination of the water in growing areas does bring various types of closures and restrictions on growing and harvesting filter feeder shellfish, including oysters. Sources of this contamination include individual dwellings, industries, farms, wild animals, and municipal sewage systems.

PRESENT STATUS

Production

In Canada, Pacific oyster landings amount to an average of 80.2% of all shellfish culture harvests for 2004-06 (MAFF 2007). Average annual oyster production for this period amounted to 7,867 t, with a landing value of C\$8.1 M. Oysters are grown on the bottom, in bags and trays, and on lines suspended from rafts. Some local seed is available from Pendrell Sound and Ladysmith Harbour (British Columbia), but spatfall intensity can be unpredictable. Most seed is imported from hatcheries located in the United States, mostly in Washington State and California. Most of the oysters are marketed as shucked meat. There is an increasing high-end market for half-shell oysters and some high-end producers cannot meet demand for specialty products. There are approximately 1,000 hectares of good oyster growing bottom owned by the Crown in British Columbia. The system of long-term tenure provides an element of stability but leaves growers at the mercy of Government with regards to annual leasing fees and long-term security of tenure.

In the United States, the Pacific oyster has been the most important cultivated oyster since 1977 (Chew 1991). The 2004 production was 44,018 t, about 99% of the oyster production of the Pacific Coast (FAO 2005). Most of the seed comes from hatcheries which can deliver the reliable supply required for production planning and infrastructure investments. Commercial production of triploids on the West Coast of North America began in 1985 (Nell 2002). In 1999/2000, triploid Pacific oysters made up 30% of all Pacific oysters farmed on the West Coast of North America.

Washington State is by far the larger producer. The Pacific Coast Shellfish Growers Association estimated that this state provided 81.7% of US West Coast oyster landings in 2005 (<http://www.pcsga.org/pub/uploads/production.pdf>). Favourable environmental conditions of good oyster growing areas like Willapa Bay, Oakland Bay and Samish Bay may explain part of that success. Another success factor is an administrative system which allows for ownership of tidelands (Beattie 1982). Ownership has allowed growers to modify substrates to improve oyster productivity and to practice predator control. Over time, tilling, discing, gravelling and rolling have been used to modify

oyster beds to improve on-bottom production, and to restrict predators and pests (Beattie et al. 1982). In addition to bottom culture, several off-bottom methods have been utilized; these include rack and bag, and floating and suspended net culture methods (Nosho 1989). Starting in 1994, the exploitation of privately owned tidal lands became more complicated as a result of Court decisions upholding harvesting rights granted to Washington native tribes under federal treaties of 1854 and 1855 (Toba 2002).

In the State of Alaska, oyster culture is practiced in several areas, including the regions of Prince William Sound, Kachemak Bay, and Southeast Alaska, and typically involves suspended culture systems using nets or trays. A recent quality study (Oliveira et al. 2006) found that cultivated oysters from these areas had high condition indices, an indication of a high quality product, even at these northern latitudes. A key advantage is that the low water temperatures inhibit spawning, hence providing growers the unique opportunity to offer year-round supply of fresh product to restaurants and live shellfish markets (RaLonde et al. 2008). However, McLaughlin & Martinek (2004) highlighted adverse effects of rising water temperatures on oyster culture in Alaska. The permitting system is perceived as a hindrance to industry growth (RaLonde et al. 2008).

In Mexico, Pacific oyster culture takes place mainly in the northern portion of Baja California, Baja California Sur and Sonora. Oysters are grown in bags, on racks and on the bottom. Production was in the order of 1,237 tons in 2004 (FAO 2005). The industry employs approximately 1,800 workers. Seed is imported from the United States. Since 1997, there have been mortality problems involving seed, juveniles, and adults. One possible cause of these mortalities is the Gill Necrosis Virus (GNV) (Vasquez-Yeomans et al. 2004).

Knowledge base and Research

The scientific literature on *Crassostrea gigas* covers many aspects of the biology and physiology of the species as well as its adaptations to new environments and culture methods.

In Canada, the Pacific Biological Station at Nanaimo, British Columbia has long been very involved in oyster science. Its library contains numerous publications on the biology, ecology, culture and diseases of oysters. Its WAVES library

system provides access to extensive bibliographies on line. Recently, several new funding initiatives, often based on research networks or partnerships, have emerged (DeJager 2005). These offer an opportunity for research institutions and industry to collaborate on finding solutions for problems or pushing forward with cutting edge technologies. In British Columbia, the Malaspina University College is expanding its research facilities to launch a new research program to support and advance aquaculture.

In the United States, invaluable information has been generated at the School of Aquatic and Fisheries Science and its predecessors at the University of Washington, Seattle, USA. The University's library is an excellent source of information; it also provides access to an extensive bibliography on line.

First Nations

The First Nations of the United States and Canada who live along the Pacific Coast have a long-standing interest in shellfish harvesting and production, as part of their cultural and ceremonial heritage. The Lummi First Nation has operated a shellfish hatchery since the 1970's. More recently the Suquamish Indian Tribe of the Port Madison Reservation located in Kitsap County, Washington State has engaged in a culture revitalization project through bivalve aquaculture including oysters (Barry & Williams 2004). In Canada, First Nations have organized into the Aboriginal Aquaculture Association to assist in the development of First Nations Aquaculture (Harry 2004). Traditionally, First Nations have been pre-occupied with respect for the environment and with long-term sustainability. Their emerging force may, over time, increase oyster supply in the market place. It may also bring a renewed interest in cleaner growing areas for oysters along the Pacific Coast of the United States and Canada.

Public support

There is increasing recognition that shellfish culture is environmentally benign and may indeed be beneficial. Recently, support has been coming from such public advocacy bodies as the Audubon Society, the Monterey Bay Aquarium's Seafood Watch, and Eco-Fish. In addition, Courts of Law and Government Departments have started to rec-

ognize the value of shellfish culture and rendered decisions favourable to the industry. An example is a US\$20,000 penalty imposed on forestry giant Weyerhaeuser Company by the Washington State Department of Ecology for a fecal coliform release which suspended oyster harvesting in July 1997 (<http://www.ecy.wa.gov/news/1997news/97-150.html>).

Bivalve shellfish culture in general, and oyster culture in particular, ought to be better known and understood by the general public and by politicians for their numerous benefits to society and to the environment. When used for oyster culture, large mud flats can generate employment and income in rural areas where jobs are scarce. A bivalve farm supports a self-regulating biomass. If a farm is overloaded beyond the carrying capacity of local waters, shell growth and meat quality suffer, and the farmer will have to reduce the biomass to maintain quality and consumer satisfaction. Oysters remove suspended solids from the water column. Undigested particles are deposited in the form of pseudo-feces. Oysters remove nitrogen and other nutrients from their environment, thereby reducing eutrophication, and help control and prevent algal blooms. Oyster beds increase biodiversity by providing support, shelter and food to small invertebrates and fish. On the West Coast of the USA, the culture of *Crassostrea gigas* is improving damaged environment. These improvements, combined with the use of modern hatchery technology, are bringing a resurgence of the Native oyster (Shumway et al. 2003).

THE FUTURE

Challenges

Governments can assist oyster farmers, but policies can also be harmful. Tideland tenure is not a problem in Washington State because oyster farms belong to their operators. In Canada, tidelands are owned by the Government which leases them to growers. Leases can be cancelled or may not be renewed upon expiry of initial terms. Leasing fees can change at the discretion of government officials. These uncertainties tend to limit investments and can leave growers at the mercy of rule changes over which they have no power.

First Nations are showing a renewed interest in shellfish culture. Their success may increase the volume of product available with a negative effect

on prices, unless a corresponding marketing effort increases demand at the same time as supply. First Nations often place great emphasis on environmental preservation. Their increased presence in the field may well bring improved conservation measures which would benefit oyster growers.

Bivalve shellfish growers can do a better job of integrating their operations into local ecosystems. Shellfish farms located in beautiful remote areas often find themselves in opposition to recreational or retiree property owners who value the beauty of the scenery and the peaceful setting. Oyster farmers are increasingly challenged to make their operations blend with the surrounding environment, to control their industrial refuse, and to avoid any pollution, including noise. Dialogue is needed to conciliate these conflicting interests.

Growers would benefit from better cohesion among themselves to share information, defend their common interests with government officials, and to engage in market development and public relation efforts.

Opportunities

Multi-species culture already generates diverse sources of income streams and this is reflected in new licensing initiatives in Canada (MAFF 2007). Hatcheries already produce seed of several species. There may be an opportunity to farm shellfish in conjunction with fish to improve both the environment and financial returns. As the oyster meat market shows sign of saturation, product diversification from meat only to a mix including half-shell and specialty products offers an option to generate a diversified revenue stream. The human population of America's Pacific Coast is very diverse in its origins and cultural backgrounds. The pursuit and development of market niches to serve diverse ethnic groups is another opportunity. Finally, the full extent of the high-end markets has not been fully explored; there may just be a market for a reliable supply of young, small, deeply cupped, 'meaty' Pacific oysters.

In a wider context, market demand for seafood produced in a sustainable manner from healthy waters is very favourable at this time (Anonymous 2008). A recent industry survey shows that chain restaurants, retailers and wholesalers foresee growth in the sustainable seafood portion of their business. A majority of respondents in these same sectors of the food industry are concerned about

the health of the ocean and its impact on their business. At the consumer level, seafood is marketed as healthy food, and consumption increases with the belief that diet is important for health (Trondsen et al. 2004).

CONCLUSION

The Pacific oyster *Crassostrea gigas* has adapted very well to the North American West Coast. Its initial success was greatly helped by the knowledge of scientists from the Imperial University of Tokyo and the persistent entrepreneurship of early growers in North America. Japanese oyster seed producers and shippers working together with the Pacific Oyster Growers Association ultimately made the introduction the success that we see today.

From its introduction to Washington State and British Columbia at the beginning of the last century, the Pacific oyster has provided food and generated wealth to countless people. Its vitality, resilience to adverse conditions, disease resistance, its ability to feed heavily and utilise food efficiently (Bayne 2002) and its reproductive capacity make it an ideal choice for countries seeking to introduce a non-native oyster.

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On the Some Observation of the Tropical Oysters Collected from Bali Island, Indonesia

Nobuyoshi NISHIKAWA

Kyowa Concrete Industry CO. LTD. Administrative Div. of Ocean Industry, Sapporo, Japan

ABSTRACT: Seventeen specimens, ranging in size from 5.1cm width×6.5cm length to 8.9cm×13.0cm were collected on Indonesia's Bali island, Indonesia on 11 July 2007. Thirteen of them were identified as *Saccostrea sp.*. They had a promyal chamber, a deep umbonal cavity, and weak chomata. They also had alternate deposition of green/brown conchiolin and white calcareous layers on the shell interior. As the other three specimens did not have an obvious conchiolin layers, it is appeared they belonged to genus *Saccoostrea* rather than *Crassostrea*. One specimen is of an unknown because it is an empty shell while being collected.

RESULTS

Specimens were collected from Bali on muddy shores of a mangrove area on 11 July 2007. Most of the collected oysters were of shell heights ~10 cm and with thick, rock-like shaped shells different from the temperate oysters.

Observations were made on promyal chamber in the soft body and umbonal cavity and chomata on both valves. All the observed features were shown in Table 1.

Promyal chamber (Fig.1) was observed on fifteen specimens except on an individual with broken soft (No.2) and a dead shell (No.6).

Weak chomata (Fig.2) was observed on the right valves near the hinge ligament of all specimens.

Deep and large umbonal cavity was present in the shell interior of left valves on all specimens (Fig.3).

Furthermore, color in the shell interior have also been examined (Fig.4). Most of the specimens had olive to yellowish green conchiolin and white

Table 1. Characteristics of shell and soft part of specimens

| No. | width in cm | length in cm | weight in g | promyal chamber | chomata | umbonal cavity |
|-----|-------------|--------------|-------------|-----------------|---------|----------------|
| 1 | 8.9 | 13.0 | 283.6 | * | week | * deep |
| 2 | 6.4 | 8.6 | 82.8 | unknown | week | * |
| 3 | 5.1 | 6.5 | 45.1 | * | week | * |
| 4 | 6.0 | 6.3 | 53.1 | * | week | * deep |
| 5 | 6.0 | 9.8 | 179.5 | * | week | * deep |
| 6 | 6.2 | 9.2 | dead shell | - | unknown | * |
| 7 | 4.6 | 7.0 | 53.2 | * | week | * deep |
| 8 | 7.4 | 8.9 | 123.2 | * | week | * deep |
| 9 | 7.5 | 10.1 | 188.8 | * | week | * deep |
| 10 | 6.7 | 10.1 | 150.0 | * | unknown | * deep |
| 11 | 6.5 | 9.2 | 121.0 | * | week | * deep |
| 12 | 9.0 | 12.8 | 209.7 | * | week | * |
| 13 | 9.0 | 8.5 | 144.0 | * | week | * deep |
| 14 | 9.6 | 9.1 | 132.6 | * | week | * deep |
| 15 | 7.3 | 9.0 | 144.9 | * | week | * deep |
| 16 | 6.0 | 11.4 | 216.2 | * | unknown | * deep |
| 17 | 7.3 | 9.9 | 216.2 | * | unknown | * deep |

(-: not observed, *: present.)

calcareous layers on the shell interior. Specimen No.10, 16, 17 did not have obvious conchiolin layers.

DISCUSSION

There are many scientific papers on genus *Ostrea* and genus *Crassostrea* in temperate oysters. The taxonomy of tropical oysters, however, remains confusing. Therefore identification for the specimens in this study was very difficult.

Oysters of three genus *Ostrea*, *Crassostrea*, and *Saccostrea* have been extensively studied and cultured. Main characteristics of these genus can be summarized in Table 2 according to Angell, 1986.

The most reliable way to accurately identify specimens requires examination of the hinge region of the internal shell or the soft part anatomy. Of further interest is shell characters and color of deposit conchiolin and white calcareous layers on the shell interior.

The shell of all specimens showed a tendency to form rustic or cornucopia-like shapes. Color of shell interior was olive to yellowish green and dark purple. Those specimens with such characteristics are most likely to be *Saccostrea* (Lam 2003).

Promyal chamber was observed in the soft part of all specimens. This chamber is an additional discharge area for outgoing water (Quayle and Newkirk 1989) and fulfill the expulsion of pseudofaeces function. Genus *Ostrea* lack a promyal chamber and do not produce pseudofaeces. It is suggested that mangrove oysters adapted to inhabit muddy shores which allowed growing and surviving in high turbidity waters.

A characteristic feature of genus *Ostrea* and *Saccostrea* is the presence of chomata. Chomata was recognized at most of specimens in this study, but could not able to confirm on specimens No.10,16 and 17.

The most useful character for identification was the size of the umbonal cavity, which was large in *Saccostrea cucullata* and *Saccostrea commercialis* (Day AJ. et al.2000). Left valve of specimens were generally deep, with umbonal cavity being deeper and larger in specimen No.2, 3 and 12.

Lam (2003) and Lam and Morton (2004, 2006) have presented an illustrated guide to morphological variations observed in common species of oysters from Indo-West Pacific, with detail description of the shell characters and DNA sequences of genus *Saccostrea*. The external surfaces of both

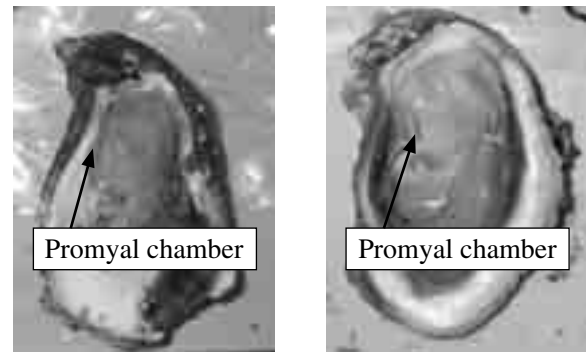


Fig.1. Specimen No.5 (left), No.16 (right) with right valve removed to show promyal chamber.

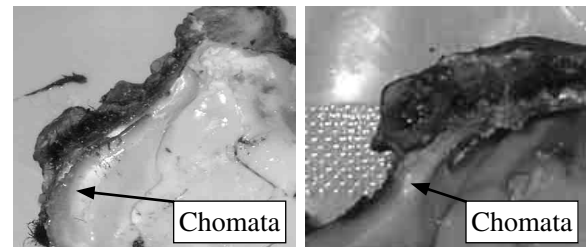


Fig.2. Specimen No.1 (left), No.4 (right) with right valve removed to show chomata.

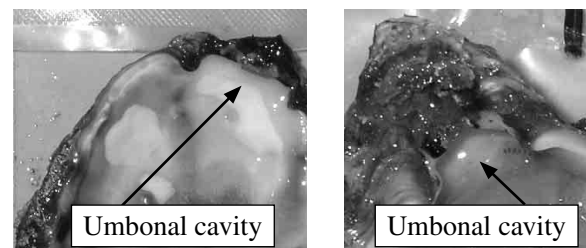


Fig.3. Specimen No.5 (left), No.8 (right) with right valve removed to show the umbonal cavity.



Fig.4. Interior of both valves, specimen No.9

Table 2. Comparative features of three oyster genera

| Characteristics | Genus | | |
|---------------------|----------------------------|-------------------------------|----------------------------|
| | <i>Ostrea</i> | <i>Crassostrea</i> | <i>Saccostrea</i> |
| Chomata | present | absent | present |
| Promyal chamber | absent | present | present |
| Umbonal cavity | absent | moderate | deep |
| Shape | subcircular, fulat | somewhat elongated and cupped | cornucopiate or rudistform |
| Shell margins | crenulated in some species | not crenulated | crenulated |
| Valves | equal | upper valve smaller | upper valve smaller |
| Size | small to moderate | may be large | small to moderate |
| Spawnig mode | larviparous | oviparous | oviparous |
| Sexual development | protandrous hermaphrodite | dioecious | dioecious |
| Salinity preference | stenohaline | euryhaline | stenohaline |

alter C.L..Angell.1986

left and right valves on genus *Saccostrea cucullata* are white to lilac with a dark purple coloration at shell margins, and interiors of both valves are usually iridescent bluish green or opalescent white with patches of bluish green, or olive to yellowish green. This description is common in most of specimens from Bali Island.

In conclusion, the internal and external shell features and some characteristics of every specimens as showed in Table1, suggested that most of the specimens were *Saccostrea* sp. However, specimen No.10, 16, 17 can be *Saccostrea* or *Crassostrea* sp. because they do not have obvious conchiolin layers (Lam,K. personal communication).

For accurate identification of the taxonomic status of the oysters, genetic identification is a must apart from morphological studies.

ACKNOWLEDGEMENT

The author is grateful to Dr. Katherine Lam, The University of Hong Kong for the invaluable advices and critical reading of the manuscript.

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Nursing and Growing Hatchery-Reared Big Oyster (*Crassostrea belcheri* Sowerby 1871) in the Intertidal Mangrove Area

Suwat TANYAROS¹, Kunchai ANAN² and Lawrence D. KITT³

¹ Department of Marine Science, Faculty of Science and Fisheries Technology, Rajamangala University of Technology Srivijaya, Trang campus, Trang, Thailand. 92150.

² Sakaew Fishery Research and Development Center, Department of Fisheries, Sakaew, Thailand. 27000.

³ Advisory Board of the Rajamangala University of Technology Srivijaya, Trang campus, Trang, Thailand. 92150.

ABSTRACT: Nursing hatchery-reared juvenile oysters (*Crassostrea belcheri* Sowerby 1871) in the intertidal mangrove area were carried out for four months. The initial average width, length and weight of the seed were 2+0.05 mm, 2+0.05 mm and 0.29+0.05 g, respectively. At the end of the experiment, the oyster seed were 3.38+0.39 cm, 3.54+0.89 cm and 3.22+2.01 g for average width, length and weight, respectively. Mean increase daily growth rate in shell width and length ranged from 0.13 to 0.29 mm/individual and 0.12-0.38 mm/individual, respectively. Mean increase in live weight ranged from 0.16-0.39 g/individual. The mean survival rate was 22.52 % at the end of the nursing experiment. For the growing experiment, 5 cm or bigger hatchery-reared oysters from the nursing experiment, were used to grow up in flipping pouches in the intertidal zone for 6 months. At the end of the experiment, mean increase in shell width and length were shown to be non-significantly different among the treatments ($p>0.05$). Significant differences were shown in survival and the amount of barnacle attachment among the treatments ($p<0.05$). High survival rate was found in all densities with aerial exposure of 5 hrs at low tide during spring tide. The highest number with attachment of barnacles was found at the aerial exposure of 1 hr at lowest tide during spring tide.

INTRODUCTIONS

Thailand has abundant oyster resources from natural beds. The oyster beds are located in the shallow coastal waters of the intertidal mangrove areas. Oyster culture in Thailand started in the year 1942. *Crassostrea belcheri* (Sowerby) is one of the commercially important bivalves and has been studied for many years (DOF, 1994). The natural distribution of this species is normally found throughout the area from the river mouths to the coast. The species is capable of tolerating a wide range of salinities (Angell, 1986) This is a desirable characteristic in a species for aquaculture. The spat is always available, either naturally or from spawnings in a hatchery. Recently, *C. Belcheri* is mainly grown using natural seeds, but the number of oyster seed from wild sources is limited and insufficient to supply the grow-out farms. Oyster seed production from hatcheries has been continuously developed and likely to be more interesting than in Europe and North America (Tanyaros et. al., 2000). Nursing of *C. Belcheri* spat production from the

hatchery is one of the critical stages, it depends on several factors as well as the food and water qualities (Tantikulratana, 1990). Mangroves serve as valuable nursery areas for many fish and invertebrates (Aksornkoae, 1999). So, this area has high potential for nursing and growing hatchery-reared juvenile oyster. Only a few researchers have considered the feasibility of nursing hatchery-reared oyster seed in intertidal mangrove areas although these habitats provide a rich source of food. Thus, the potential for nursing and growing hatchery-reared big oyster in the intertidal mangrove area is the main purpose of the present study.

MATERIALS AND METHODS

Study site

This study was conducted over a period of four months for nursing and six months for growing, at the Faculty of Science and Fishery Technology, Rajamangala University of Technology Srivijaya, Trang campus, located on the southern coast of the

Andaman sea.

Nursing experiment

The experiments were started with *Crassostrea belcheri* seeds that were produced from the Muka Head hatchery in Penang Malaysia. The average length (Anterior-Posterior axis) of the seed was 2 mm (SD = 0.5). The seed were placed in nursing bags and tied to PVC frames (1.2 m x 1.2 m) as shown in figure 1. The PVC frames were made from 0.75 inch diameter PVC pipe. The nursing bags were divided into three types depending on mesh size. Green bags of mesh size 0.5 mm, red bags of mesh size 2 mm, and blue bags of mesh size 5 mm. The selected hatchery-reared seed were put into the green bags at the beginning. After two months, seed grading was done by using sieves of mesh sizes 5 and 10 mm. The seed smaller than 5 mm were put back into the green bags. 5-10 mm seed were put into the red bags and seed bigger than 10 mm were put into the blue bags. Stocking densities of 1000, 500 and 200 individuals per bag were used for the green, red and blue bags, respectively. 30 randomly collected oysters were collected from each experimental nursing bag at monthly intervals for the determination of shell length (Anterior-Posterior axis), shell width (dorsal-ventral axis) and shell weight. Dead individuals were counted to determine survival rate.

Growing experiment

In this experiment, the flipping pouch culture method was used for grow out of oysters. The flipping pouches are made from plastic mesh size of 1 cm. The size of each flipping pouch was 50 cm

in width, 50 cm in length 50 and 10 cm in depth. Styrofoam was placed on the top of the flipping pouches to cause the pouches to flip up during high tides. The shape of the flipping pouch is illustrated in figure 2. The hatchery-reared oysters from the nursing experiment of sizes bigger than 5 cm (Anterior-Posterior axis) were used in this experiment. The oysters were put in the flipping pouches at four densities ranging from 10 to 40 individuals per pouch. The pouches were hung in the intertidal mangrove area at different air exposures from 0, 1, 3 and 5 hrs at low tide during spring tide. The experiment was set up using a 4 x 4 factorial in a completely randomized design (CRD) with each treatment in four replications.

Statistical analysis

One-way ANOVAs, with experimental period as factors, were applied for the growth parameters (shell length, shell width, and shell weight) and survival rate for the nursing experiment. Two-factor ANOVAs, with density and time of aerial exposure (hours) as factors, were applied, for the various growth parameters (shell length, shell width, and shell weight), survival rate, and increases in fouling for the growing experiment. If significant effects were present, the data was then subjected to Duncan's Multiple Range Test to determine which treatments were significantly different.

RESULTS

Nursing experiment

Nursing hatchery-reared juvenile oysters in the intertidal mangrove area were cultured for four



Figure 1. Nursing bag tied to PVC frame



Figure 2. Flipping pouch culture method

months. The initial mean values of shell width, length and weight of oyster seed were 2 ± 0.05 mm, 2 ± 0.05 mm and 0.29 ± 0.05 g, respectively. After 4 months of culturing the mean shell width, length and weight were 3.38 ± 0.39 cm, 3.54 ± 0.89 cm and 3.22 ± 2.01 g, respectively. (Figures 3 and 4). The statistical analysis of mean growth rate of shell

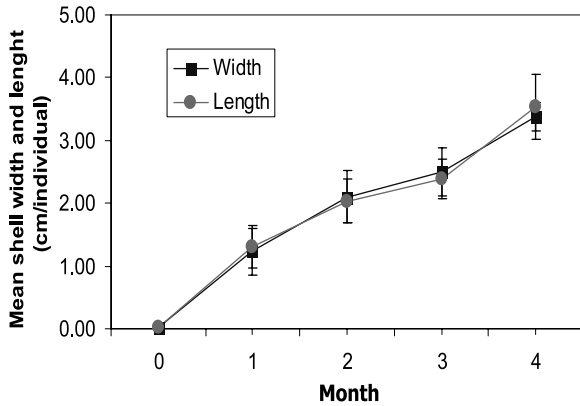


Figure 3. Cumulative growth of shell width and length of hatchery-reared seeds.

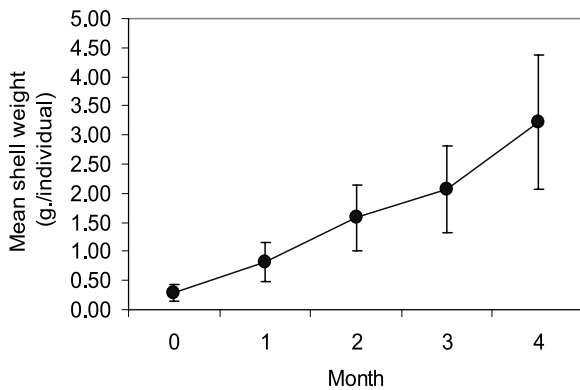


Figure 4. Cumulative growth of shell weight of hatchery-reared seeds.

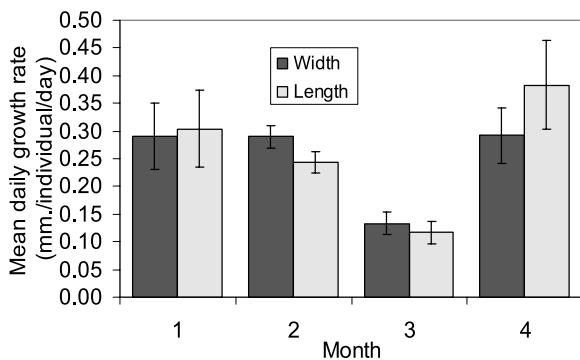


Figure 5. Mean growth rate of shell width and length of hatchery-reared seeds.

width and length in each month showed significant differences ($p < 0.05$), but the results showed that statistical analysis of mean shell weight in each month was non-significantly different ($p > 0.05$). Lowest mean growth rate of shell width and length were found in the 3rd month of the experiment period. The mean growth rate by shell width was 0.29 ± 0.11 mm/individual/day, 0.29 ± 0.03 mm/individual/day, 0.13 ± 0.03 mm/individual/day, and 0.29 ± 0.09 mm/individual/day and shell length was 0.30 ± 0.11 mm/individual/day, 0.24 ± 0.03 mm/individual/day, 0.12 ± 0.03 mm/individual/day, and 0.38 ± 0.14 mm/individual/day at the 1st, 2nd, 3rd and 4th month, respectively (Figure 5). The mean growth rate of shell weight was 0.18 ± 0.11 g/individual/day, 0.25 ± 0.15 g/individual/day, 0.16 ± 0.10 g/individual/day, and 0.39 ± 0.15 g/individual/day in the 1st, 2nd, 3rd and 4th month, respectively. (Figure 6). After four months the mean survival rate was 22.52%. (Figure 7).

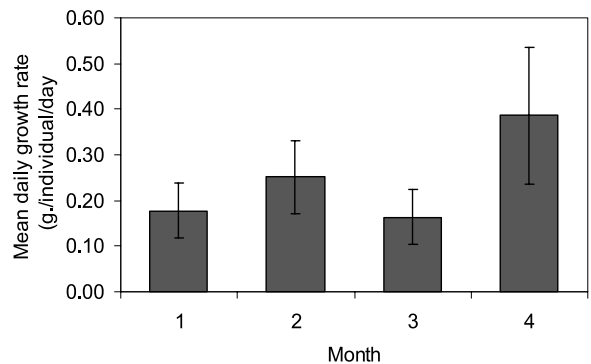


Figure 6. Mean growth rate of shell weight of hatchery-reared seeds.

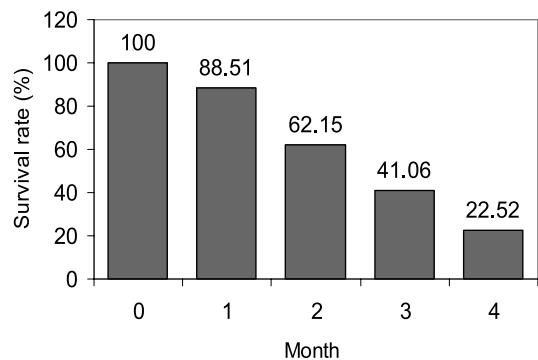


Figure 7. Mean survival rate of hatchery-reared seeds.

Growing experiment

In this experiment, the hatchery-reared oysters of sizes of 5 cm or bigger from the nursing experiment were used to grow out in flipping pouches in the intertidal zone. After 6 months, the statistical analysis of mean increase in shell width and length were shown to be non-significantly different among the treatments ($p>0.05$). Significant differences were shown in survival rate and the number of barnacles attached on the oyster shells among the treatments ($p<0.05$). Highest survival rate was found at a stocking density of 10 and 20 oysters/pouch with aerial exposure of 5 hrs at low tide during spring tide, while the lowest survival rate was found at all stocking densities immersed in water. The highest number of barnacles attached on the oyster shells was found with the aerial exposure of 1 hr at low tide during spring tide when compared

among treatments ($p<0.05$) as shown in Table 1.

DISCUSSION

Nursing experiment

Nursing hatchery-reared seeds of juvenile oyster in the intertidal mangrove area. The experiment was carried out for four months. High growth and survival rate were obtained when compared with other experiments (Titikularatana, 1990; Pinkaew and Srinual-adj, 1997). However, the growth and survival rate (22.5%) found in this experiment was rather low as a result of the small size of initial seeds used. The size of oyster seed from settlement to 3 cm is the critical stage for nursing *C. Belcheri* (Sahavatcharin et. at., 1990), The hatchery-reared seeds of juvenile oyster used in this experiment were smaller than 5 mm. Simultaneously, in the 3rd

Table 1. Mean shell width, length, survival rate and number of attachment of barnacles on oysters grown at different stocking densities and air exposure over the culture period (6 months)

| Treatments | Width(cm) | Length(cm) | Survival rate (%) | Number of Barnacle attachment/individual |
|------------|-----------|------------|-------------------|--|
| L1 × D1 | 7.0+0.5a | 9.8+0.7a | 56.6+2.5a | 19+3a |
| L1 × D2 | 7.5+0.5a | 9.4+0.6a | 56.6+2.4a | 10+4a |
| L1 × D3 | 7.7+0.6a | 10.6+0.5a | 64.4+4.2a | 11+5a |
| L1 × D4 | 7.2+0.6a | 10.6+0.7a | 55.8+6.1a | 5+8a |
| L2 × D1 | 8.0+0.4a | 10.7+0.7a | 73.3+2.1b | 82+12b |
| L2 × D2 | 7.4+0.5a | 9.9+0.6a | 71.6+1.8b | 61+8b |
| L2 × D3 | 7.5+0.5a | 11.0+0.5a | 72.2+2.6b | 52+7b |
| L2 × D4 | 6.9+0.6a | 10.3+0.5a | 61.6+3.4a | 21+6a |
| L3 × D1 | 7.2+0.4a | 10.1+0.4a | 86.6+2.1b | 22+7a |
| L3 × D2 | 7.4+0.6a | 10.4+0.5a | 78.3+2.2b | 9+8a |
| L3 × D3 | 7.6+0.6a | 10.6+0.6a | 76.6+1.8b | 22+9a |
| L3 × D4 | 7.2+0.6a | 10.5+0.5a | 70.8+2.4b | 25+9a |
| L4 × D1 | 7.6+0.6a | 10.2+0.4a | 90.0+1.8c | 19+12a |
| L4 × D2 | 8.1+0.4a | 10.4+0.4a | 98.3+2.5c | 16+8a |
| L4 × D3 | 7.4+0.4a | 10.1+0.4a | 83.3+1.9b | 13+8a |
| L4 × D4 | 7.5+0.5a | 9.9+0.5a | 83.3+2.3b | 9+5a |

Remark : values designated by the same latter were considered to be non-significantly different of means ($p>0.05$) by Duncan's Multiple Range Test. Vertical comparison only.

L1 = Submerged in water; L2 = Aerial exposure 1 hr during low tide at spring tide; L3 = Aerial exposure 3 hrs during low tide at spring tide; L4 = Aerial exposure 5 hrs during low tide at spring tide
D1 = Stocking density at 10 oysters/pouch; D2 = Stocking density at 20 oysters/pouch
D3 = Stocking density at 30 oysters/pouch; D4 = Stocking density at 40 oysters/pouch

month of the experiment, there was heavy rainfall and freshwater run-off into experimental site. The water salinity dropped lower than 10 ppt. High mortality occurred during this period. The tolerant oyster seeds remained, but low growth and survival rate were found during this time. These oysters (*C. belcheri*) are capable of tolerating a wide range of salinities both as larvae (12-24 ppt). (Tan and Wong, 1996) and as adults (10-35 ppt) (Titikulrattana and Wongvivattana, 1984). Nevertheless, cultured oysters in the intertidal mangrove areas where there are fluctuations of salinity during the rainy season often exert significant physiologic impacts (Tirard et al. 1997). The response of oysters to changes in environmental salinity has been investigated. The results suggest a complex physiological change. A study of the oyster *C. virginica* found that changing water salinities affected the sensitivity and activity of cilia and cirri on the ctenidia (Dean and Paparo, 1983). The oysters exposed to changing salinities there showed very rapid valve movements and significant valve closure (Hand and stickle, 1977). In addition, marine bivalves contain very high levels of free amino acids (FAA). An increase or decrease in salinity often results in an increase or decrease of FAA level in the tissues (Ellis et al. 1985). They are often monitored as a stress indicator in oyster *C. virginica* (Powell et al. 1984) and *C. gigas* (Lee. et. al, 2004).

Growing experiment

A low mortality was observed of oysters grown in flipping pouches at all stocking densities and with aerial exposure of 5 hrs at lowest tide during spring tide. The survival rate of oysters from the present study was higher than the oysters grown on trays submerged beneath floating plastic pipe pontoons and grown on conventional intertidal trays reported for *C. commercialis* by Wisely et al., (1979). While low survival rate was found at all stocking densities submerged in water because of the colonisation of submerged shells by aquatic organisms dominated by the boring organisms (e.g. polychaetes and sponges), bryozoan and barnacle. The biofouling is a particular problem in bivalve culture, resulting in reduced growth rates and survival (Taylor et al., 1997; Kaehler and McQuaid, 1999). Thus biofouling has to be addressed in oyster culture, given the relatively long culture period (Acosta-Salmon et al., 2004). A significantly high number barnacles attached on the oyster shells was

found at the aerial exposure of 1 hr at lowest tide during spring tide when compared among treatments. The oyster exposure to sunshine during low tide affected the attachment of barnacles on the oysters. The increasing temperature has a direct effect on barnacle larval development (Thiyagarajan, et al., 2003) and the ultraviolet radiation from sunshine has an indirect effect on attachment of barnacles (Hung et al., 2005). The feasibility of heat treatment as an antifouling option has been studied (Rajagopa et al., 2005). In this experiment, it was observed that increasing settlement of barnacles on oyster shell increased with age similar to the result reported by Guenther et al., (2006). The negative effects of biofouling on cultured oysters are minimised by regular manual cleaning, which contributes significantly to the operational expenses in oyster farming. Cleaning procedures are costly with regard to labour and equipment and may contribute up to 30% of the operational expenses in bivalve farming (Claereboudt et al., 1994). Actually, the flipping pouch culture technique in this experiment was designed to minimize cleaning, but the results demonstrate that the loss of oysters increased significantly with age. These results imply that under culture conditions by this culture technique, the oyster should be cleaned more frequently to counteract increased settlement of fouling organisms. The cultured oysters in the intertidal mangrove area should be cleaned every 1 to 2 weeks.

ACKNOWLEDGEMENT

I gratefully acknowledge and thank the Faculty of Science and Fisheries Technology, Rajamanagala University of Technology Srivijaya for research site support and to Ms. M. B. Athersmith for editing the manuscript.

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Antioxidant Activity of *Crassostrea gigas* Meat Extract in Diabetic Mice

Mitsugu WATANABE

Watanabe Oyster Laboratory Co., LTD.

ABSTRACT: When compared to other shellfish, the Japanese oyster *Crassostrea gigas* contains more than ten times the amount of zinc and more than seven times the amount of copper, and is also rich in selenium.

Zinc, copper and selenium are indispensable for the functional expression of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), enzymes that remove active oxygen, and it has been reported that decreases in the biological concentrations of these metals result in organ and tissue damage due to oxidative stress. Oxidative stress in diabetes causes diabetic nephropathy, one of the three major complications of diabetes.

Subsequently, the antioxidant activity of oyster meat (soft-body extract of *C. gigas* :SEC) was investigated in diabetic mice on a zinc-, selenium- and chromium-deficient diet.

The results showed that in mice with spontaneous diabetes, 28-day administration of SEC reduced urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), and the suppressive effects of SEC on DNA oxidation were confirmed.

SEC significantly lowered renal thiobarbituric acid reactive substances (TBARS), thus suggesting suppressive effects of SEC on renal lipid oxidation. In addition, SEC lowered renal TBARS in a dose-dependent manner, thus suggesting the dose-dependent suppressive effects of SEC on renal lipid oxidation.

The renal GSH-Px activity for the 150-mg SEC groups was significantly higher than that for the 0-mg SEC group, thus confirming that SEC administration significantly increases the activity of active oxygen-removing enzymes.

KEY WORDS: oyster, antioxidant activity, 8-OHdG, TBARS, glutathione peroxidase

INTRODUCTION

Oyster meat is referred to as the “milk of the sea”, and is a high-protein food containing a good balance of essential amino acids. Oyster meat is a good source of glycogen and trace elements, such as zinc and copper.

When compared to other shellfish, *Crassostrea gigas* contains more than ten times the amount of zinc and more than seven times the amount of copper, and is also rich in iron and selenium. These metals are indispensable for human survival and are referred to as essential trace elements.

In appropriate doses, active oxygen produced by normal metabolic processes in the body is involved in important physiological functions, such as detoxification and sterilization. However, when more active oxygen is produced than required, oxidative stress results. Excess active oxygen causes DNA denaturation, enzyme degeneration and lipid oxidation, which lead to various diseases and aging (Anthony, P.B. & JOHN, A.M. 1995, S.S. Wallace. 2002, Mitra, S., T.Izumi, I. Boldogh, K.K. Bhakat,

J.W.Hill & T.K.Hazra. 2002).

Superoxide O_2^- is metabolized by superoxide dismutase into H_2O_2 , which is then metabolized by glutathione peroxidase into water to eliminate active oxygen. Zinc, copper and selenium are indispensable for the functional expression of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), enzymes that remove active oxygen, and it has been reported that decreases in the biological concentrations of these metals result in organ and tissue damage due to oxidative stress.

As *Crassostrea gigas* meat is rich in zinc, copper, iron and selenium, it is considered to be useful in removing active oxygen. However, to the best of our knowledge, there have been no animal studies investigating the antioxidant activity of *Crassostrea gigas* meat. Therefore, confirming the antioxidant activity of *Crassostrea gigas* meat will improve its food value.

The present study investigated the trace element supplementation and antioxidant activities of *Crassostrea gigas* meat in type II diabetes. The reasons for targeting type II diabetes were two-

fold: first, in diabetes, hyperglycemia induces excessive active oxygen production and causes clear oxidative stress (James, V. H., A. B. Mark & Malcolm, J. M. 1993, Shi, D. Y., A. M. Schmidt, G. M. Anderson, J. Zhang, J. Brett, Y. S. Zou, D. Pinsky & D. Stern. 1994), and thus the antioxidant activity of *Crassostrea gigas* meat could be confirmed more readily in diabetic subjects under clear oxidative stress; secondly, serum levels of Zn and Se in type II diabetics are significantly lower when compared to healthy individuals (Chausmer, B. 1998, Navarro-Alarcon, M., H. Lopez-G de la Serrana, V. Perez-Valero & C. Lopez-Martinez. 1999).

The two main factors for low serum levels of Zn and Se are as follows: 1) in diabetes, there is a positive correlation between fasting blood glucose and urinary zinc excretion, and the tendency is that higher blood glucose leads to higher urinary zinc excretion. In other words, abnormal hyperglycemia is one of the factors for low serum zinc (Tarui, S. 1963). The same tendency also applies to selenium and chromium (Ghosh, Debjani, Basudev Bhattacharya, Biswajit Mukherjee, Byomkesh Manna, Mitali Sinha, Jyothi Chowdhury, Subhankar Chowdhury. 2002) and 2) because of caloric intake restrictions, the contents of trace elements in diabetic diets are low, and it has been reported that long-term diabetic diet consumption is one of the factors for low serum trace elements in diabetics (Suzuki, K. 1993, Murphy, S. P. & D. H. Calloway 1986).

Oxidative stress in diabetes also induces angiopathy, and oxidative stress in the kidney causes diabetic nephropathy, one of the three major complications of diabetes (Metz, T. O., N. L. Aldeason, S. R. Thorpe & J. W. Baynes. 2003, Ulrich, P. & A. Cerami. 2001). Subsequently, the antioxidant activity of *Crassostrea gigas* meat was investigated in diabetic mice on a zinc-, selenium- and chromium-deficient diet.

METHODS

1. Diets

AIN-93 powder (Oriental Yeast Co., Ltd.) (control diet) and AIN-93G powder deficient in zinc, selenium and chromium (deficient diet) were used. The zinc, selenium and chromium contents of the deficient diet were close to zero.

2. Extract

In order to extract low molecular-weight compounds, such as trace elements and amino acids from *Crassostrea gigas* meat, soft bodies were placed in hot water (extraction temperature: room temperature to 95°C), and were then placed in ethanol (extraction temperature: room temperature to 95°C). The hot water and ethanol extracts were combined and concentrated, and the resulting dried extract was used (soft-body extract of *Crassostrea gigas* : SEC). Table 1 shows the composition of 100 g of SEC.

3. Preliminary study

Six-week-old male KKAY/Ta mice (spontaneous diabetic mice) were purchased from Clea Japan. Eight mice each were assigned to the control- and deficient-diet groups, and the mice had free access to control- or deficient-diet for two weeks. Blood samples were collected at the end of the study (Figure 1).

Body weight and food intake were measured between 10:00 AM and 12:00 PM everyday. Each mouse was separately housed in a plastic cage and was maintained at 24±1°C and 55±5% humidity. Mice had free access to water and food.

4. Main study

Forty-eight KKAY mice were fed the deficient diet for 14 days and were then divided into three groups of 16 mice each: the 0-mg group (physiological saline, 0 mg/kg/10mL of SEC), the 50-mg group (50 mg/kg/10mL of SEC) and the 150-mg group (150 mg/kg/10mL of SEC). SEC was administered orally using a gastric tube once daily for 28 days. During the oral administration period, mice had free access to the deficient diet (Figure 1).

5. Blood sampling and plasma trace element measurement

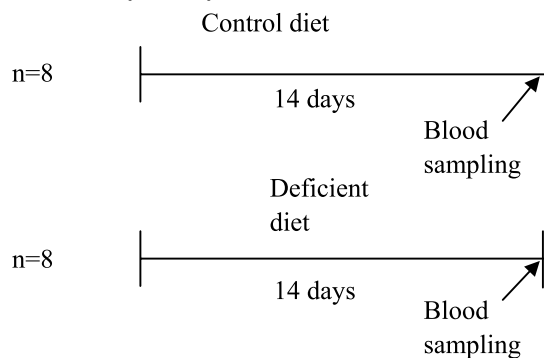
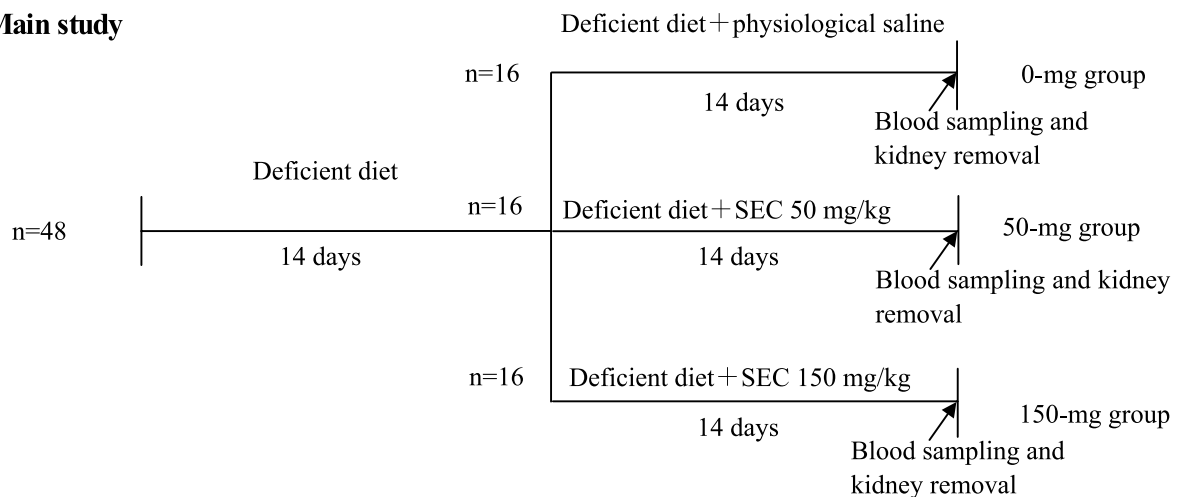
At the end of the 14-day preliminary study and the 28-day main study, mice were fasted for 18 hours, and 50 mg/kg of pentobarbital sodium was administered for collection of blood samples from the abdominal vena cava using a heparinized tube. Samples were centrifuged (4°C, 5,000 rpm, 15 minutes), and the plasma was used for analysis. Next, the left and right kidneys were removed and stored at -80°C. Levels of plasma zinc, selenium and chromium were measured by atomic absorption photometry in the conventional manner.

Table 1. Composition of soft-body extract *Crassostrea gigas*

| | | | | | | | |
|--------------|-----------|-------------------------|-------------|------------------------|-------------|--------------------------|-------------|
| Moisture | ··· 1.4g | Composition of Vitamin | | Composition of mineral | | Chromium | ··· 19.0μg |
| Protein | ··· 27.6g | Vitamin A | ··· 25132IU | Potassium | ··· 1.44g | Selenium | ··· 160.0μg |
| Fat | ··· 1.4g | Vitamin B ₁ | ··· 278.8mg | Calcium | ··· 178.0mg | Composition of the other | |
| Carbohydrate | ··· 62.7g | Vitamin B ₂ | ··· 95.1mg | Phosphorus | ··· 786.7mg | Glycogen | ··· 37.6g |
| Mineral | ··· 6.8g | Vitamin B ₆ | ··· 58.1μg | Magnesium | ··· 150.0mg | Glycosaminoglycan | ··· 114mg |
| Fiber | ··· 0.1g | Vitamin B ₁₂ | ··· 56.3μg | Manganese | ··· 3.4mg | linoleic acid | ··· 422.6mg |
| Total | 100.0g | Folic acid | ··· 248.6μg | Iron | ··· 17.4mg | EPA | ··· 75.7mg |
| | | Vitamin E | ··· 624.0μg | Zinc | ··· 64.9mg | DHA | ··· 35.0mg |
| | | Alpha tipe | ··· 549.1μg | Copper | ··· 10.0mg | Lecithin | ··· 604.0mg |
| | | Biotin | ··· 871.1μg | Iodine | ··· 5.9mg | | |

per 100g soft-body extract *Crassostrea gigas*

The soft bodies of *Crassostrea gigas* were placed in hot water, and were then placed in ethanol. The hot water and ethanol extracts were combined and concentrated, and the resulting dried extract was used.

Preliminary study**Main study****Fig.1.** Experimental protocols

6. Measurement of urinary 8-OHdG and 15-isoprostane F2t

Starting at 24 days after the start of test diet administration, 24-hour urine samples were collected for three days, and after removing contaminants, the samples were frozen at -80°C until testing. After thawing under refrigeration, a commercially available ELISA kit was used (Yin, H., C. M. Havrilla, L. Gao, J. D. Morrow & N. A. Porter. 2003).

7. Measurement of renal lipoperoxides

After washing each renal tissue using cold physiological saline, wet weight was measured, and the tissue was homogenized to measure its protein content by Lowry's method. In addition, levels of thiobarbituric acid reactive substances (TBARS) in the homogenate were measured by TBA, and the levels of TBARS per protein (mg) were calculated.

For calculations, only measurements for solutions processed using TBA-EDTA malondialdehyde derivatives (TBA reactions are independent of conditions) were used. The malondialdehyde assay kit (Japan Institute for the Control of Aging, NIKKEN SEIL Co., Ltd) was used.

8. Measurement of renal tissue SOD and GSH-Px activities

SOD activities were measured as follows. The right kidney was thawed in ice water and was then washed using cold physiological saline. Next, by adding the buffer (pH 7.0) included in the kit, the kidney was homogenized, and according to the manual (Randox Laboratories Ltd.), SOD activity was measured at a wavelength of 505 nm. Lowry's method was used to quantify the amount of protein in each tissue homogenate.

GSH-Px activities were measured as follows: The left kidney was thawed in ice water and then washed using cold physiological saline. Next, by adding Tris buffer (pH 7.5), the kidney was homogenized, and according to the manual, GSH-Px activity was measured at a wavelength of 340 nm. Lowry's method was used to quantify the amount of protein in each tissue homogenate.

9. Statistical analysis

Means \pm standard error were calculated, and after one-way ANOVA, Fisher's PLSD was used to compare the groups. Regression analysis was also performed for 8-OHdG and TBARS. The Excel statistics 2006 for windows (Social Survey Research Information Co., Ltd.) was used for the

calculations.

RESULTS

1) Changes in plasma trace elements

Table 2 shows the levels of plasma zinc, selenium and chromium in KKAY mice in the preliminary study. Plasma levels of zinc, selenium and chromium in the deficient-diet group were significantly lower than those in the control-diet group.

Tables 3 shows the levels of plasma zinc, selenium and chromium in KKAY mice in the main study. No significant differences were found in the plasma levels of zinc, selenium and chromium among the 0-mg, 50-mg and 150-mg groups.

2) Changes in urinary 8-OHdG and 15-isoprostane F2t in KKAY mice

Table 4 shows the changes in urinary 8-OHdG and 15-isoprostane F2t for each group. Urinary 8-OHdG for the 150-mg group was $30.44 \pm 2.05 \text{ ng/mg crea}$, which was significantly lower than that for the 0-mg group at $43.66 \pm 2.24 \text{ ng/mg crea}$ ($p < 0.01$). No significant differences were in urinary 15-isoprostane F2t between the 0-mg, 50-mg and 150-mg groups.

3) Relationship between SEC dose and urinary 8-OHdG in KKAY mice

Figure 2 shows the relationship between SEC dose and urinary 8-OHdG. A significant negative correlation was found between SEC dose and urinary 8-OHdG in KKAY mice (regression equation: $y = 44.4047 - 6.8339x$, $r = -0.5545$ and $P = 0.00005$), thus confirming significant dose dependency.

4) Changes in TBARS, SOD and GSH-Px in renal tissue of KKAY mice

Table 5 shows the changes in TBARS, SOD and GSH-Px in the renal tissue of KKAY mice. Renal TBARS for the 150-mg group was $0.482 \pm 0.044 \text{ nmol MDA/mg protein}$, which was significantly lower than that for the 0-mg group at $0.707 \pm 0.075 \text{ nmol MDA/mg protein}$ ($p < 0.05$).

No significant differences were found in renal SOD activity between the 0-mg, 50-mg and 150-mg groups. Renal GSH-Px activity for the 150-mg group was $0.719 \pm 0.124 \text{ mU/mg protein}$, which was significantly higher than that for the 0-mg group at $0.387 \pm 0.079 \text{ mU/mg protein}$ ($p < 0.05$).

Table 2. Plasma levels of zinc, selenium and chromium in KKAY mice at the end of preliminary study.

| | Groups | |
|----------------------|-------------------|-----------------------------|
| | Control (n=8) | Deficiency (n=8) |
| ($\mu\text{g/dL}$) | | |
| Zinc | 111.88 \pm 2.79 | 91.63 \pm 5.87 ** |
| Selenium | 60.09 \pm 1.51 | 48.58 \pm 2.31 ** |
| Chrome | 0.06 \pm 0.01 | 0.03 \pm 0.00 ** (n=4) |

Values are means \pm SEM.

Control diet: AIN-93 powder (Oriental Yeast co., Ltd) were used.

Deficiency diet: AIN-93G powder (Oriental Yeast co., Ltd) were used. The Zinc, selenium and chromium content of the deficient diet were close to zero.

** $p < 0.01$: significantly different from the P. Control.

Table 3. Plasma levels of zinc, selenium and chromium in KKAY mice at the end of main study.

| | Groups | | |
|----------------------|--------------------------|--------------------------|--------------------------|
| | 0mg/kg (n=8) | 50mg/kg (n=8) | 150mg/kg (n=8) |
| ($\mu\text{g/dL}$) | | | |
| Zinc | 83.25 \pm 4.34 | 83.50 \pm 3.58 | 80.75 \pm 3.89 |
| Selenium | 55.21 \pm 1.20 | 56.98 \pm 1.77 | 55.76 \pm 1.84 |
| Chrome | 0.09 \pm 0.05 (n=6) | 0.05 \pm 0.00 (n=7) | 0.05 \pm 0.01 (n=5) |

Values are means \pm SEM.

0mg/kg: the 0-mg group (physiological saline, 0mg/kg/mL of SEC), n=8

50mg/kg: the 50-mg group (50mg/kg/mL of SEC), n=8

150mg/kg: the 150-mg group (50mg/kg/mL of SEC), n=8

SEC: Soft-Body Extract of *Crassostrea gigas*

Table 4. Urinary 8-hydroxy-2'-deoxyguanosine and 15-isoprostane F_{2t} in KKA^y mice.

| | Groups | | |
|-----------------------------|------------------|-------------------|---------------------|
| | 0mg/kg (n=16) | 50mg/kg (n=16) | 150mg/kg (n=16) |
| (ng/mg crea.) | | | |
| 8-hydroxy-2'-deoxyguanosine | 43.66 \pm 2.24 | 38.16 \pm 2.12 | 30.44 \pm 2.05 ** |
| 15-isoprostane F_{2t} | 3.11 \pm 0.43 | 2.39 \pm 0.25 | 2.72 \pm 0.29 |

Values are means \pm SEM.

0mg/kg: the 0-mg group (physiological saline, 0mg/kg/mL of SEC), n=16

50mg/kg: the 50-mg group (50mg/kg/mL of SEC), n=16

150mg/kg: the 150-mg group (50mg/kg/mL of SEC), n=16

SEC: Soft-Body Extract of *Crassostrea gigas*

** $p < 0.01$: significantly different from the 0mg/kg.

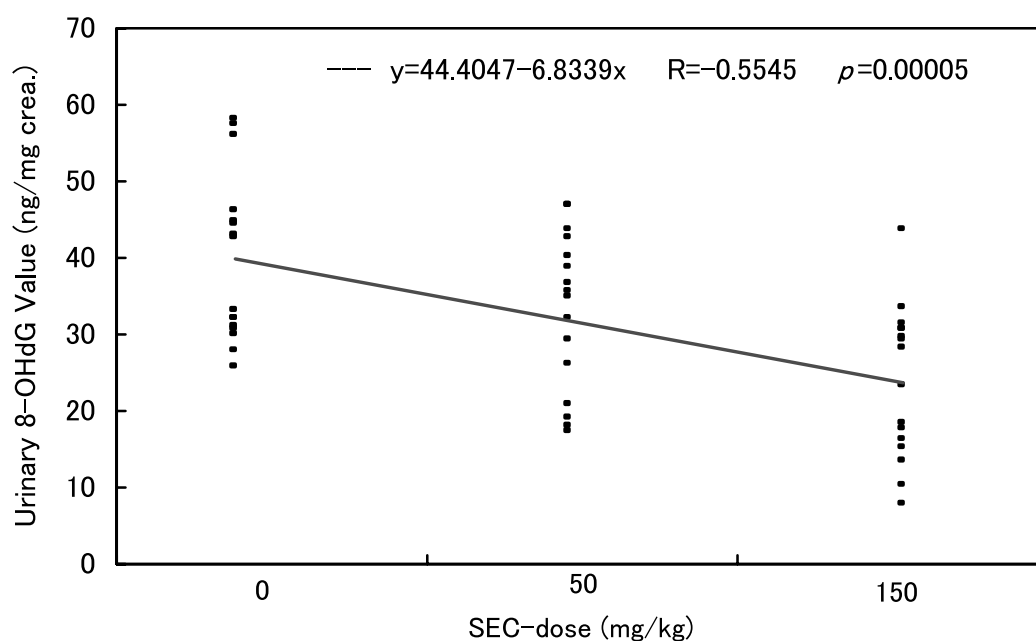


Fig. 2 Relationship between SEC-dose and urine 8-hydroxy-2'-deoxyguanosine concentration in KKA^y mice. 0: the 0-mg group (physiological saline, 0mg/kg/mL of SEC), n=8; 50: the 50-mg group (50mg/kg/mL of SEC), n=8; 150: the 150-mg group (50mg/kg/mL of SEC), n=8
SEC: Soft-Body Extract of *Crassostrea gigas*; a significant negative correlation was found between SEC dose and urine 8-OHdG (regression equation: $y = 44.4047 - 6.8339x$; $r = -0.5545$; $P = 0.00005$)

Table 5. TBA-reactive substance and SOD and GSH-Px activity of kidney in KKA^y mice.

| | Groups | | |
|----------------------------------|-----------------|------------------|-------------------|
| | 0mg/kg (n=8) | 50mg/kg (n=8) | 150mg/kg (n=8) |
| (nmol MDA/mg of protein) | | | |
| TBA-reactive substance of kidney | 0.707 ± 0.075 | 0.586 ± 0.054 | 0.482 ± 0.044 * |
| (units/mg protein) | | | |
| SOD | 1.489 ± 0.105 | 1.793 ± 0.163 | 1.877 ± 0.151 |
| GSH-Px | 0.387 ± 0.079 | 0.369 ± 0.148 | 0.719 ± 0.124 * |

Values are means ± SEM.

0mg/kg: the 0-mg group (physiological saline, 0mg/kg/mL of SEC), n=8

50mg/kg: the 50-mg group (50mg/kg/mL of SEC), n=8

150mg/kg: the 150-mg group (50mg/kg/mL of SEC), n=8

SEC: Soft-Body Extract of *Crassostrea gigas*

* $p < 0.05$: significantly different from the 0mg/kg.

5) Relationship between SEC dose and renal TBARS

Figure 3 shows the relationship between SEC dose and renal TBARS concentration of kidney in KKAY mice. A significant negative correlation was found between SEC dose and renal TBARS (regression equation: $y=0.6865-0.1017x$, $r=-0.4653$ and $P=0.02527$).

DISCUSSION

In the preliminary study, plasma levels of zinc, selenium and chromium in the deficient-diet group were significantly lower than those in the control-diet group. This confirmed that diabetic mice with low trace elements, resembling human diabetes, were prepared.

In the main study, there were no significant differences in the plasma levels of zinc, selenium and chromium among the 0-mg, 50-mg and 150-mg groups, and SEC did not increase the plasma levels of zinc, selenium and chromium. These results indicate that the markedly deficient diet (close to zero zinc, selenium and chromium) caused severe trace element deficiency, and that the trace elements included in SEC were not sufficient to

significantly increase the plasma levels of zinc, selenium and chromium.

Urinary 8-OHdG for the 150-mg group was significantly lower than that for the 0-mg group, thus confirming significant suppressive effects of SEC on DNA oxidation. In addition, a significant negative correlation was found between SEC dose and urinary 8-OHdG in KKAY mice, thus indicating significant dose-dependent suppressive effects of SEC on DNA oxidation.

Lipid oxidation in the kidney damages renal capillaries and induces diabetic nephropathy (Metz, T. O., N. L. Aldeason, S. R. Thorpe & J. W. Baynes. 2003, Ulrich, P. & A. Cerami. 2001). Therefore, the TBA method was used to determine the changes in renal TBARS due to SEC administration.

The results showed that TBARS in the 150-mg group was significantly lower than in the 0-mg group, thus confirming the suppressive effects of SEC on lipid oxidation in the kidney. Furthermore, a significant negative correlation was found between SEC dose and renal TBARS, and as the SEC dose increased, TBARS in the kidney decreased. In other words, the results indicate significant dose-dependent suppressive effects of SEC on lipid oxidation in the kidney.

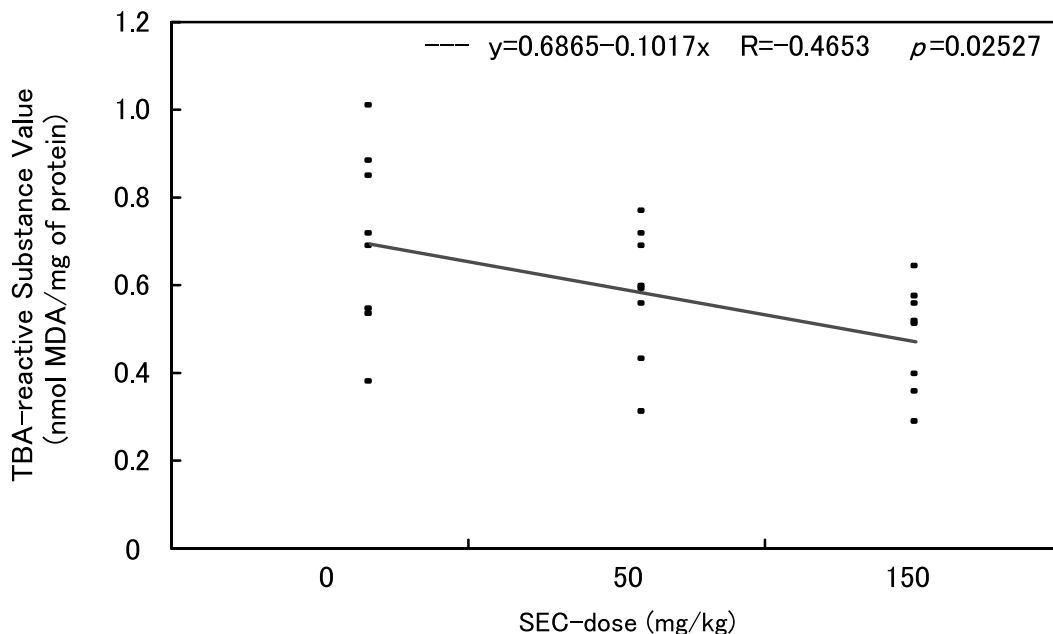


Fig. 3 Relationship between SEC-dose and TBA-reactive substance concentration of kidney in KKAY mice.

0: the 0-mg group (physiological saline, 0mg/kg/mL of SEC), n=8; 50: the 50-mg group (50mg/kg/mL of SEC), n=8; 150: the 150-mg group (50mg/kg/mL of SEC), n=8

SEC: Soft-Body Extract of *Crassostrea gigas*

a significant negative correlation was found between SEC dose and renal TBA-reactive substance (regression equation: $y=0.6865-0.1017x$; $r=-0.4653$; $P=0.02527$)

No significant differences were found in urinary 15-isoprostane F2t between the 0-mg, 50-mg and 150-mg groups, and no suppressive effects of SEC on phospholipid oxidation in KKAY mice were found. However, SEC suppressed lipid oxidation in the kidney, thus suggesting that the suppressive effects of SEC on lipid oxidation may be organ specific.

GSH-Px, an enzyme that removes active oxygen, plays an important role in the removal of lipoperoxides (Little, C. & P. J. O'Brien. 1968, Christophersen, B. O. 1969, Sunde, R. A. & W. G. Hoehstra. 1980). In the present study, the GSH-Px activity for the 150-mg group was significantly higher than that for the 0-mg group, and the TBARS for the 150-mg group was significantly lower than that for the 0-mg group. Subsequently, the SEC-induced increase in GSH-Px activity is one of the factors for the low renal lipoperoxides in the 150-mg group.

SEC contains trace elements and antioxidants, such as vitamin A and vitamin E, and these compounds appear to be involved with its oxidant activity. Further investigations are necessary in order to elucidate the antioxidant activity of SEC.

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Clinical Effects of an Activated Oyster Meat Extract against Refractory Diseases in Pharmacies

Hideki YAMASAKI

Ishindou Pharmacy

Among people who come to pharmacy to talk to pharmacists, a lot of them complain about their symptoms that are less likely to be cured by western medicine, such as allergies, skin disease, diabetes, liver disease, insomnia, depression. These symptoms are more from people were given their birth after 1960's and the elderly. When analyzing the relationship of these diseases to the Japanese diet, we consider that people began eating more instant and processed food starting in the 1960s. and that the elderly may develop circulatory diseases and insomnia because they eat less and take more medications¹⁾²⁾. And also it is reported that zinc is consumed by excessive stress³⁾.

To think lack of trace element causes these diseases, using an activated oyster meat extract product by Watanabe Oyster Laboratory (Watanabe Oyster) has favorable results as expected.

Chinese classic book "Compendium of Materia Medica" says "Eating boiled oyster meats will make you release from your mental anxiety, conditioned yourself, remove your rose and make women's blood circulation better. If you eat raw oysters with vinegar, you can care your rose, lower your fever after drinking and satisfy your thirst. Broiled oysters are very delicious and will make your skin smoother and brighter."⁴⁾ And also we know they had used oysters to care insomnia and depression from Chinese classic book "The Classic of Food by Cui Yuxi" (Chinese name: *Cui Yuxi Shi Jing*) says "Eating oysters will care insomnia and mental anxiety."⁵⁾ The research of Drs. Kiyoshi Kimura and Joji Kumura announced in 1985 shows clinical effects of oyster meats extract containing zinc on Hebephrenic Schizophrenia with scientific supports⁶⁾.

Case1: A 34-year-old woman

She gave birth to a boy in March 1997 and had lived away from her parents. After she was pointed out that her baby was not gaining his weight very much at his third-month screening, she got child-care neurotic. From July of the same year downward, she became to be constantly in and out of hospital and take sleeping pills habitually. Her mother-in-law come to my pharmacy on the 10th of January in 1998 being at a loss to see her daughter-in-law could not do house work nor childcare. I advised her to make her daughter-in-law take Watanabe Oyster to supply zinc she lacked with the aim of making her operation of her brain active.

We could see she changed after 3 or 4 days she started to take 6 Watanabe Oyster tablets a day. She did not care changing her clothes even stripping herself in front of people before, but after taking the tablets, she became trying to avoid people closing the sliding door when she changed her clothes. 20 days later, she became to be ready to pick her child up and a month later, she re-started to do childcare at home.

Case2: A 43-year-old man

After he had testicular cancer operation, he became insomnia caused by anxiety of the relapse and stress from his work. He was taking paroxetine, nitrazepam, zopiclone, flunitrazepam and losartan potassium.

He was expressionless and lethargic, had constipation and palpitations, lost appetite and feel irritable at that time.

At the same time of restoring his condition using Chinese herbal medicines, he took Watanabe Oyster to supply his lack of zinc caused by a lot of use of psychotropic agents. I advised him to take more than 6 tablets a day. A week later, his bowel movement became better and he had his facial expression back. Another 11 days later, even he had stopped to use psychotropic agents at all, he became to be able to sleep well and his facial expression became brighter. After this day, his disease never had returned only using Watanabe Oyster.

Case3: A 76-year-old man

He came to my pharmacy complaining of his skin itching and ruses. Even he used steroidal ointment containing antihistamine drugs, his condition had just seesawed for 5 months and did not improve. However, taking from 4 to 6 tablets of Watanabe Oyster a day cured his condition and it has not recurred.

Case4: A 57 year-old- man

He suffered from insomnia, was going to the hospital for 2 months and taking medicines to cure it. He was also under a lot of stress and menopausal disorder. At the same time, he began to lose his sexual desire. I made him to take Chinese herbal medicines and 6 Watanabe Oyster tablets a day. Because a month later, we could see improvement in his condition, he took only Watanabe Oyster and *Eleutherococcus senticosus* extract, but his symptoms did not worsen. After 2 months he was able to sleep well with taking only Watanabe Oyster.

Even though there are few cases from the above, the facts explain symptoms that are less likely to be cured with curatives can be improved by supplying missing trace elements.

We maintain our lives by eating habits and keep our health. Nutrition (especially trace elements) is essential to necessary enzymatic reaction for life activities and satisfying nutrition is also needed to make effects of medicines better.

The rates of Modern Japanese nutritive suf-

iciency show appearance of diseased symptoms caused by a lack of nutrition like zinc, such as decrease in an intake of trace elements caused by a change in our eating habit, increased stress, increase in an intake of food additive containing chelate compounds and excreting trace elements caused by various medicines.

Satisfying nutrition using an oyster meat extract raises effects of treatment and makes it better.

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The 1st & 2nd International Oyster Symposiums



Photo 1-3 : The 2nd International Oyster Symposium, Photo 4-6 : The 1st International Oyster Symposium
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