水研機構研報，第50号，115－121，令和3年
Bull. Jap. Fish. Res. Edu. Agen. No. 50, 115－121, 2021

Comparative study of the impact of environmental changes on oyster culture between USA and Japan, as collaborative research under UJNR

Natsuki HASEGAWA*1, Brett R. DUMBAULD*2, Masakazu HORI*3, Satoshi WATANABE*4, Michael RUST*5, and Zachary FORSTER*6

Abstract: Several oyster species are cultured globally, and the Pacific oyster, *Crassostrea gigas*, is a widely cultured species in both the USA and Japan. In Japan, aquaculture production of the Pacific oyster is decreasing slightly for multiple reasons including large die-offs of adults during and after the reproductive season due to a delay in the reproductive season and poor post-spawning recovery, poor wild spat collection, and a labor shortage for both operation of aquaculture and post-harvest processing. These problems may be aggravated by environmental changes such as global warming and oligotrophication around Japan's coastal areas. The Japan Fisheries Research and Education Agency is investigating the causes of the die-off during the reproductive season and attempting to establish countermeasures. The Pacific oyster is native to Japan and was introduced to the USA for aquaculture in the early 1900's. Nonetheless, there are large differences in the culture system, habitats and environmental conditions between the two countries. A comparative study was initiated to evaluate oyster reproduction in the two countries in order to understand the effects of habitat and the environment on future success of aquaculture given predicted environmental changes. Oyster culture experiments were conducted in intertidal and subtidal zones inside and outside of seagrass habitat in Hiroshima Bay in Japan and Willapa Bay in USA during the reproductive season (March to June 2019 and February to July 2019, respectively). We focused on elucidating the effects of habitat and the environment on the energy allocation of the oyster between reproduction and somatic growth.

Key words: Pacific oyster *Crassostrea gigas*, aquaculture, die-off, energy allocation, reproduction

Background

Oyster species are cultured globally, and the Pacific oyster, *Crassostrea gigas*, is the most widely cultured species in the USA, Japan, and world. Aquaculture production of the Pacific oyster (Pacific cupped oyster in FAO, Cultured Aquatic Species Information Programme) globally exceeds 572 × 10^3 ton yr^{-1}; Japan's production was 174 × 10^3 ton yr^{-1} in 2017, and the production in USA was 26 × 10^3 ton yr^{-1} (FAO, Fishery Statistical Collections). Pacific oysters have the second highest production volume after *Crassostrea virginica* (American cupped oyster, 112 × 10^3 ton yr^{-1}) in the USA (FAO, Fishery Statistical Collections). The Pacific oyster has its origins in East Asia and has been cultivated for centuries. It has been cultured on the west coast of the USA since the 1920s, and in France since 1966. It has been widely introduced as an alternative species to indigenous oysters that had been severely depleted by overfishing and disease, and to create new industries.

2020年12月11日受理（Accepted on December 11, 2020）
*1 Hokkaido National Fisheries Research Institute, Kushiro Laboratory, 116 Katsurakoi, Kushiro, Hokkaido, 085-0802, Japan
*2 US Department of Agriculture, Agriculture Research Service, Hatfield Marine Science Center, Newport, OR 97365, USA
*3 National Research Institute of Fisheries and Environment of Inland Sea, 2-17-5, Maruishi, Hatsukaichi, Hiroshima, 739-0452, Japan
*4 National Research Institute of Aquaculture, 422-1 Nakatsuhamaura, Minamiise, Mie, 516-0193, Japan
*5 NOAA Office of Aquaculture, 1315 East-West Highway, Silver Spring MD. 20910, USA
*6 Washington Department of Fish and Wildlife, Ocean Park, WA. 98640, USA
E-mail: hasena "at" affrc.go.jp

There are two large traditional production areas in Japan: Hiroshima and Miyagi Prefectures, and there are many small production areas all over Japan. Production areas are distributed from the temperate zone (western part of Japan, Kyushu) to the cold-temperate-zone (northern Island of Hokkaido). In Japan, wild spats are collected with scallop shells hung in open water areas and are widely used for aquaculture. Suspended culture from rafts or floating longlines in offshore area are the main culture system. Most of the harvested oysters are shucked for commercial distribution.

In the USA, hatchery produced spats are used as widely as wild spats. Bottom and off-bottom culture in the intertidal area (tidal flats where seagrass beds may be present) are the main culture systems. Pacific oyster culture is mainly operated in the northwestern part of the USA, primarily in the State of Washington.

In Japan, production of the oyster is decreasing gradually for multiple reasons. In addition to insufficient wild spat collection and a labor shortage for both operation of aquaculture and post-harvest processing, large die-offs of adults during and after the summer reproductive season is a major problem. This problem may be aggravated by environmental changes such as global warming and oligotrophication of coastal waters. The Japan Fisheries Research and Education Agency is investigating the causes of the die-off during the reproductive season and attempting to establish countermeasures (Hasegawa and Sakami, 2019).

Summer die-off of the oyster has also been a recognized problem on the west coast of the USA since the mid 1950's, and some of these incidences are related to stress during the reproductive season combined with environmental factors (Cheney *et al.*, 2000). However, occurrence of high mortality is less frequent in the USA than Japan. Koganezawa and Goto (1972) reported that oysters from relatively oligotrophic waters were characterized by a smaller growth rate and reduced amount of spawning eggs than oysters from eutrophic waters in Japan. Production of a large amount of eggs associated with some environmental conditions that prolong spawning may increase mortality risk. Akashige *et al.* (2006) concluded that there was a high risk of large die-offs in years with high water temperature and small amounts of rainfall, especially during the spawning season when metabolic activities are devoted to gamete production (Akashige *et al.*, 2005). Thus, excessive energy allocation to gonad development is speculated to be one of the major causes of the large die-offs.

Comparative Culturing Experiments in the USA and Japan

Elucidating the effects of habitat, environment and culture system on the energy allocation of the Pacific oyster between reproduction and somatic growth would contribute to an understanding of the reasons oysters experience challenges during the spawning season under climate change and would support establishment of countermeasures. For this purpose, comparative oyster culture experiments were conducted in high oyster production areas in the USA and Japan, where there are large differences in the culture system, habitats and environmental conditions. This study was developed and conducted as collaborative research through the UJNR bilateral. The Japan site examined was Hatsukaichi in Hiroshima Bay, a part of Seto-Inland-Sea (HI), and the USA sites were Nahcotta (NA) and Bay Center (BC) in Willapa Bay, Washington State (**Fig. 1**). In these sites, mesh bags including ten tagged oysters (oyster bag) were set up in tidal flats at intertidal and sub-tidal levels (**Fig. 2**). Oyster bags were also set up inside seagrass beds at the experimental tidal flats to evaluate the effect of seagrass, which are common around oyster culture tidal flats in the USA. In addition to the experiments around tidal flats, oyster bags were also suspended in docks and kept submerged throughout the experiments to mimic suspended culture in the subtidal zone, which is a common oyster culture system in Japan.

Gametogenic development of the Pacific oyster progresses at ambient temperature above 10℃ and reaches the peak in sexual maturation at 600℃・days heat summation (*i.e.* cumulative daily temperature exceeding 10℃; Mann, 1979 and Oizumi *et al.*, 1971). Oyster culture experiments were conducted from March to June 2019 in Japan and from February to July 2019 in the USA, which corresponds to the

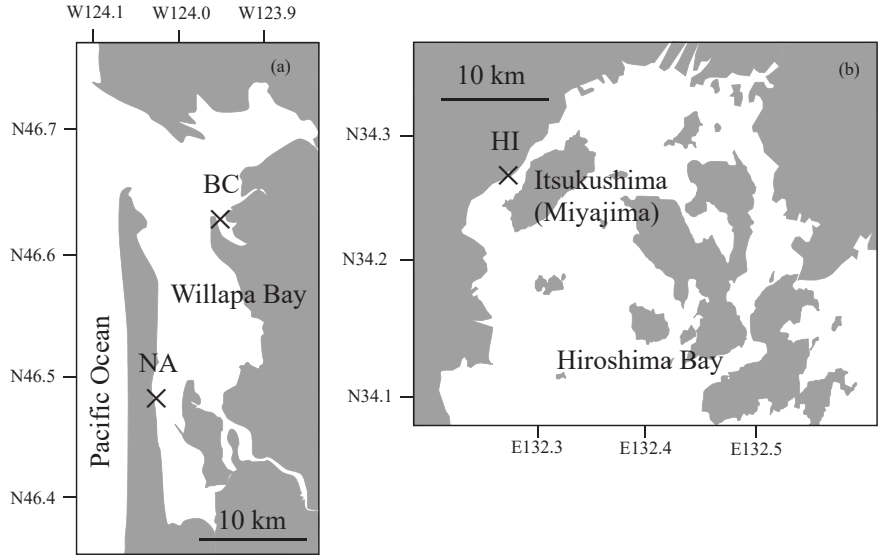

Fig. 1. Study sites for oyster culture experiments in Willapa Bay, USA (a) and Hiroshima Bay, Japan (b).

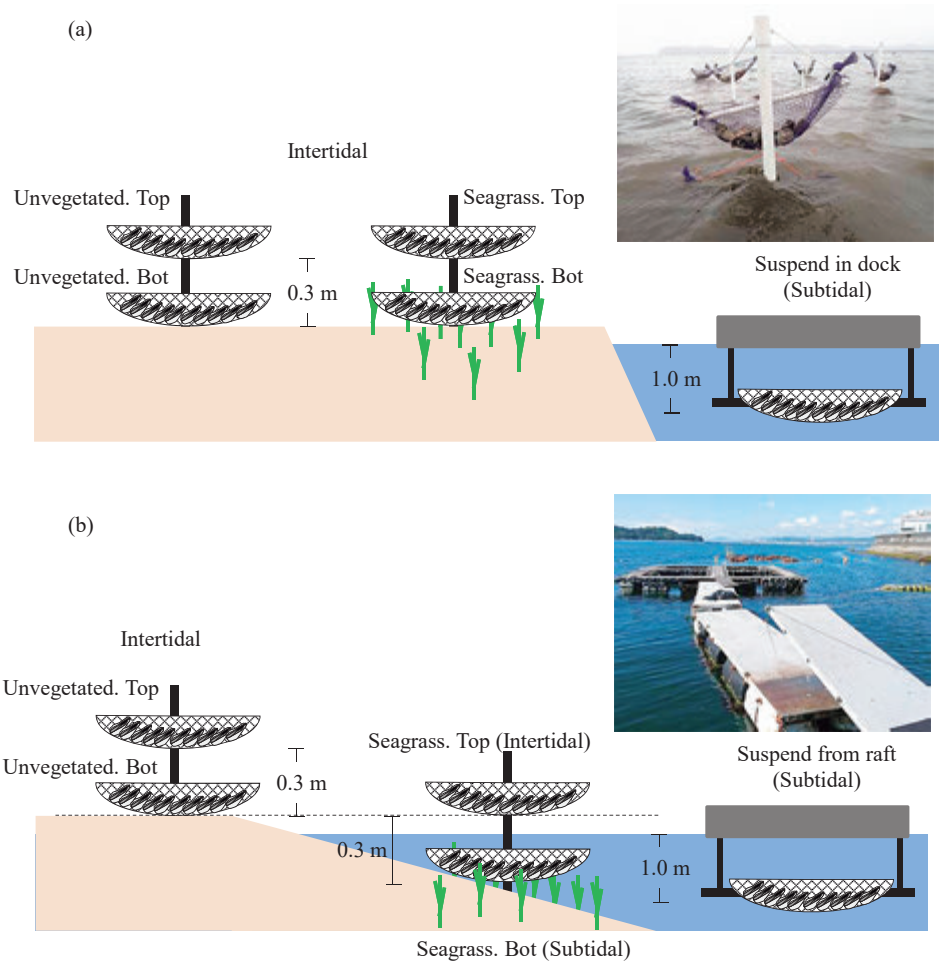

Fig. 2. Design of oyster culture experiments in Willapa Bay, USA (a) and Hiroshima Bay, Japan (b). Oyster bags, which were mesh bags including ten tagged oysters in each, were set around the tidal flat.

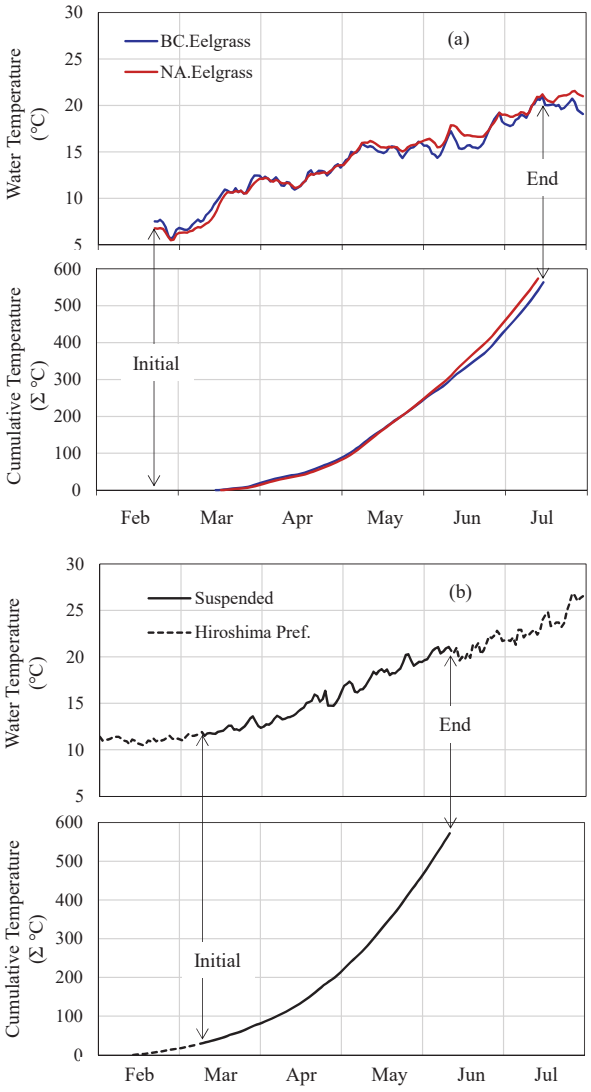

Fig. 3. Change of daily mean water temperature (during submerged times) and cumulative temperature at oyster culture experiments in Willapa Bay, USA (a) and Hiroshima Bay, Japan (b). Water temperature before and after the culture experiments obtained from Hiroshima Prefectural Fisheries and Marine Technology Center.

periods between beginning and peak (just before spawning) of sexual maturation. Peak in sexual maturation was one month later in the USA than Japan, but duration until sexual maturity (600℃・days) was similar between USA and Japan: about 4 months (**Fig. 3**).

Oysters for the USA experiments were harvested from tidal flats at the NA site in Willapa Bay, and market-size suspended oysters were purchased from oyster farmers in Hiroshima Bay for the Japan

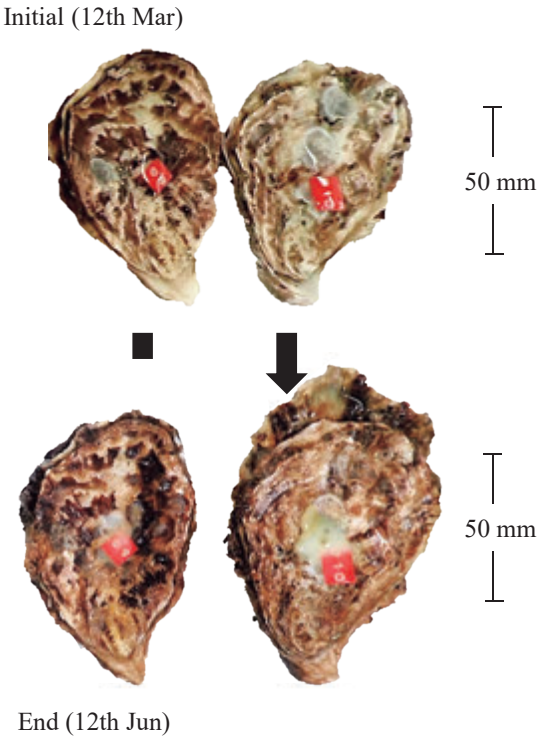

Fig. 4. Example of tagged oysters at the beginning and end of oyster culturing experiments in Willapa Bay, USA.

experiments. At the beginning of the experiments, the total wet weight inclusive of shell and shell height were measured, and the oysters were tagged for individual identification (**Fig. 4**). Initial total wet weight of oysters (shell + tissue + seawater) ranged from 77.6 to 274.6 g and from 61.9 to 162.4 g for the USA and Japan experiments, respectively. At the end of the experiments, oysters were measured for total wet weight and shell height and were also dissected. After measuring the whole tissue wet weight, the tissue was separated into the outer somatic parts (mantle, gill, and adductor muscle), inner somatic parts, and reproductive parts.

Vertical histological sections of the inner somatic and reproductive parts were prepared (**Fig. 5**). Outer somatic parts and one side of the inner parts were weighed and stored frozen at −20℃. Frozen samples were then freeze-dried and homogenized for nutrient component analysis (crude carbohydrate, protein and fat). The other side of the inner parts were weighed and fixed in Davidson's fixative, dehydrated in tissue dehydration solutions and embedded in paraffin. The paraffin blocks were

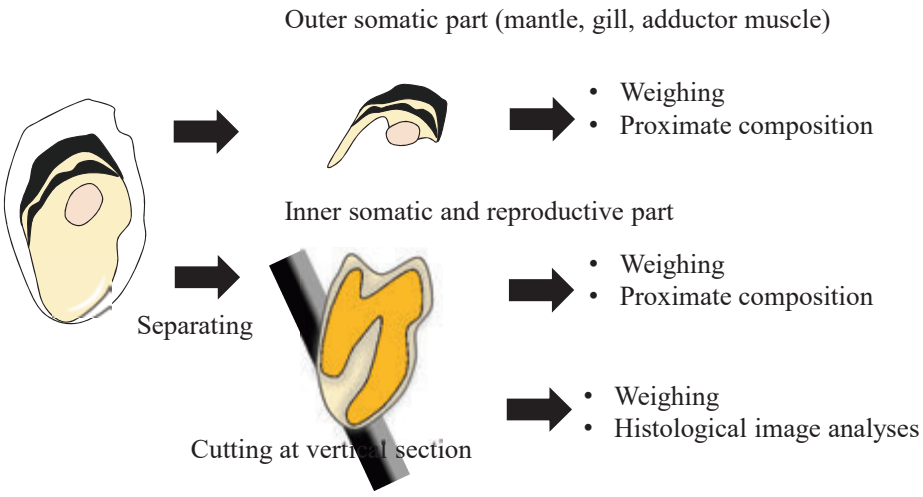

Fig. 5. Workflow of oyster sampling for evaluating energy allocation to the gonads.

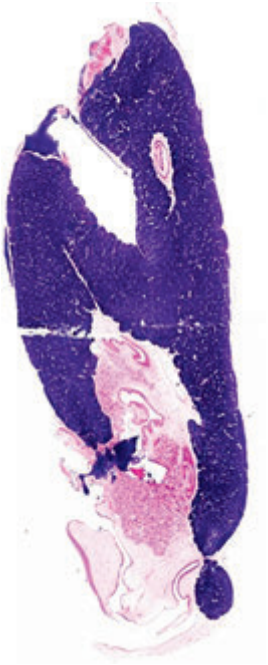

Fig. 6. Example of tissue section of oyster inner parts (male) stained with hematoxylin and eosin.

sliced at 5–8 μm thickness and stained with hematoxylin and eosin (**Fig. 6**). Sectional area ratio of gonad to total inner part were histologically determined and volume ratio, which are substitutes for weight ratio, were calculated. Total carbohydrate content of dried sample was determined using the phenol-sulfuric acid method (Dubois *et al.*, 1956). The amount of total nitrogen in samples was measured using an elemental analyzer (Flash EA1112, Thermo-Finnigan) and nitrogen content was converted to protein content by multiplying the conventional conversion factor of 6.25. Crude fat content was determined by the modified gravimetric method (Ichihara *et al.*, 2011) based on the method originally described by Bligh and Dyer (1959). For total lipid extraction, tert-butyl methyl ether was used instead of chloroform. Energy contents of each tissue part were calculated as the sum of the energy content in each nutrient component estimated by the following factors: carbohydrate (17.2 kJ/g DW), protein (23.9 kJ/g DW) and lipid (39.8 kJ/g DW) (Ansell, 1974). Finally, energy allocation to the gonads was collectively estimated with the obtained data.

Relative growth rate (RGR) of shell height during culture experiments was calculated as follows:

$$\mathrm{RGR} = \frac{Ln\ (Shell\ height_{end}) - Ln\ (Shell\ height_{initial})}{Culture\ period\ (day)}$$

Condition index (CI) was calculated as follows;

$$\mathrm{CI} = \frac{TSW}{TTW - SLW} \times 10^2$$

where TSW is the tissue weight (gWW), TTW is the total weight with shell (filled with water, gWW) and SLW is the shell weight (gWW).

This is a progress report of the UJNR collaborative study. At the moment, the culture experiments and sample analysis have been finished, and collected data are being organized and analyzed. The results

are planned to be published in future reports.

Acknowledgements

We are grateful to A. J. Trimble, A. Bolm, and B. McIntyre for assistance with field experiments and Y. Fuchigami, S. Tanaka, and T. Kawabuchi for assistance with analysis. This study was supported by the international joint research project of Japan Fisheries Research and Education Agency.

References

Akashige S., Hirata Y., Takayama K., and Soramoto K., 2005: Seasonal change in oxygen consumption rates and filtration rates of the cultured Pacific oyster *Crassostrea gigas*. *Nippon Suisan Gakkai Shi,* **71**, 762–767. (in Japanese with English abstracts)

Akashige S., Hirata Y., Takatsuji Y., and Aida S., 2006: Occurrence of mass mortality in oyster culture with relation to seawater temperature and rainfalls in Hiroshima. *Bulletin of the Hiroshima Prefecture Fisheries and Marine Technology Center*, **1**, 9–13. (in Japanese)

Ansell A. D., 1974: Seasonal changes in biochemical composition of the bivalve *Lima hians* from the Clyde Sea Area. *Mar. Biol.*, **27**, 115–122.

Bligh E. G. and Dyer W. J., 1959: A rapid method of total lipid extraction and purification. *Canadian J. Biochem. Physiol.,* **37**, 911–917.

Cheney D. P., MacDonald B. F., and Elston R. A., 2000: Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. *J. Shellfish Res.*, **19**, 353–359.

Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A., and Smith F., 1956: Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**, 350–356.

FAO: Cultured Aquatic Species Information Programme, *Crassostrea gigas* (Thunberg, 1793), FAO Fisheries and Aquaculture Department, Rome. http://www.fao.org/fishery/culturedspecies/Crassostrea_gigas/en (Cited 31 March 2020)

FAO: Fishery statistical collections, Global aquaculture production, FAO Fisheries and Aquaculture Department, Rome. http://www.fao.org/fishery/statistics/global-aquaculture-production/en, (Cited 31 March 2020)

Hasegawa N., and Sakami. T., 2019: Change in wild spat collection of oysters in Japan and new wild spat collection method for single-seeds. *Aquaculture Business*, **56(5)**, 14–17. (in Japanese)

Ichihara K., Yoneda K., Takahashi A., Hoshino N., and Matsuda M., 2011: Improved methods for the fatty acid analysis of blood lipid classes. *Lipids*, **46**, 297–306.

Koganezawa A., and Goto K., 1972: Ecological studies at a seed oyster production area- I. Characteristics of mother oyster populations in Sendai. Bay. *Nippon Suisan Gakkai Shi,* **38**, 1–8. (in Japanese with English abstracts)

Mann R., 1979; Some biochemical and physiological aspects of growth and gametogenesis in Crassostrea gigas and Ostrea edulis grown at sustained elevated temperatures. *J. Mar. Biolog. Assoc. U.K.,* **59**, 95 –110.

Oizumi S., Ito S., Koganezawa A., Sakai S., Sato R., and Kanno H., 1971: Techniques of oyster culture, in "Aquaculture in. shallow seas: progress in shallow sea culture (Revised edition)" (ed. by Imai T.), Koseisha Koseikaku, Tokyo, pp 153–189. (in Japanese)

Annotated Bibliography of Key Works

(1) Akashige, S., Hirata Y., Takatsuji Y., and Aida S., 2006: Occurrence of mass mortality in oyster culture with relation to seawater temperature and rainfalls in Hiroshima. *Bulletin of the Hiroshima Prefecture Fisheries and Marine Technology Center*, **1**, 9–13.

Mass mortality in the Pacific oyster, *Crassostrea gigas* occurred in 1979, 1994, 2001 and 2002, and relationships with surface water temperature or rainfall in Hiroshima Bay were analyzed using a dataset from 1970 to 2004. Mass mortality occurred in years with high water temperature (*i.e.* the number of days over 20℃ was greater than the mean+SD) and small rainfall (*i.e.* cumulative total rainfall from July to October was smaller than the

mean－SD) except in 2001. Spat introduced from Miyagi Prefecture experienced extraordinary mass mortality in 2001, which has a colder climate than Hiroshima Prefecture. Therefore, there is high risk of mass mortality of local oysters in years with high water temperature and small rainfall.

(2) Akashige, S., Hirata Y., Takayama K., and Soramoto K., 2005: Seasonal change in oxygen consumption rates and filtration rates of the cultured Pacific oyster *Crassostrea gigas*. *Nippon Suisan Gakkaishi*, **71(5)**, 762–767.

The oxygen consumption rate (OCR) and filtration rate (FR) of cultured Pacific oysters, *Crassostrea gigas*, of different sizes were measured in still water systems at different seawater temperatures in various seasons. The OCR had clear relationships with water temperature (t ℃) and dry body flesh weight (W_d g) expressed by the formula: OCR (mg O^2 hr^{-1} ind^{-1}) = $(0.072t - 0.64)$ $W_d^{0.75}$ and there was no difference among seasons. The FR was expressed by the formula: FR (L/h/ind) = $(0.70t - 6.6)$ W_d in non-spawning seasons. However, in the spawning season, the FR was expressed by the formula: FR = 4.9 W_d, which was lower than the value expected by the formula applied during the non-spawning season. These results clearly showed that feeding activities declined during the spawning season, but oxygen demand (weight base and temperature dependent) was constant throughout the year. Oysters would be especially vulnerable during the spawning season when metabolic activities would be devoted to gamete production.

(3) Cheney, D.P., MacDonald B.F., and Elston R.A., 2000: Summer mortality of Pacific oysters, *Crassostrea gigas* (Thunberg): Initial findings on multiple environmental stressors in Puget Sound, Washington, 1998. *Journal of Shellfish Research*, **19**, 353–359.

Summer mortality has been a recognized problem for oyster aquaculture on the west coast of the USA since the mid 1950's, and these authors related some of these incidences to stress during the reproductive season combined with environmental factors. Nonetheless triploid oysters experienced higher mortality than diploids.