The Third International Symposium on Manila (Asari) Clam

-International Collaboration for Manila clam (Asari) Studies-

Ust-Tsu, Tsu, Mie, Japan,
June 1 – 2, 2015

Co-organized by
Fisheries Research Agency
Graduate School & Faculty of Bioresources, Mie University
Mie Prefectural Government

Programme

Symposium

June 1, 2015 (Monday)
Venue: Ust-Tsu, Tsu, Mie, Japan
9:30 - 9:45: Registration
9:45 - 10:00: Welcome Remarks and Opening Addresses
Fuminari Ito (Executive Director, Fisheries Research Agency)
Yoshihiro Komada (President, Mie University)
Eikei Suzuki (Governor, Mie Prefecture)
10:00-10:10: Outline of the Symposium
10:10-12:00: Session I, Manila clam fisheries in Asian countries

1. Stocks and fisheries of asari in Japan. (Mitsuharu Toba, Tokyo University of Marine Science and Technology, Japan)
2. Perkinsus (Lester and Davis 1981) infection in the Manila clam (*Ruditapes philippinarum*) in Korea; species identification, impacts and spatio-temporal distribution (Kwang-Sik Choi, Jeju National University, Korea)
3. Development of Manila clam industry in China (Fang Jianguang, Yellow Sea Fisheries Research Institute, China)

12:00-14:00: Lunch Break and Poster Session
14:00-15:45: Session II, Manila clam fisheries in Western countries

4. Asari clam in France: 1972 - 2015 (Xavier de Montaudouin, University of Bordeaux, France)
5. Manila clam introduction in the Sacca di Goro Lagoon (Northern Italy): ecological and economic implications (Marco Bartoli, Parma University, Italy)
6. Status of Manila clam, *Ruditapes philippinarum*, culture and fisheries on the West coast of North America (Brian C. Kingzett, Vancouver Island University, Canada)

15:45-16:00: Coffee Break
16:00-17:10: Session III, Genetic diversity of Manila clam and fishery ground restoration

7. Mapping the stranger: genetic diversity of Manila clam in European coastal lagoons (Stefania Chiesa, University of Aveiro, Portugal)
8. An a-priori evaluation model for restoration of fisheries population of the Mania clam and a countermeasure example (Hisami Kuwahara, Fisheries Research Agency, Japan)

17:10-18:00: General Discussion
19:00-21:00: Banquet (Restaurant Le Vert)

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Keynote Lectures

Stocks and fisheries of asari in Japan

Mitsuharu Toba*

Tokyo University of Marine Science and Technology, Konan, Minato, Tokyo, Japan,
*Corresponding author: mtoba00@kaiyodai.ac.jp

Asari is one of the most important fishery resources in Japan. Populations of asari are naturally maintained in the coastal bay areas throughout the country. They have been utilized not only as a food but also as a recreational harvest species at least since eighth century AD. Asari is popular among Japanese people because it is a popular seafood, and is abundant in shallow sandy coasts near urbanized areas and thus can be harvested easily.

Major harvested areas of asari are Tokyo Bay, Mikawa Bay, Ise Bay, Seto Inland Sea and Ariake Bay in Japan. Habitats (harvested zone) of asari stretch from intertidal to shallow subtidal areas with muddy or sandy bottoms. Fishermen harvest asari using a hand rake or a basket rake in tidal areas, and a long-handled basket rake, a vessel-trawled dredge or by helmet diving in deeper areas. Annual production of asari in Japan been in decline since reaching its maximum of about 160,000 mt in the mid 1980’s, and the production has stayed below 40,000 mt after the 1990’s. The production decreased in all major harvested areas mentioned except Mikawa Bay.

Stock decline of natural asari is remarkable and seriously problematic for coastal fisheries in Japan. A number of factors are thought to be responsible for the clam decline, including a decrease of suitable habitat areas, deterioration of water and substrate quality due to coastal development, physical destabilization of bottom substrate by strong wave disturbance, newly arrived invasive predators and parasites, oligotrophication of seawater with strict regulation of waste water quality control, and overexploitation. The extent and intensity of these factors seem to differ among harvested areas based on a number of field and laboratory investigations.

Various countermeasures have been taken to restore the natural stocks of asari, mainly manipulations of habitat environment, including control of strong wave action or bottom substrate movement by artificial structures, improvement of bottom substrate quality by spreading of sand or cultivation, improvement of seawater exchange with training dikes or dredging of channels. While positive results were obtained in some cases, particularly with spreading of sand, there were certain cases that showed little or no effects.

Recently, challenges are being set to integrate several techniques into one; for example, countermeasures for improvement of physical environment of habitat, control of predation and competition and transplantation of seed clams are to be performed in the same area at the same time. While these subtechniques are not novel, integrated techniques are characterized by being planned and performed under comprehensive investigation of the population ecology of asari in relation to the environmental conditions in the entire bay area. The investigation includes determination of possible contribution of local asari populations to larval supply, locations of sources and sinks of clam larvae, natural mortality and causative factors for juvenile mortality, and so on.

On the other hand, trials of asari culture using on-site facilities are being developed in many areas. These operations do not focus mainly on restoration of asari stocks but on economic feasibility. One successful example is suspended culture using oyster rafts and plastic containers. Although production is limited, suspended culture has become important for local industry because the asari produced are of high quality (more meat content), and thus fetch higher prices.

Recent topics in the related fields are extreme oligotrophication of water in some areas, mass outbreaks of parasitic sea spiders (*Nymphonella tapetis*) and increasing production of the exotic hard clam (*Mercenaria mercenaria*) in Tokyo Bay. Dissolved inorganic nutrients (DIN and DIP) in the Seto Inland Sea frequently decrease to levels below those in the open ocean from fall to winter. Low nutrients directly affect the growth of the macroalgae nori (*Porphyra*) that is cultured widely in the area. Decreased nutrient levels is reported to cause bay-wide depression of primary production, which in turn may result in lower egg production and retarded growth of asari.

Mass mortality of asari by the parasitic infection of sea spiders has seriously hampered the asari fishery in Tokyo Bay since 2007. The origin and cause of the sudden outbreak of the parasite are still unknown. Measures attempted for controlling the parasite infection (e.g. extermination of free-living adult sea spider by net trawling) had been ineffective. The asari production in Kisarazu area (Tokyo Bay) has decreased by more than 90% due to the parasite outbreak.
Natural colonization of the hard clam was confirmed in 1998 in Tokyo Bay. The hard clam is considered to be introduced to Japan unintentionally with ballast water of ships from North America. The hard clam production is increasing steadily, exceeding 1,000 mt in 2014, and has become an important local fishery resource to substitute asari in Tokyo Bay. The hard clam, however, is classified as an invasive alien species. Although there is no legal restriction, artificial enhancement or culture of the hard clam should be avoided from the viewpoint of biodiversity conservation.

＜要約＞日本のアサリ資源と漁業（鳥羽光晴）
アサリは日本全国の内湾沿岸に自然分布する重要な水産資源である。レジャー対象種としても古くから利用されており、８世紀には潮干狩りの記録がある。アサリの主要生産海域は、東京湾、伊勢・三河湾、瀬戸内海西部、有明海で、主な生息域は潮間帯から浅い潮下帯の砂泥域である。潮間帯では手掘りや鋤簾、潮下帯では大型の鋤簾、底引き、潜水器などで漁獲されている。日本のアサリ生産量は 80 年代に 16 万トンに達したが、三河湾を除く全ての主要海域で生産は減少し、90 年代以降は 4 万トン以下にまで低下しており、近年の沿岸漁業の重要かつ深刻な問題の一つである。減少原因とされる要因は数多く、沿岸開発による生息場の減少や水底質の悪化、貧酸素、強い波浪による底質の物理的な不安定化、新たな食害生物や病虫害、水質規制による海域の貧栄養化、過剰な漁獲などがある。各要因の影響の強さと広がりは海域によって異なっている。アサリの資源回復を図るために、さまざまな漁場環境の改善努力が行われてきた。構造物による海水の流動や底質の移動緩和、覆砂や耕耘による底質改善，導流堤や削澪による海水交換促進などである。これらの環境改善は一定の効果があり、とくに覆砂では稚貝の増加が認められる例が多いが、期待した効果が得られない場合もある。近年、生息場所の物理環境の改善、食害生物や競合生物の除去、稚貝の移植などの技術を組み合わせた意欲的な手法が試みられている。個々の技術は以前からあるものであるが、対象海域全体のアサリの個体群生態と環境条件の網羅的な調査を行い、最も効果的な実施条件を判断した上で統合的な手法を適用している。一方、資源増殖策とは別に、全国的に試行例が増えているのは採算性を重視した施設養殖事業である。代表的なものは海面イカダでプラスチック容器に入れたアサリを垂下飼育する養殖事業である。生産量は少ないが、身入りが良く高品質のアサリが生産できることから高価格で取引され、地域の重要な産業になっている。アサリに関連する近年の話題として、海域の貧栄養化、カイヤドリウミグモの寄生による大量死、外来種ホンビノスガイの増加がある。近年は排水の水質規制が進んだために栄養塩（無機溶存態窒素、同リン）が減少している海域があり、特に瀬戸内海では冬季には栄養塩が外洋より低いレベルにまで減少することがある。これは直接的には海藻（ノリ）の著しい生育不良を起こしてノリ養殖業にとって最大の問題になっている。さらに、栄養塩量の減少は海域全体の一次生産力の低下につながっており、アサリにおいても成貝の産卵量の低下、成長の悪化などとの関係が指摘されている。東京湾では 2007 年に寄生性節足動物カイヤドリウミグモの大量発生があり、その寄生によっ

てアサリが大量死亡した。カイヤドリウミグモの由来と発生原因は不明である。そりネットによる自由生活成体の除去など様々な防除対策が試みられたが十分な効果を上げていない。カイヤドリウミグモの寄生は続いており，木更津地区ではアサリの生産量は発生前の 1/10 以下まで低下している。1998 年以降、東京湾で外来種のホンビノスガイが自然発生している。由来は北米地域からの船舶のバラスト水などによる非意図的移入であると言われている。ホンビノスガイの漁獲量は年々増加し、2014 年には千トンを越えており、地域の重要な漁獲対象種となっている。一方でホンビノスガイは、侵略的外来種として区分されており、法的規制はないものの積極的な増殖や養殖は控えるべきとされている。

Perkinsus (Lester and Davis 1981) infection in the Manila clam (_Ruditapes philippinarum_) in Korea: species identification, impacts and spatio-temporal distribution

Kwang-Sik Choi*

School of Marine Biomedical Science, Jeju National University, Jejudaehakno, Jeju, Republic of Korea
*Corresponding author: skchoi@jejunu.ac.kr

Asari or Manila clam *Ruditapes philippinarum* (=*Venerupis philippinarum*) is commonly distributed on the west and south coast of Korea, supporting the local shellfisheries industry. Studies examined Manila clams in Korean waters have reported Perkinsosis, a parasitic disease caused by protozoan belonging to the Genus *Perkinsus*. PCR analysis of the Perkinsosis agent indicated that *P. olseni* is the only Perkinsosis agent in Manila clam in Korea's waters, while *P. houshuensis*, first discovered in Japan from Japanese Manila clam, detected from *R. variagata* in coastal Jeju Island.

Several surveys carried out in Korea's waters for the past decade revealed that infection intensity of *P. olseni* over 1×10^6 cells/g tissue caused necrosis and lesion of the gills and the mantle tissues. Heavy infection with *P. olseni* also retarded reproductive process of the clams, including slow gonad maturation and decrease in the reproductive effort. Clams survived from mass mortality event occurred during post-spawning period on the west coast also showed high infection prevalence and intensity of *P. olseni*, suggested that *P. olseni* is closely linked to the mass mortality event.

Despite the high infection intensity, some clam populations on the coastal Yellow Sea where the phytoplankton biomass was relatively higher, showed relatively higher condition factor, suggested that high level of food supply may compensate the energetic loss caused by high load of the parasite in Manila clam.

〈要約〉韓国のアサリのパーキンサス病：種の同定と時空間分布と影響（崔光植）

アサリは韓国の西岸と南岸に広く分布しており地元の水産業を支えている。韓国水域のアサリには、*Perkinsus* 属の原虫寄生によるパーキンサス病が報告されている。PCR 法により、韓国では *P. olseni* 一種のみが報告されていたが、日本で最初に発見された *P. houshuensis* が済州島沿岸で発見された。*P. olseni* の密度が組織 1g あたり百万細胞を超えると組織が壊死してエラや外套膜に障害が起きることが知られている。重篤の場合は、生殖腺発達異常など生殖に影響を及ぼす。韓国西岸で繁殖シーズン後に起きた大量斃死の際に *P. olseni* の感染が確認され、大量斃死の原因であると考えられた。アサリの餌となる植物プランクトンに富む黄海沿岸では、感染があってもアサリの栄養状態が良いことが知られており、感染症による栄養消費を豊富な餌の供給が助けている可能性がある。

Development of Manila clam industry in China

Fang Jianguang*

Yellow Sea fisheries Research Institute, CAFS. Qingdao, P. R. China
*Corresponding author: fangjg@ysfri.ac.cn

Over 2 million tons of Manila clams *Ruditapes philippinarum* (Adams et Reeve) were produced each year on the coast of Yellow Sea, Bohai Sea and East China Sea of China. Most of this production occurs in Liaoning, Shandong and Fujian Provinces. In addition, a significant Manila clam seed production industry has developed with pond production facilities in Fujian province. Before 1970's, all farmed seeds of Manila clam relied on natural. Farmers collected seeds of Manila clam from natural inhabit. By the late of 1970's, the seed production technology of Manila clam in ponds was developed rapidly and achieved great success in Fujian Provinces because the natural seed was not enough to meet the demand from farming.

Since 1990's, the Manila clam industry has developed rapidly in China prompted by the demand from domestic and abroad. In order to meet the increasing demand of seed from whole China, the seed production of Manila clam in reclamation areas became the major sources of seed of Manila clam for farming in China. In 2013, the total seed production in Fujian province was 7952 tones with shell length about 1 cm. Meanwhile, the Manila clam is also the major species farmed with shrimp, fish, crab in form of pond integrated multi-trophic aquaculture that is practiced in commercial scale in Zhejiang and Fujian Provinces.

In history, the farming of Manila clam was mainly carried out in tidal zone and subtidal zone before 1980's. An unfilled domestic and overseas demand was pushing attempts to increase production of this clam from 1990's. The farming areas of the clam has extended from intertidal zone to shallow sea with the depth of about 10 m in Shandong and Liaoning provinces. Because of longer time immersing in water and richer supply of phytoplankton, the growth rate and suitable harvest time of the clam cultivated in deeper water is much higher and longer than that of clam growing in natural inhabits. The main problems for such farming are the predation from crab, starfish and sea snails.

The farming space decreasing mainly due to sea reclamation along the coast, and the pollution from lands are being the major threat to Manila clam industry in China. However, the impact of climate change, such as acidification, sea storm and extreme weather on development of Manila clam should be studied systematically in the world.

<要約>中国のアサリ産業の発展（方建光）
中国では黄海、渤海、東シナ海の沿岸、主に遼寧省、山東省、福建省でアサリが年間約２百万トン以上生産されてきた。70 年代以前は天然種苗に依存してきたが、需要の増大のため 70 年代以降養殖池での種苗生産技術が発達し、福建省で成功を収めた。90 年代以降国内外の需要の増加によりアサリ産業は急激に成長した。種苗需要の増大のために造成場での種苗生産が主な供給源となった。2013 年の福建省での種苗生産量は 7952 トンであった（殻長 1cm）。福建省と浙江省では、養殖池でのアサリ、エビ、魚、カニの複合養殖が商業規模で行われている。80 年代前のアサリ養殖は主に潮間帯と浅い潮下帯で行われたが、90 年代以降の需要増加のため山東省と遼寧省では、水深 10m にまでエリアが拡大した。深い水深帯では干出がなく餌となる植物プランクトンが豊富なためアサリの成長がよく収穫期も長いが、カニ、ヒトデ、巻き貝類による食害が問題となっている。漁場面積は沿岸の埋め立てにより減少し陸域からの環境汚染が問題となっている。温暖化や酸性化の影響に関してはさらに研究を進める必要がある。

Asari clam in France: 1972 - 2015

Xavier de Montaudouin*

OASU, Station Marine d'Arcachon, Université de Bordeaux, 2 Rue du Professeur Jolyet, Arcachon, Cedex, France
*Corresponding author: x.de-montaudouin@epoc.u-bordeaux1.fr

Asari clam was introduced to France in 1972 for aquaculture purpose. At this time, France underwent an oyster crisis and urgently needed to diversify its aquaculture. All adults and spat originated from the same area (Puget Sound, WA, USA). After a promising start, Asari clam culture rapidly faced a series of concomitant handicaps: firstly, there was a lack of suitable ground availability due to certain reluctance from oyster farmers to share their fields and/or diversify their activity. Indeed, Asari clam introduction was concomitant with the introduction of Pacific oyster (*Crassostrea gigas*); secondly, economical competition with Italy rapidly became tough, exacerbated by the occurrence of neonaturalized Asari clam populations. In this context, European regulation was unfavorable to France due to contrasted rules between the Mediterranean Sea and the Atlantic Ocean; finally, several diseases impacted clam populations. At the end of the 80's, mass mortality occurred due to Brown Ring Disease and related to a prokaryote (*Vibrio tapetis*).

Today, the French production remains low (2-3000 tons) and most of it is provided by harvesting the neonaturalized populations in two sites (Arcachon lagoon and Morbihan Gulf). In Arcachon Bay, an exhaustive population dynamics study highlighted the shared responsibility of fishing activity and environmental characteristics. A management model proposed different scenarios based on fishing effort reduction (i.e. fishing area, and/or fishing licences number, and/or fishing period). The implementation of these recommendations provided some encouraging results. However, Asari clam fitness remains poor. A genetic impoverishment due to population isolation was argued by fishermen, but transplant experiment demonstrated that these bivalves kept their plasticity, at least in terms of growth and condition index. Thus, several environmental factors were investigated as possible fey factors explaining clams low performances. Again, pathologies were pointed out and in particular the high pressure exerted by the protist *Perkinsus* sp. (perkinsosis). Besides, in 2005 a new pathology was discovered in Arcachon bay, the Brown Muscle Disease. The etiological factor is not yet discovered, although viral origin is suspected.

A meta-analysis comparing Asari clam characteristics in Arcachon Bay with the international literature pointed out that one of the most important factor explaining their bad condition index in this French lagoon was certainly a pool of unfavorable factors (diseases, trace elements, …). However, 30% of the differences of condition index among sites over the world were explained by chlorophyll a concentration.

Finally, marine ecologists must deal with a conflictual goal: on the one hand Asari clam is an important exploited bivalve in France, on the other hand it remains an exotic species which needs careful attention (European regulations).

＜要約＞フランスのアサリ 1972-2015（グザビエ・デ・モントドゥアン）
アサリは 1972 年に養殖目的でアメリカ、ワシントン州のピュージェット湾からフランスに持ち込まれた。この時フランスではカキ養殖の不振のために養殖の多角化が求められていた。アサリ養殖は順調なスタートを切ったが、いくつもの問題に直面することとなった。まず、カキ

養殖業者が経営の多角化や養殖場の共有に積極的でなかったため､養殖場の不足が問題となった。実際にアサリの導入はマガキと同時に行われた。次に、アサリ販売はイタリアとの商業的競争が熾烈であったが、さらに天然繁殖アサリが増えるにつれて収益性が低下した。これに関連して､欧州での規制が大西洋と地中海で対照的であることがフランスにとって不都合であった。最後に、疾病がアサリに打撃を与えた。1980 年代の終わりにブラウンリング病またそれに関連したウイルス性疾病による大量斃死が問題となった｡今日のフランスでのアサリ生産量は低位水準（2000～3000 トン）であり、ほとんどがアルカション湾とモルビアン湾の天然資源に依存している。アルカション湾では、徹底的な個体群動態に関する研究により、資源量に対する漁業と環境の両方の影響が指摘されている。異なる漁獲圧の低減方法（漁場面積、免許制限、漁期制限）を想定した管理モデルが提唱されており、これらの導入により良好な結果が得られている。しかし、アサリの生産力は低いままである。漁業者から、隔離による遺伝的脆弱性の可能性が指摘されたが､移殖実験により少なくとも成長や肥満度に関しては多様性が保たれていることが示された。アサリの生産性に影響を与える環境要因に関して調査が行われ、寄生虫病、とくにパーキンサスの影響が取りざたされた。さらに 2005 年にアルカション湾で新しくブラウンリング病が発見され、ウイルス感染が疑われているが、まだ明らかとなっていない｡アサリの様々な特性をアルカション湾での調査と文献値とで比較したメタアナリシスにより、フランスの低生産には、疾病や重金属などの複合的要因が考えられた。一方で、クロロフィル濃度（餌の量）は生産性の差の３０％しか説明しなかった。アサリがフランスにおいて重要な漁業対象二枚貝であると同時に、扱いに注意が必要な外来種（欧州規制）であるという対立する要素を持った生物であるという難しい問題と対峙する必要がある。

Manila clam introduction in the Sacca di Goro Lagoon (Northern Italy): ecological and economic implications

Marco Bartoli*

Department of Life Sciences, Parma University, Parma, Italy
*Corresponding author: marco.bartoli@unipr.it

Monitoring activities in the Sacca di Goro Lagoon (Po River Delta, northern Italy) started in 1988, when summer dystrophic events associated to macroalgal bloom and collapse seriously impacted the most relevant local economic activity (clams farming). The manila clam was introduced a few years before and represented an incredible economic success and somehow a paradox, as this species is exotic.

From 1988 to 1994 an intensive, monthly-based monitoring plan of water column physio-chemical parameters was performed, aiming at analyzing salinity, dissolved oxygen, chlorophyll *a* and nutrient distribution in a grid of sampling stations, with respect to hydrodynamics and freshwater inputs.

Later on, monitoring of spatial distribution and biomass evolution of the dominant macroalgal community was started (1990-1996), together with experiments focusing on primary production and nitrogen uptake by macroalgae and on the theoretical role of clams in coupling benthic and pelagic processes. Regular monitoring was afterwards flanked by a more experimental approach aiming at more detailed analyses of how clams affect benthic processes, in particular aerobic and anaerobic respiration, the regulation of denitrification and the analysis of nitrogen cycling within farmed areas (1993-2005).

More recently different European projects focused on socio-economic aspects associated to clams farming. In this review I summarize the main outcomes of these research activities with the overall aim of quantifying at the lagoon scale the role of farmed organisms, as regulators of some of the multiple processes involved in N cycling. Special emphasis is given to how clams affect nitrogen uptake, dissipation and recycling pathways, with respect to external loads and net export to the adjacent coastal area, and to how the introduction of this species altered and improved the local economy.

＜要約＞イタリア北部サッカディゴロ湾のアサリ導入（マルコ・バルトリ）
夏季の赤潮による栄養障害がイタリア北部ポ川の三角州サッカディゴロ湾のアサリ養殖産業に大打撃を与えた 1988 年にアサリのモニタリングが始まった。アサリはこの数年前に移殖され商業的に大成功を収めていたと同時に移入種であることが問題視されていた。1988 年から 1994 年にかけて海水の塩分、溶存酸素、クロロフィル、栄養塩に関する物理化学的モニタリングが海洋の動態と淡水流入との関係を調べるために毎月行われた。1990 年から 1996 年に優占海藻群落の空間分布と生物量の推移に関する調査が行われ、同時に海藻の生産量と栄養塩吸収およびアサリが水柱と底質との関わりに与える影響に関する実験が行われた。その後、1993 年から 2005 年まで、この定期調査は、アサリが底質の嫌気呼吸、好気呼吸、脱窒、窒素循環に与える影響に注目してより詳細に行われた。近年のヨーロッパでは、アサリ養殖の社会経済学的な要因に関するプロジェクトが行われている。本公演では、これらの調査の結果に関して、養殖が湾の窒素循環に与える影響を網羅的かつ定量的に評価することを目的としてまとめる。とくに、アサリが窒素の吸収、消費、再利用の経路に与える影響のうち外部からの負荷と近隣の沿岸域への流出に関して、また、アサリがどのように地域経済を改善したかに関して紹介する。

Status of Manila Clam, *Ruditapes philippinarum*, culture and fisheries on the West coast of North America

Brian C. Kingzett*

Centre for Shellfish Research, Vancouver Island University, Nanaimo, British Columbia, Canada
*Corresponding author: brian.kingzett@viu.ca

The Manila clam *Ruditapes philippinarum* was inadvertently introduced into the Northeast Pacific Coast in the early 1900's with seed shipments of Pacific oysters (*Crassostrea gigas*). The species was first detected on the east coast of Vancouver Island in 1930 and spread rapidly. It is now found from the Central Coast of British Columbia to California. Production from aquaculture and commercial fisheries is approximately 5938 tonnes from British Columbia, Washington and California.

Commercial fisheries for Manila clams began in Washington State in the 1940's and the 1960's in British Columbia. Aquaculture of Manila clams supplemented by hatchery produced seed began in 1972 in Washington State; clam aquaculture was permitted since 1981 in California and 1985 in British Columbia. Washington State is unique in that tidelands may be privately held and this has promoted the development of intertidal shellfish aquaculture. Tidelands are leased from the State and the Provincial governments in California and British Columbia respectively.

Aboriginal groups in British Columbia and Washington State have differential access to wild stocks of Manila clams. Under federal treaties of 1854 and 1855, Washington tribes reserved the right to harvest fish and shellfish at all usual grounds and stations. Recent court decisions have upheld the Puget Sound tribes' rights to up to 50 percent of the sustainable yield from natural shellfish beds which exclude aquaculture sites. In British Columbia there are 597 Aboriginal Commercial Fishery licenses which are held by various First Nations (aboriginal communities) which subsequently distribute them to members. British Columbia First Nations' traditional harvest for Food Social and Ceremonial purposes may occur coast-wide where areas are open for harvest. In both Canada and the USA aboriginal groups who previously had strong cultural ties to shellfish are exploring or becoming increasing involved in clam aquaculture.

Clam aquaculture in North America utilizes conventional techniques included the use of hatchery produced seed to augment natural recruitment and predator netting to protect seeded stocks. Most harvesting is conducted by hand raking although the adaption of mechanical digging machines is being used at certain sites in Washington State and tested in British Columbia.

Social conflicts with clam aquaculture are increasing within the Strait of Georgia (British Columbia) and Puget Sound (Washington State) where upland urbanization is increasing. Controversy includes the concerns over the use of intertidal areas for shellfish cultivation, and licensing of new farms. These include increasing opposition to the use of predation netting which is believed to cause negative effects on wildlife, benthic ecosystems, increasing mechanization in the marine environment and public use and enjoyment of the marine areas. Non-point source pollution in these areas as a result of population increase have resulted in downgraded water quality and increased restrictions on harvesting in certain areas. Depuration of clams harvested from areas with decreased water quality is practiced in British Columbia only. Social conflicts are one of the drivers of research examining ecosystem interactions of clam aquaculture. Recent research projects have examined the ecological impacts that clam aquaculture may have in the marine environment as well as the ecosystem services that it may provide.

＜要約＞北米西海岸のアサリ漁業と養殖（ブライアン・キンゼット）

アサリは 1900 年代にマガキ種苗に混入して北米に移植された。1930 年にバンクーバー島東岸で発見され、その後急速に分布を拡大した。現在ではブリティッシュコロンビア州中部からカリフォルニア州にまで分布している。ブリティッシュコロンビア州、カリフォルニア州、ワシントン州の養殖と漁業生産は合計で 5938 トンである。アサリ漁業はワシントン州で 1940 年代に始まりブリティッシュコロンビア州では 1960 年代に始まった。人工種苗による養殖は 1972 年にワシントン州で始まり、カリフォルニア州では 1981 年、ブリティッシュコロンビア州では 1985 年に養殖が許可された。ワシントン州では個人所有の干潟が多いために養殖の開始が早かった。ブリティッシュコロンビア州とカリフォルニア州の干潟は州からの借地である。ブリティッシュコロンビア州とワシントン州の先住民には特別許可が与えられている。ワシントン州では、1954 年と 1955 年の国との協定によりワシントン州の先住民には全ての場所で魚類と二枚貝類の採捕が認められている。近年の裁判では、ピュージェット湾の、養殖場を除く持続可能な二枚貝資源の 50%を先住民が採補する権利を認めた。ブリティッシュコロンビア州では先住民グループが 597 の先住民漁業許可を有しており、それぞれ所属メンバーに配付している。ブリティッシュコロンビア州の「先住民グループ食用および儀式のための伝統的採捕」は、漁業が許されている全ての地域に広がっている。カナダとアメリカの両方で採貝と文化的に強いつながりも持ってきた先住民グループによる養殖が増えている。北米の二枚貝養殖は、人工種苗を用いた種苗数の補強と被覆網による食害防除を組み合わせた手法で従来行われている。収穫は手堀が主だが、ワシントン州の一部では機械堀りを用いており、ブリティッシュコロンビア州でも試験的に導入されている。ジョージア海峡とピュージェット湾では、無計画な都市化が進んでいる地域で養殖と住民との間に社会葛藤が生じており、潮間帯での二枚貝養殖や新規養殖場の許可が問題となっている。被覆網が自然生物や底生生態系へ与える悪影響、機械化の促進、海の娯楽利用などが問題点となっている。人口増加による非特定汚染源負荷が水質悪化を招き、採貝が禁止されている場所もある。水質が悪化した地域での貝の浄化処理は、ブリティッシュコロンビア州でのみ行われている。社会葛藤のために養殖と環境との関係に関する研究が求められている。近年の研究プロジェクトでは、二枚貝養殖が環境へ与える影響や生態系サービスへの役割が調べられている。

Mapping the stranger: genetic diversity of Manila clam in European coastal lagoons

Stefania Chiesa[1*], Livia Lucentini[2], Rosa Freitas[1], Francesco Nonnis Marzano[3], Silvia Breda[4], Etelvina Figueira[1], Nathalie Caill-Milly[5], Roger J.H. Herbert[6], Amadeu M.V.M. Soares[1] and Emanuele Argese[4].

[1]Department of Biology & CESAM, University of Aveiro, Campus de Santiago, Aveiro, Portugal; [2]Department of Chemistry, Biology and Biotechnologies, University of Perugia, Via Elce di Sotto, Perugia, Italy; [3]Department of Life Sciences, University of Parma, Viale delle Scienze, Parma, Italy; [4]Department of Molecular Sciences and Nanosystems, Ca' Foscari University of Venice, Dorsoduro, Venice, Italy; [5]Ifremer, Laboratory Halieutic Resources from Aquitaine, UFR Sciences and Technics, 1 allée du Parc Montaury, Anglet, France; [6]Centre for Conservation Ecology and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Christchurch House, Fern Barrow, Poole, Dorset, United Kingdom.

[*]Corresponding author: stefania.chiesa@ua.pt

Manila clam *Ruditapes philippinarum* - synonym *Venerupis philippin*arum (Adams and Reeve, 1850) is one of the most successful marine invaders worldwide and represents almost 20% of worldwide mollusc total production. Originally distributed in the Indo-Pacific region, the species was introduced for aquaculture and fisheries in North America, Polynesia, the US Virgin Islands, as well as in Atlantic and Mediterranean coasts of Europe. Due to the commercial value of the species, population genetics and stock composition are of primary importance both in natural and invaded environments. Moreover, molecular genetics can provide valuable data in invasion biology, as, for example, the structure of the exploited populations, the origin of source populations and the routes of invasions. Furthermore, molecular data might be useful for the tracking of seafood products, as requested by the European Commission for European seafood products (see EC laws n°178/2002, 509/2006, 510/2006 and their improvements). Despite all these considerations genetic data are lacking, especially for introduced populations, and should be urgently provided.

In a framework of an European network of researchers involved in Manila clam studies, clams were collected in coastal lagoons and estuaries in both Mediterranean (Northern Adriatic Sea, Italy) and Atlantic (England, France, Spain and Portugal) coasts of Europe. Both mitochondrial and nuclear molecular markers were applied to investigate the genetic structure of populations, specifically by the direct sequencing of *16S* rDNA and *COI* gene fragments and by the genotyping of 7 microsatellite markers previously used in native populations. Results demonstrated the occurrence of multiple haplotypes for both mtDNA markers. Singletons were occurring, but most of the samples were grouped into 1-3 main haplotypes (depending on the mtgene) with high frequencies. Regarding *16S* rDNA, one main haplotype was identified in the European analyzed populations, but few sequences were available in Genbank to make comparisons with native populations. As for *COI*, even if new haplotypes were described for Europe, the main frequent haplotypes identified in European samples were already described in native Japanese and Chinese populations, allowing the identification of source and possible routes of invasion. Concerning the microsatellite data, the genotyping of Manila clam populations showed low genetic diversity among them, considering the Fst values, and a lack of genetic structuring when analysed by Structure software. Both these data suggest the absence of geographic differentiation among the introduced populations of Manila clam, as a result of introgression due to translocations and other human activities. The levels of genetic diversity within introduced populations were comparable to those described from native regions, suggesting that multiple introduction events and mixed source populations can counterbalance the loss of genetic diversity caused by the founder effect. However, it must be remarked that microsatellites showed a strong occurrence of null alleles when tested by Micro-checker, as previously observed in other bivalves, suggesting that the problem is differently distributed among Manila clam populations or specific loci, thus microsatellites should be carefully checked before used in extensive population studies. In the near future, population genetics and stock identification of this species on a large scale should be assessed using new powerful markers such as those obtained by NGS (Next Generation Sequencing) methods. In conclusion, this research represents the very first extensive genetic investigation on Manila clam's European populations, confirming the usefulness of molecular studies for comparing native and introduced populations, defining the levels of genetic diversity that will help to inform management practices.

＜要約＞ ヨーロッパ沿岸のアサリの遺伝的多様性（ステファニア・チエサ）
アサリは世界的に最も成功した移入種のひとつであり、世界の貝類生産の 20%を占める。原産地はインド太平洋で、北米、ポリネシア、バージン諸島、ヨーロッパの大西洋と地中海沿岸に養殖目的で移殖された。商業価値が高いため原産地と移殖先の両方において、集団遺伝と資源組成の解明は重要である。また分子生物学的手法は、移入元、移殖経路、資源構成に関して重要な情報を与え、欧州委員会の欧州水産物のトレーサビリティーにも有用である。しかし、移殖種に関するこのような情報は少なく、緊急に入手すべきである。欧州でのアサリ研究ネットワークでは、地中海沿岸（北アドリア海、イタリア）と大西洋沿岸（イギリス、フランス、スペイン、ポルトガル）でアサリを採集している。ミトコンドリアと染色体マーカー、特に 16SrDNA と COI 遺伝子とマクロサテライトを用いた資源の集団遺伝構造が調査された。これらの結果から、16SrDNA と COI 遺伝子の両方で、遺伝子多型が認められた。単一遺伝子も出現したが、ほとんどは 1 から 3 のハプロタイプにグルーピングされた。16SrDNA に関しては、一つの大きなハプロタイプが認められたが、比較するための原産地からの遺伝子情報が少ない。COI に関しては、欧州で確認された主なハプロタイプが日本や中国から報告されており、移殖経路を知るために利用できる。マイクロサテライトからは、遺伝的多様性が少ないことが示され、人的に移殖されたアサリには地理的隔離が起こっていないことが考えられる。集団内での多様性に関しては、原産地と同様に豊かで、移殖が複数回にわたって行われたこと、複数の原産地が混ざっていることなどが創始者効果を打ち消していると思われる。マイクロサテライトに関しては他の二枚貝同様に無効対立遺伝子の出現が強く、使用には注意が必要である。今後アサリの大規模な集団遺伝と資源判別を次世代シーケンサー等強力なツールを用いて行われるべきである。

An a-priori evaluation model for restoration of fisheries population of the Manila clam and a countermeasure example

Hisami Kuwahara[1*], Ryogen Nambu[2], Junya Higano[3], Satoshi Watanabe[3], Natsuki Hasegawa[3], Yoshimi Fujioka[3], Yuka Ishihi[3], Kazuhiro Hanyu[4], Dai Miyawaki[5], Yoshio Tanaka[6], Masayuki Saito[6], Tomoaki Kudo[7] and Hiroshi Okamoto[8]

[1]Fisheries Research Agency, Yokohama, Kanagawa, Japan; [2]National Research Institute of Fisheries Engineering, Fisheries Research Agency, Kamisu, Ibaraki, Japan; [3]Natioanl Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [4]Mie Prefectural Fisheries Institute, Suzuka, Mie, Japan; [5]Aichi Fisheries Research Institute, Minamichita, Aichi, Japan; [6]Tokyo Kyuei, Kwaguchi, Sitama, Japan; [7]Kaiyo Engineering, Suzuka, Mie, Japan; [8]Nippon Aspi, Kashiwa, Chiba, Japan.

[*]Corresponding author: kwhr@affrc.go.jp

The national production of the Manila clam, *Ruditapes philippinarum*, fishery has been continuously declining since the mid 1980s in Japan. The production of the clam in Ise Bay has also been declining, which was around 10,000 t per year from 1980 to 1995 and then sharply dropped to the current level of less than 5,000 t per year. Fisheries Agency of Japan has been implementing stock enhancement programs, including fisheries management, fishing ground development and reseeding as countermeasures for the production decline. In order to attain successful stock enhancement, it is first of all necessary to elucidate the causes of stock depletion in different stages in life cycle of the clam. Then, one must make a plan to remove or reduce the effects of the elucidated causes taking into account what measures should be applied to what location to what extent. In the present study, we introduce an a-priori evaluation model for stock restoration of the clam, which we developed under a Fisheries Agency project conducted in Ise Bay. An example of a countermeasure is also introduced.

The a-priori evaluation model takes into account the life cycle characteristics of the clam and describes broad area larval network, juvenile mortality to the instability of the sediment due to wave action, fishing mortality, and so on. In addition, the model makes it possible to evaluate the efficacy of nursery ground and spawning ground for stock restoration. The model estimated clam catch well agreed with the actual catch in Ise Bay. It was also predicted that spawning grounds should be developed in inner parts of the bay and nursery grounds in middle parts of the bay or near the bay mouth.

The population level of the clam juvenile is relatively stable in Ise Bay every year; however, they often fail to recruit into marketable size due to adverse effects of wave actions and strong river runoffs. In order to cope with this problem, we have established an effective collection method of juveniles from unfavorable habitats, and we are trying to develop a nursery ground where the collected juveniles can be released and grow up to marketable size with high growth and low mortality. We have succeeded in collecting 3.8 million juveniles by using developed device in only four days and 5 workers at Kushida estuary, Mie prefecture. Nursery grounds were developed by capping on the seabed within a radius of 50m using river sand (median grain size: 0.75mm) and gravel (median grain size: 5mm), respectively, in October 2016 in cooperation with Mie prefectural government. Their attempts were expected efficiency to improve sediment without silt ratio and stability for wave action on seabed, and their efficacy has been investigated.

＜要約＞アサリ資源回復のための事前評価モデルと対策事例の紹介　（桑原久実）

我が国のアサリ漁獲量は、1980年の後半から減少傾向にあり、回復の兆しが見えない状況にある。伊勢湾のアサリ漁獲量も同じ傾向にあり、1980年～1995年は、おおよそ1万トン程度であったが、その後急激に減少し、現在は、半分より少ない状況が続いている。このため、水産庁は、資源管理、漁場造成および種苗放流などの資源回復の取り組みが実施している。このような対策の成果をあげるためには、まず生活史のどの段階で何が制限になり資源が回復しないのか明らかにする必要がある。次に、その制限要因を除去や緩和するために、どのような対策をどの場所でどの規模で実施すべきか明らかにする必要がある。本発表は、筆者らが水産庁事業において伊勢湾のアサリを対象に実施している調査研究の中から、１．アサリ資源回復にための事前評価モデル、２．対策事例について紹介する。<u>１．アサリ資源回復にための事前評価モデル：</u>本モデルは、アサリの生活史を考慮し、広域な幼生ネットワーク、波浪による底質の安定性からみた稚貝の死亡、漁獲の影響などを表現したものとなっている。また、資源回復のために実施する成育場、母貝場などの対策がこのモデルに組み込まれ、これらの効果を事前に評価できるようになっている。母貝場造成は、母貝を増やしてアサリ漁場への幼生の供給量を増加させる場をつくること、成育場造成は、アサリ幼生が着底した後、稚貝の死亡が少なく成長が早く数多くの成貝が成育する場をつくることが目的である。漁獲量の計算結果は、実際の漁獲量と良好な一致を示した。資源の回復には、湾奥部で母貝場、湾中央部～湾口部で成育場の設置が効果的であることが示された。<u>２．具体的な対策事例：</u>　伊勢湾のアサリは、毎年、比較的安定して稚貝は発生するが、その後、波浪や河川出水によって、漁獲サイズまで成長できずに死亡する場合が多い。このため対策としては、放置しておけば死んでしまうアサリ稚貝を効率よく回収すること、回収したアサリ稚貝を放流して成貝まで安定して成長できる成育場をつくることが重要で、これらの技術開発について取り組んでいる。前者については、アサリ稚貝回収装置を開発し、三重県松阪海域で現地実用試験を実施した。2013年5月27日～31日（29日のぞく）の4日間で380万個体の稚貝回収に成功した。後者については、三重県庁の協力を得て、川砂覆砂（シルト含有率の少ない）と砕石覆砂（中央粒径=5mm）共に50m×50mを2014年10月に施工し、成育場としての機能を調査中である。川砂覆砂は、アサリが生息する底質環境の改善、砕石覆砂は波浪に対する底質の安定性の増加しアサリが定着できる環境に改善する効果を期待している。

Poster Presentations

Marine microalga *Rhodomonas* sp. as a novel live food for bivalves

Satoshi Yamamoto[1], Masanori Okauchi[2] and Takao Yoshimatsu[1*]

[1]Graduate School of Bioresources, Mie University, Tsu, Mie, Japan; [2]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan
[*]Corresponding author: takaoyos@bio.mie-u.ac.jp

Recently, many species of bivalves, including the asari clam have become more popular as seafood all-over the world. In spite of the increasing demands of bivalves, the natural resources are becoming scarce year by year. Therefore, stable aquaculture production of bivalves is highly required to substitute the natural resources. Marine microalga *Rhodomonas* sp. is widely distributed in Japan's coastal waters. This species has a high content of highly unsaturated fatty acids (HUFAs) that enhance the survival and growth of bivalves. For this reason, *Rhodomonas* sp. is expected to play an important role as feed for bivalve aquaculture. In this study, suitable conditions for mass culture of *Rhodomonas* sp. in terms of temperature, salinity and light intensity were investigated. A series of rearing experiments was conducted with one of the three factors altered at a time. In temperature, higher cell increase rate was observed between 16 and 24°C, and the maximum cell density (435.8×10^4 cells ml^{-1}) was observed at 24°C. In salinity, higher cell increase rate was observed between 14 and 35 psu, and the maximum cell density (373.9×10^4 cells ml^{-1}) was observed at 21 psu. In light intensity, higher cell increase rate was observed between 35 and 80 μmol m^{-2} s^{-1}, and the maximum cell density (360.1×10^4 cells ml^{-1}) was observed at 80 μmol m^{-2} s^{-1}.

2

Observation and monitoring of asari clam predators in a population-collapsed habitat using Field Server

Naoaki Tezuka[1*], Naoto Kajihara[1], Tokihiro Fukatsu[2], Kazumasa Oguri[3] and Takehisa Yamakita[3]

[1]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Hatsukaichi, Hiroshima, Japan; [2]National Agriculture and Food Research Organization Agricultural Research Center, Tsukuba, Japan; [3]Japan Agency for Marine-Earth Science and Technology, Yokosuka, Kanagawa, Japan
[*]Corresponding author: tezukan9@fra.affrc.go.jp

The Nakatsu tidal flat, located in western Seto Inland Sea, Japan, is known as an asari clam, *Ruditapes philippinarum,* population-collapsed habitat. A recent study observed low clam survival rates, < 15%/yr, from post-settlement until fishable size in this flat. Based on previous netting/caging experiments, predation has been suggested as a major cause of clam mortality. However, we do not have enough information on predator species composition or when and how often the predators appear on the flat. We employed the Field Server, originally developed for agricultural field monitoring equipped with a web-camera and sensors, to observe the asari clam predators on the Nakatsu tidal flat. Field Server observations, comprised web-camera images retrieved every 30 min via the internet, revealed that ducks (*Anas acuta*) often appear on the monitoring site during low tide in winter. These results suggest that predation by ducks may be a significant cause of asari clam mortality during winter in the Nakatsu tidal flat.

This study was supported by the Ministry of Agriculture, Forestry and Fisheries of Japan.

3

Seasonal variations of carbon and nitrogen stable isotope ratios in Manila clam, *Ruditapes philippinarum*, in Wajiro intertidal sand flat, Japan

Masashi Kodama[1*], Machiko Yamada[2], Sayaka Yamazaki[2] and Norihiro Ikeda[2]

[1]Japan International Research Center for Agricultural Sciences, Tsukuba Japan; [2]Fukuoka Women's University, Fukuoka Japan
[*]Corresponding author: mkodama@affrc.go.jp

Seasonal variations of carbon and nitrogen stable isotope ratios, $\delta^{13}C$ and $\delta^{15}N$, in soft body tissue of the Manila clam, *Ruditapes philippinarum*, and their potential food sources were investigated in the Wajiro intertidal sand flat, Fukuoka, Japan. The $\delta^{13}C$ and $\delta^{15}N$ of *R. philippinarum* varied between -15.0‰ and -19.1‰ and between 10.2‰ and 14.0‰, respectively, and they showed a seasonal cycle that was higher from summer to autumn and lower from winter to spring. Carbon to nitrogen (C/N) ratio of the soft body tissue, which is related to glycogen content, also showed a seasonal cycle which became higher in spring and lower in winter, and C/N ratio exhibited a significant negative correlation with $\delta^{13}C$. Comparison of the yearly mean stable isotopic signatures of *R. philippinarum* and those of potential food sources indicates that *R. philippinarum* utilize particulate organic matter in the water column and benthic micro algae as main food sources. These results suggest that the stable isotopic signatures of *R. philippinarum* reflect not only the isotopic signature of assimilated food but also the fluctuation of their body condition related to seasonal storage cycle of glycogen.

4

Predator-prey relationship among 3 species (Manila clam, kuruma prawn, tiger puffer) for stock enhancement program at tidal flat in Japan

Ayumi Yoshida[1], Hikaru Yamane[1], Hideki Yamazaki[2], Atsushi Itou[2], Kazutaka Sakiyama[3] and Yoshitaka Sakakura[1*]

[1]Graduate School of Fisheries Science & Environmental Studies, Nagasaki University, Nagasaki, Japan; [2]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Hiroshima, Japan; [3]Japan Sea National Research Institute, Fisheries Research Agency, Kyoto, Japan

[*]Corresponding author: sakakura@nagasaki-u.ac.jp

Hatchery-reared Manila clam (*Ruditapes philippinarum*), kuruma prawn (*Marsupenaeus japonicus*) and tiger puffer juveniles (*Takifugu rubripe*s) are released into the same tidal flat, however, they can be in predator-prey relationship at the nursery area. If this is the case, we need to reconsider a releasing strategy. However, it is unclear whether predator-prey relationship occurs among these seedlings. We examined Exp.1) size-related predator-prey relationship between kuruma prawn and Manila clam, Exp.2) size-related predator-prey relationship between tiger puffer and kuruma prawn and Exp.3) predator-prey relationships among 3 species. Exp.1) One kuruma prawn and 5 - 10 Manila clams were placed into a container. We identified shell-length of Manila clam ingested by kuruma prawn after collecting Manila clam survivors after 24 hours. Kuruma prawn (23.1 - 141.2 mm BL) preyed on Manila clam (1.6 - 10.4 mm shell length). Size ratio between prey and predator ranged 1.3 - 9.5%. The size of second maxilliped of kuruma prawn coincided with the maximum size of ingested clam. Exp.2) One tiger puffer and 10 kuruma prawns were placed into a tank (43 cm diameter, 30 cm water depth). After 24 hours, we identified BL of kuruma prawn ingested by tiger puffer after collecting survivors of kuruma prawn. Tiger puffer juvenile (25.7 - 100.3 mm SL) preyed on kuruma prawn (18.6 - 52.2 mm BL). Size ratio between prey and predator was 20.7 - 118.4%. Tiger puffer preyed on kuruma prawn 25 individuals at maximum in 24 hours. Exp.3) Tiger puffer, kuruma prawn and 2 size classes of Manila clam (9.0 cm and 3.2 cm shell length) were placed into a tank (167 × 75 cm, 30 cm water depth) and we counted survival individuals twice a day for 5 days. Next, we set 5 partition nets (4 × 4 m) in a mesocosm, and 3 species were transferred into 3 compartments, and 3 species and black seabream (*Acanthopagrus schlegeli*) were transferred into 2 other compartments. We counted survived individuals 5 days later. Kuruma prawn and Manila clam survived at 43% and 69.5% in the experimental tanks, respectively. In the mesocosm, Manila clam (3.2 mm shell length) greatly decreased in the 3 species mixed experiment. On the other hand, kuruma prawn greatly decreased and Manila clam (3.2 mm shell length) survived when 3 species and black seabream coexisted.

5

Correlation between water nutrient level and catch of asari, *Ruditapes philippinarum*, in fishing grounds in Japan

Motoharu Uchida[1*], Mutsumi Tsujino[1], Naoaki Tezuka[1], Toshihiro Shigeta[1], Yoshitake Takada[2], Kentaro Niwa[3] and Yuka Ishihi[4]

[1]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Hatsukaichi, Hiroshima, Japan; [3]Japan Sea National Research Institute, Fisheries Research Agency, Chuo-ku, Niigata, Japan; [3]Yokosuka Station, National Research Institute of Aquaculture, Fisheries Research Agency, Yokosuka, Kanagawa, Japan; [4]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan
[*]Corresponding author: uchida@affrc.go.jp

Catastrophic decrease of catch of the asari clam, *Ruditapes philippinarum,* has been observed for the last three decades in Japan's coastal waters. Many factors including overfishing, disease, habitat loss, competition with invasive species, global warming, and altered trophic cascade are suggested to be involved. However, major factor is still not well characterized to explain the widespread and long-term decrease of the catch. We conducted comprehensive comparison of environmental and biological characteristics among 17 asari fishing grounds. Significant relationship was observed between water nutrient level (i.e., total nitrogen and chlorophyll *a*) and the asari catch. Significant relationship was also observed between the benthos biomass (macrobenthos, meiobentos, and nematodes) and the asari catch. Stable carbon isotope ratio of asari was found to be a useful indicator representing not only the primary production but also the asari catch per unit area.

6

Long-term fluctuations in stock abundance of the asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan

Kazuhiro Hanyu*

Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan
*Corresponding author: hanyuk00@pref.mie.jp

Commercial catch of the asari clam (*Ruditapes philippinarum*) decreased markedly in Japan after the 1980's, and catch in Mie prefecture decreased after the 1990's. In addition, catch in Ise region, southern part of Ise Bay, Mie prefecture, decreased after the 1990's. Mie Prefecture Fisheries Research Institute conducted a survey on the abundance of *R. philippinarum* in a no-fishing area in the southern part of Ise Bay from 1954 to 2000 by direct estimation. The present study identified cohorts of *R. philippinarum* and analyzed long-term fluctuations in the abundance of clams in two size classes (small, shell length of 2 – 7 mm and large, shell length of ≥20 mm) in each cohort in the no-fishing area from 1957 to 2000 based on the data collected by Mie Prefecture Fisheries Research Institute. Forty cohorts were identified in the no-fishing area during the period. The maximum density of small clam in cohorts in the 1950's, 1960's, 1970's, 1980's, 1990's, and 2000's were 6408, 1120, 644, 514, 332 and 331 individuals/m^2, respectively, showing a gradually decreasing decadal trend. The maximum density of large clam in the 1950's, 1960's, 1970's, 1980's, 1990's, and 2000's were 2.8, 5.0, 6.5, 2.7, 3.3 and 2.0 kg/m^2, respectively. The density of large clam decreased markedly after the late 1970's. Therefore, the decreasing trend in the no-fishing area did not synchronize with those in Ise region and Mie prefecture (i.e., 1990's). These results indicate that fishery catch data may not correctly predict actual fluctuation pattern of stock abundance.

7

Effects of small clam abundance, rainfall and sediment on recruitment success of the asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan

Kazuhiro Hanyu*

Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan
*Corresponding author: hanyuk00@pref.mie.jp

The abundance of large (shell length of ≥ 20 mm) asari clam (*Ruditapes philippinarum*) in a no-fishing area in the southern part of Ise Bay, Japan decreased markedly after the 1970's. The present study examined effects of small clam (shell length of 2 – 7 mm) abundance, rainfall pattern and sediment on the recruitment success of the cohorts in the no-fishing area from 1957 to 2000 by using logistic regression analysis. The recruitment success was defined as large clam abundance being higher than the median of the entire set of data (355 individuals/m^2). Small clam abundance and rainfall significantly influenced the recruitment success of the cohort, whereas the effect of sediment (i.e. ratio of very fine silt, silt and clay to total weight) was not significant. The logistic regression analysis revealed that the recruitment success was most strongly affected by the maximum monthly rainfall within three months of the peak occurrence of the small clams. The heavy rainfall and consequent river flood seemed to decrease the recruitment success of the clam to the no-fishing area. However, heavy rainfall and river flood (e.g. maximum monthly precipitation of over 500 mm) were often observed not only after the 1970's but also before the 1970's, and the maximum small clam abundance showed a decreasing decadal trend from the 1960's to 2000's, so the decline in stock abundance after the 1970's was considered to be primarily caused by a decrease in abundance of small clams after the 1960's. The very high abundance of small clams in Shitomo River in the western part of Ise Bay during the 1990's was determined to be a result of abundant larval supply (Miyawaki and Sekiguchi 1999, 2000). Therefore, larval supply from spawning grounds to the western and southern part of Ise Bay may have decreased since the 1960's, and this may have contributed to the decline in stock in the no-fishing area and the entire Ise Bay after the 1970's.

8

Sampling design for abundance estimation of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan

Kazuhiro Hanyu[1*], Hideki Kokubu[1], Naotsugu Hata[1], Tomomi Mizuno[1], Takahiko Inoue[2], Tomoaki Kudo[3], Natsuki Hasegawa[4], Yuka Ishihi[4], Satoshi Watanabe[4], Yoshimi Fujioka[4], Junya Higano[4], Ryogen Nambu[5], and Hisami Kuwahara[6]

[1]Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan; [2]Tokyo Kyuei, Kawaguchi, Saitama, Japan; [3]Kaiyo Engineering, Suzuka, Mie, Japan; [4]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [5]National Research Institute of Fisheries Engineering, Fisheries Research Agency, Kamisu, Ibaraki, Japan; [6]Fisheries Research Agency, Yokohama, Kanagawa, Japan
[*]Corresponding author: hanyuk00@pref.mie.jp

To elucidate the formation process and to develop maintenance techniques of populations of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan, large-scale surveys were undertaken to estimate the abundance of large and small clams in many local populations in the bay. In order to design an adequate sampling technique for estimating the clam abundance on a large scale, we conducted surveys in 7 fishing grounds in Ise Bay and analyzed the data by using a two-stage sampling technique, which is a common method used for estimating the mean, standard deviation and standard error of fishery and ecological data. In the present study, the standard deviations positively correlated with the means both in primary units (sampling points randomly selected in a given fishing ground) and in secondary units (replicates of each sampling point). In addition, the standard deviations in the primary units were larger than those in the secondary units. If we diminish the ratios of the standard error to the mean (i.e., coefficient of variation of the abundance, which is one of the precision index), a sample size of 2 in the secondary units may be sufficient and an as-large-as-possible sample size in the primary units may be adequate. In practice, in the present surveys with a sample size of > 0.18 points per hectare in the primary units and a sample size of 2 replicates per point in secondary units, the coefficient of variations were not small (i.e., 0.2 – 0.8), but we were able to discriminate the fishing grounds and seasons with large abundance from those with small abundance.

This study was supported in part by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

Discovery of a spawning ground of the asari clam (*Ruditapes philippinarum*) in Ise Bay, Japan

Kazuhiro Hanyu[1*], Hideki Kokubu[1], Naotsugu Hata[1], Tomomi Mizuno[1], Takahiko Inoue[2], Yoshio Tanaka[2], Tomoaki Kudo[3], Natsuki Hasegawa[4], Yuka Ishihi[4], Satoshi Watanabe[4], Yoshimi Fujioka[4], Junya Higano[4], Ryogen Nambu[5] and Hisami Kuwahara[6]

[1]Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan; [2]Tokyo Kyuei, Kawaguchi, Saitama, Japan; [3]Kaiyo Engineering, Suzuka, Mie, Japan; [4]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [5]National Research Institute of Fisheries Engineering, Fisheries Research Agency, Kamisu, Ibaraki, Japan; [6]Fisheries Research Agency, Yokohama, Kanagawa, Japan
[*]Corresponding author: hanyuk00@pref.mie.jp

Abundance of small (shell length of 2 - 7 mm) asari clam (*Ruditapes philippinarum*) in the southern part of Ise Bay, Japan, explosively increases once in several years. However, the location of the spawning grounds, as well as the reason for the large fluctuation in abundance is yet to be discovered. In order to locate the spawning grounds and elucidate the mechanisms of the fluctuations, we conducted surveys of the clam abundance in several regions of Ise Bay, Japan from November 2012 to November 2014. We discovered a high abundance of large clams (shell length of ≥ 15 mm) at a water depth of 5 – 10 m in a non-fishing ground, offshore of Suzuka region, northern part of Ise Bay in November 2013. We subsequently observed a high abundance of small clams inshore of Matsusaka region, southern part of Ise Bay in May 2014. Therefore, this ground may be a spawning ground for the populations in the southern part. The occurrence of such ground with high abundance in the northern part was negatively correlated with the occurrence of inflow of hypoxic water masses during the survey period. The inflow of hypoxic water masses to the ground was often observed by Mie Prefecture Fisheries Research Institute from 2002 to 2009, during which the occurrence of inflow negatively correlated with commercial catch of the clam (shell length of ≥ 32 mm) in the southern part two years later. This supports the hypothesis that the ground discovered in the northern region may be a spawning ground for the populations in the southern part, and the occurrence pattern of hypoxic water masses in the northern region may help understand the population dynamics of the clam in the southern part of Ise Bay.

This study was supported in part by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

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Ontogenetic changes in ingestion and digestion abilities against diatom cells of the Manila clam

Shouji Houki[1*], Tomohiko Kawamura[1], Nobuhiro Ogawa[1], Nam-Il Won[2] and Yoshiro Watanabe[1]

[1]Atmosphere and Ocean Research Institute, University of Tokyo, Kashiwa, Chiba, Japan; [2]KWI, Korea Water Resources Corporation, Jeonmindong, Yuseonggu, Daejeon, Korea
[*]Corresponding author: houki@aori.u-tokyo.ac.jp

Planktonic diatoms have long been considered as one of the main food sources for the Manila clam. Whereas, recent studies using stable isotope analyses suggested that benthic diatoms, which often grow at high densities on the tidal flat and are suspended by waves or tidal currents into the water column, were mainly assimilated by the clams. To assimilate the diatom cell contents, clams need to physically break the silica cell walls of diatoms. In the preceding studies on the feeding of abalone species, the physical strength of the cell walls is an important factor determining the dietary value of diatoms for juvenile abalone. Diatom cells cannot be efficiently broken in the abalone gut, and only highly breakable diatoms when they are eaten, such as those with structurally weak cell walls, have high dietary values for abalone. For the Manila clam, detailed mechanisms in the ingestion and digestion of diatoms have never been investigated, and whether the clam can break the diatom cells in the gut is still totally unknown. In the present study, ontogenetic changes in the ingestion and digestion abilities against diatoms of the Manila clam were examined using several diatom species with different hardness and size of cells. Two planktonic and three benthic diatom species were isolated and cultured from a tidal flat in Tokyo Bay, and the physical strength of their cell walls was measured using the PicoIndenter® (Hysitron). Each diatom species was fed to newly settled (200 - 300 µm) and juvenile clams of 3, 5 and 10 mm shell length (SL) respectively, and the cell walls of diatoms in the tissue section of the digestive tract and feces of the clams were observed using a SEM or TEM. To evaluate the dietary value of each diatom species for newly settled juvenile clams, growth rates of juveniles were measured for a month. The percentage of crushed diatom cells in the ingested cells was measured. The physical strength of cell walls was significantly different among the diatom species, for example, that of the pelagic *Chaetoceros* species was low, and that of the benthic *Navicula* species was high. The newly settled juvenile clams did not crush the benthic diatoms with hard cell walls, and did not ingest large diatoms. Consequently, small pelagic diatoms with fragile cell walls were considered to be suitable foods for the newly settled juveniles. The clams of 3 - 5 mm SL partly crushed the benthic diatoms with hard cell walls. Clams of 10 mm SL ingested large diatoms, and crushed over 90% of ingested diatom cells regardless of the physical strength of the cells.

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Reproductive biology of Manila clam *Ruditapes philippinarum* in the coastal waters along eastern Hokkaido, Japan

Yasuko Konda[1*] and Masayoshi Sasaki[2]

[1]Kushiro Fisheries Research Institute, Hokkaido Research Organization; [2]Mariculture Fisheries Research Institute, Hokkaido Research Organization
[*]Corresponding author: konda-yasuko@hro.or.jp

In eastern Hokkaido, there is a potential for a further increase in production of Manila clam, *Ruditapes philippinarum*, but there is insufficient information about the reproductive biology of the clam necessary for appropriate resource management. Therefore the reproductive biology was studied from June to September 2011 in the coastal waters off Takase, Nemuro in eastern Hokkaido. The condition factor increased during June, and the population maturity rate increased from late June to mid-July. Both variables maintained high value until late August to early September and decreased thereafter. Gonads in active stage and/or partially spawned stage of both females and males were histologically observed in late June, and gonads in these stages were continuously observed during the spawning peak from late August to early September. These results indicate that the spawning of the Manila clam starts before late June and peaks in late August in eastern Hokkaido. Shell length at which 50% of individuals mature was estimated to be 23.6 mm by visual observation of the molluscous part, and to be 20.2 mm for females and 16.3 mm for males by histological observation. Female fecundity was estimated by counting the number of eggs under the microscope, and it was positively correlated to the shell length. The fecundity was also positively correlated to the condition factor. The maximum fecundity estimated in this study was 10^7 eggs for a female with the shell length of 50.9 mm. The current findings will be used to implement resource management proposal in eastern Hokkaido.

Food environment for bivalves around *Zostera marina* beds

Hideki Kokubu*, Hirokazu Hanyu and Naotsugu Hata

Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan.
*Corresponding author: kokubh00@pref.mie.jp

Zostera marina is a common seagrass that can be observed in Ise Bay, Japan. *Zostera* beds have important functions as spawning and nursery grounds for valuable marine resources. However, the area of *Zostera* beds in bay area drastically decreased from about 10,000 ha in 1955 to 100 ha in 2000 (one hundredth of 1945) by reclamation for industrial ground and devastation by fisheries trolling device. At the same time the catch of fisheries in Ise Bay has been decreased by about half for 50 years. For this reason, restorations of *Zostera* beds have recently been conducted in coastal environment in Ise Bay. However, few studies have focused on the function of fish-gathering around *Zostera* beds. In this study, we studied the food environment for bivalves around *Zostera* beds at Gotenba and Matsunase beach in Ise Bay. Particulate matter suspended in the water of *Zostera* beds was collected in a sediment trap, and the amount of bivalves was measured in both beaches every season in 2013. Around the margin of the *Zostera* beds, high amount of bivalves were found, and they decreased with the increasing distance from *Zostera* beds. The chlorophyll-*a* level in the particulate matter was the highest at the center of *Zostera* beds and also decreased with the increasing distance from *Zostera* beds. The results suggest that the deposition of particulate matter was increased by buffering of waves and currents by *Zostera* beds. Thus, the food environment for bivalves was improved toward the *Zostera* beds, resulting in increased amount and condition factor of bivalves around the *Zostera* beds.

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Manila clam, *Ruditapes philippinarum*, culture using *Pyropia* spheroplast in closed recirculation system

Novi Arisman*, Mohammad Nakib Dad Khan, A.M. Shahabuddin, Takao Yoshimatsu, Toshiyoshi Araki

Graduate School of Bioresources, Mie University, Tsu, Mie, Japan
*Corresponding author: novi.arisman@gmail.com

The Manila clam, *Ruditapes philippinarum*, were reared in an indoor closed recirculation system, using *Pyropia* spheroplast feed products, freeze dried spheroplast (FDS), spray dried spheroplast (SDS), and enzyme treated nori (ETN). The study aimed to investigate the compatibility of the recirculation system in order to sustain the Manila clam culture using *Pyropia* spheroplast. Water quality parameters and biological performances were evaluated within thirty days. Experimental results showed that there were no statistical differences among all treatments in water quality parameters of ammonia, nitrite, nitrate and phosphorus concentrations. Water quality on each dietary treatment was under the ambient water quality criteria for the Manila clam. On the biological performance, there were no significant different on survival, feed consumption and growth among the Manila clam feed FDS, SDS and ETN. This result shows that the recirculation system is adequate in sustaining the Manila clam culture in indoor tank.

Comparison of types of cages and substrates in suspended culture of the Manila clam, *Ruditapes philippinarum*

Naotsugu Hata[1*], Natsuki Hasegawa[2], Tomomi Mizuno[1], Yoshimi Fujioka[2], Yuka Ishihi[2], Satoshi Watanabe[2], Daisuke Asao[3], Megumu Yamaguchi[4], Hotaka Imai[4], Kazuhide Morita[5] and Junya Higano[2]

[1]Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan; [2]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [3]Uramura Asari Study Group, Uramura Branch Office, Toba and Isobe Fisheries Association, Toba, Mie, Japan; [4]Carshell Co., Ltd., Toba, Mie Japan; [5]Mie Prefectural Government Office in Ise, Ise, Mie, Japan
[*]Corresponding author: hatan00@pref.mie.jp

A series of experiments was conducted to determine optimal rearing conditions for suspended culture of the Manila clam, *Ruditapes philippinarum*, in Ounoura Bay, Mie prefecture, Japan. Comparisons of four types of cages (plastic container, cylindrical net cage, small mesh cage, curing cage used for pearl oyster) and two types of substrates (gravel and pumice) showed that the combination of plastic container with gravel and the combination of cylindrical net cage with pumice had the fastest growth rate of the clam. In terms of handling, however, cylindrical net cage with pumice was considered superior due to its lighter weight. A rearing experiment with different amount of the clam and pumice in the cylindrical net cage (45 cm diameter) showed that installing two mesh bags each containing 0.5 kg of the clam and 5 L of pumice had the best growth rate. The growth of the clam was faster in inner part of the bay than in the bay mouth at the depth of 4 m among the three culturing depths (1 m, 2 m and 4 m), and chlorophyll *a* level seemed to be one of the factors affecting the growth rate. The condition factor (CF, soft tissue weight / shell volume) of the clam cultured in cylindrical net cages ranged from 18.0 to 22.9 from December to March, which increased from 16.5 at the initiation of the experiment in October.

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Effects of feeding regimes on oocyte quality of Manila clam *Ruditapes philippinarum*

Masahiko Awaji*, Toshie Matsumoto and Junya Higano

National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan
*Corresponding author: awajim@affrc.go.jp

In the seed production of Manila clam, *Ruditapes philippinarum*, low survival and retarded growth of the larvae sometimes occur. One of the possible causes for these troubles is the inferior quality of mature oocytes (eggs) spawned to be fertilized. Insufficient feeding of the female brood stocks is expected to deteriorate the oocyte quality, but experimental studies to clarify the effects of feeding on the oocyte quality are scarce. In this study, we examined effects of three different feeding regimes on the quality of mature oocytes in Manila clam. Two hundred Manila clams (mean shell length 30 ± 1.7 mm) in the spawning season (October) were divided into three groups (group H, M and L) and reared in tanks at 22°C for 37 days. They were continuously fed with *Chaetoceros neogracile* at 6.0 x 10^4 cells/ml for the first 7 days. On day 8, the feed concentration was changed into three different levels (H, 8.0; M, 1.6; L, 0.3 x 10^4 cells/ml) and the rearing was continued for the rest of the experiment period. On days 28 and 37, a part of the clams from three groups were induced to spawn by an exposure to increasing water temperature. Females and males that started gamete release were immediately transferred to a small container individually. The eggs collected from each female were inseminated with the pooled sperm obtained from three males, and the total number of eggs from each female was counted. Then a part of the fertilized eggs from each female was transferred to 3 wells of a 24-well microplate at 60-100 eggs/well and to 24 wells of a 48-well microplate at 3-4 eggs/well, and kept at 25°C. At 4-6 hours after the insemination, eggs in the 24-well plate were fixed with 5% formalin/seawater to obtain the fertilization rate. At 22-23 hours after the insemination, larvae in the 48-well plate were fixed in the same way, and the number of veliger larvae was counted to obtain the metamorphosis rate. In addition, hinge length of the fixed larvae was measured. Average feed concentration in the waste water (H, 1.7; M, 0.5; L, 0.17x10^4 cells/ml) confirmed difference in feed availability among the experimental groups. The difference in the feeding regimes, however, did not show significant effects on the number of the spawned eggs, the fertilization rate, the metamorphosis rate, or the hinge length of the veliger larvae. These results indicate that limited feeding (i.e. group L in this study) for about one month during spawning season does not deteriorate the oocyte quality in Manila clam. Large variation of the observed parameters within each group suggests presence of other factors that affect the oocyte quality.

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Low-salinity endurances of the penshell *Atrina* sp. and clam *Trapezium liratum*

Takeo Kurihara[1*], Shoji Nakano[1], Yukihiko Matsuyama[1], Kazumasa Hashimoto[1,] Atsushi Ito[2] and Masaei Kanematsu[2]

[1]Seikai National Fisheries Research Institute, Fisheries Research Agency, Tairamachi, Nagasaki, Japan; [2]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Momoshima, Onomichi, Hiroshima, Japan
[*]Corresponding author: takeo@affrc.go.jp

Species-specific and temperature-dependent endurances against low salinity were revealed for the bivalves *Atrina* sp. and *Trapezium liratum* in a 4-day consecutive laboratory experiment with an orthogonal design: 12 levels of salinity ranging from 0 to 100 psu × 2 bivalve species × 2 temperatures, 12 and 24ºC. [Result 1] *Atrina* sp. was highly vulnerable to low salinities compared to *T. liratum* (mean survival rate in salinities ≤ 20 psu: 11% and 94%, respectively). This conforms to the interspecific difference such that, of the two species, *Atrina* sp. inhabits deeper water where abrupt decrease in salinity is relatively rare. [Result 2] Both species showed decreased survival rate and/or survival time at a higher temperature. This accords with the results of additional experiments that both species increased clearance rate at higher temperatures owing presumably to increased respiration and were likely to expose their fragile soft tissue to more low-salinity water.

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Effect of salinity on the survival, growth and settlement of full-grown larvae of the Manila clam *Ruditapes philippinarum* in rearing tanks

Masaei Kanematsu[1*], Naoaki Tezuka[1], Hideki Yamazaki[1], Atsushi Itou[1], Daisuke Ojima[1] and Atsushi Nishimoto[2]

[1]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Onomichi, Hiroshima, Japan; [2]National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama, Kanagawa, Japan
[*]Corresponding author: kanematu@fra.affrc.go.jp

The effect of salinity on the survival, growth and settlement of full-grown larvae of the Manila clam *Ruditapes philippinarum* was examined in rearing tanks. Salinities were set at 12, 21 and 30 in quadruplicate (total 12 tanks), and we repeated experiments twice. The survival rate, growth in shell length and settlement rate were measured at the end of the experimental period on day of culture 15 (exp.1), and same measurements as experiment 1 were carried out on days of culture 3, 6, 9 and 12 (exp.2). Exp.1: the highest survival rate (89.2%) and the settlement rate (84.9%) were obtained at salinity 12. Higher larval growths were observed at lower salinity. The maximum growth in shell length (32.5 μm/day) were obtained at salinity 12. Exp.2: higher larval settlement rates were obtained at lower salinities through the duration. On day 12, settlement rates were significantly different among the salinity levels. The settlement rates were 67.5%, 16.7% and 4.6% at salinity 12, 21 and 30, respectively. Salinity 12 was demonstrated to be suitable for full-grown larvae rearing of the Manila clam.

Effect of netting on survival of Manila clam *Ruditapes philippinarum* in an artificial tideland in Okayama, Japan

Koichi Izumikawa[1], Tsuyoshi Mototani[1], Fumiyasu Murayama[1], Jiro Sato[1], Atsushi Nishimoto[2,3], Hideki Yazamazaki[2], Kazutaka Sakiyama[2,4] and Atsushi Ito[2*]

[1]Research Institute for Fisheries Science, Okayama Prefectural Technology Center for Agriculture, Forestry, and Fisheries, Setouchi, Okayama, Japan; [2]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Onomichi, Hiroshima, Japan; [3]Present address: National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama, Kanagawa, Japan; [4]Present address: Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Miyazu, Kyoto, Japan.
[*]Corresponding author: itoa@affrc.go.jp

In Yorishima artificial tideland located in Seto Inland Sea, small Manila clams (*Ruditapes philippinarum)* tend to be found in spring but disappear by autumn. Therefore, these clams might suffer high mortality during summer. In this study, the effect of netting of their habitat on survival of Manila clam was examined in Yorishima artificial tideland. Prior to netting treatments, spatial distribution of Manila clam in Yorishima artificial tideland was surveyed with line transect sampling. A core sampler (10 cm in diameter and 8 cm in depth) was used to measure the density of clams. A total of 160 plots were surveyed and clam density ranged from 0 to 4318 clams/m^2. Five plots with high clam densities (2159 to 4318 clams /m^2) were assigned as netting treatment plots and covered with nets (2.5 × 2.5 m, 4 mm mesh openings) in May 2014. Net edges were buried 20 cm deep. A no-net control plot was placed adjacently to each netting treatment plot. In October 2014, the clam densities in three netting treatment plots were higher than those in no-net control plots. These results suggested that the netting treatment was effective to protect the clams from waves and/or predators in Yorishima artificial tideland.

This study was supported by the Ministry of Agriculture, Forestry and Fisheries of Japan.

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Effects of water temperature on the expression of heat shock protein (HSP) 22, 40, and 70 genes in Manila clam (*Ruditapes philippinarum*) larvae

Daisuke Ojima[1*], Masaei Kanematsu[1], Atsushi Nishimoto[1,2], Atsushi Ito[1], Hideki Yamazaki[1], Kimio Asami[1,3] and Kazutaka Sakiyama[1,4]

[1]National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Onomichi, Hiroshima, Japan; [2]Present address: National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama, Kanagawa, Japan; [3]Present address: Seikai National Fisheries Research Institute, Fisheries Research Agency, Ishigaki, Okinawa, Japan; [4]Present address: Japan Sea National Fisheries Research Institute, Fisheries Research Agency, Miyazu, Kyoto, Japan
[*]Corresponding author: ojidai@affrc.go.jp

Biomarkers are powerful tools to understand optimal environmental conditions for the rearing of target animals. The heat shock protein (HSP) family is one of the biomarkers, which responds to various stress factors. We examined *HSP 22-1*, *22-2*, *40*, and *70* expressions in the Manila clam (*Ruditapes philippinarum*) larvae in different thermal conditions. In experiment (Exp.) 1 (short-term stimulation), the fertilized eggs were collected by thermal spawning induction and were hatched at 23°C. D-shaped larvae [1 day after fertilization (DAF)] were transferred into 3 tanks at 20–22, 25, or 30°C, and were sampled after 24 h. In Exp. 2 (long-term stimulation), D-shaped larvae were reared in the same thermal conditions as described for Exp. 1, and sampled at the ambo (5–7 DAF) and full-grown stages (9–12 DAF). All samples were soaked in RNA preservative solution and stored at -80°C. Expressions of *HSP*s were quantified by real-time PCR. In Exp. 1, *HSP 22-1* and *22-2* expressions significantly increased at 25 and 30°C. *HSP 40* expression significantly decreased at 30°C, and *HSP 70* expression showed no significant changes. In Exp. 2, *HSP 22-1* expression in full-grown larvae at 25 and 30°C, and *HSP 22-2* expression in the ambo and full-grown larvae at 25 and 30°C increased significantly. *HSP 40* expression in ambo and full-grown larvae in all temperature regimes decreased significantly, and *HSP 70* expression in ambo larvae in all temperature regimes increased significantly. These results suggest that *HSP 22-1* and *22-2* are suitable biomarkers of thermal stress in Manila clam larvae.

Spatiotemporal population structure of the asari clam *Ruditapes philippinarum* juvenile in Ise Bay inferred by microsatellite marker analysis

Natsumi Sano[1*], Natsuki Hasegawa[2], Kazuhiro Hanyu[3], Dai Miyawaki[4], Akira Komaru[1] and Hisami Kuwahara[5]

[1]Graduate School of Bioresources, Mie University, Tsu, Mie, Japan; [2]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [3]Mie Prefecture Fisheries Research Institute, Suzuka, Mie, Japan; [4]Marine Resources Research Center, Aichi Fisheries Research Institute, Minamichita, Aichi, Japan; [5]Headquarters, Fisheries Research Agency, Yokohama, Kanagawa, Japan.
[*]Corresponding author: natsumi5@bio.mie-u.ac.jp

The production of asari clam *Ruditapes philippinarum* fishery has decreased severely in Ise Bay. To distinguish the spatiotemporal population structure of the clam for the better resource management, we sampled juvenile clams (shell length < 10 mm) in May and November 2013 and May 2014 at 6 to 7 sites in Ise Bay. For microsatellite analysis, total DNA was extracted from 23 to 32 individuals from each sampling location at each sampling period. The microsatellite analysis was performed on six loci (Asari 16, Asari 43, Asari 62, Asari 64, KTp8 and KTp31-1) to reveal the genetical population structure of the clam in Ise Bay. Calculations of molecular variance and pairwise F_{ST} test were done using the software ARLEQUIN. No significant population subdivision was detected between the May 2013 and May 2014 specimens in all of 49 pairs by pairwise F_{ST} test. However, significant pairwise F_{ST} differences were observed in 25 of 42 (0.015 - 0.048, $p < 0.01$) pairs between May 2013 and November 2013 and 26 of 42 (0.015 - 0.055, $p < 0.01$) pairs between November 2013 and May 2014. Assignment analyses using the software STRUCTURE identified $K = 1$ as the most probable number of population. Genetic relationship among the samples collected from different locations at each sampling period was also estimated with the software PHYLIP using the neighbor-joining method. The samples were divided into 2 clusters comprising 4 groups from November 2013 specimens and the other 17 groups while the Bootstrap value was 56. These results suggested the main sources of the juvenile clams may differ between May and November in Ise Bay.

This study was supported in part by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

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Present situation of the asari clam *Ruditapes philippinarum* resources in Mikawa Bay, Japan

Dai Miyawaki[1*], Yoshiki Murauchi[1], Manabu Hibino[2] and Shunji Okamoto[2]

[1]Marine Resources Research Center, Aichi Fisheries Research Institute, Minamichita, Aichi, Japan; [2]Fisheries Administration Division, Department of Agriculture, Forestry and Fisheries, Aichi Governmental Office, Nagoya, Aichi, Japan
[*]Corresponding author: dai_miyawaki@pref.aichi.lg.jp

The annual catch of the asari clam in Aichi prefecture amounted approximately to 16,000 tons, occupying about 70% of the national catch of Japan in 2013. The national catch of Japan has decreased by 80% in 30 years, whereas the catch in Aichi has been maintained above 10,000 tons annually for the last 30 years. We suggest the following factors contribute to the stable asari clam resources in Aichi. (1) Wider tidal flat and shallow areas for the asari clam habitats. Fishing grounds of the asari clam are reported in depths shallower than 10 m close to the flats. (2) Availability of seeds on the tidal flats around river mouths. (3) Transplantation of juvenile clams for stocking from the seed areas into various areas within the bay. (4) Planktonic larvae with high density from spring to early winter. (5) Fishery managements by the local fishery associations, including the transplantation, restriction on the shell size and amount of commercial catches, setting of no-fishing periods and areas, removal of predatory and competitive organisms, tilling of the fishing grounds, and also prohibition of stocking juveniles from the above areas. In particular, factors (1) to (4) are important for the formation, maintenance, and enhancement of the asari clam resources to maintain population network of the asari clam within the bay thorough the life cycle. The asari clam spends most of its lifecycle on tidal flats and the neighboring shallow areas, so we need to keep environmental conditions suitable for the asari clam. Currently, the supply of juvenile clams for the transplantation is dependent upon limited seed areas. We assume that juvenile clam aggregations in the seed areas are generated largely by the characteristic bottom water flow patterns at river mouth areas.

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Seasonal dynamics of the parasitic larvae and free-living adults of the sea spider *Nymphonella tapetis* infecting the asari *Ruditapes philippinarum* in the eastern coast of Chita Peninsula.

Yoshiki Murauchi[1*], Shunji Okamoto[2], Akira Hirai[2], Dai Miyawaki[1], Naoki Yamamoto[3] and Manabu Hibino[2]

[1]Marine Resources Research Center, Aichi Fisheries Research Institute, Minamichita, Aichi, Japan; [2]Fisheries Division, Aichi Prefectural Office, Nagoya, Aichi, Japan; [3]Chita Agriculture, Forestry and Fisheries Office, Handa, Aichi, Japan
[*]Corresponding author: yoshiki_murauchi@pref.aichi.lg.jp

We investigated infection of the Manila clam *Ruditapes philippinarum* with larvae of the sea spider *Nymphonella tapetis* and distribution density of free-living adults of *N. tapetis* on the eastern coast of the Chita Peninsula, Japan. The prevalence of infection and mean abundance of parasitic larvae per host decreased in May or June when many adults were released from the host and peaked in June or July when many young larvae had parasitized *R. philippinarum*. Subsequently, the prevalence of infection and mean abundance of parasitic larvae per host decreased sharply between August and October. After October, only a few larvae of *N. tapetis* invaded *R. philippinarum*. Invasion after October was considered to contribute to the main reproductive cycle that occurred from May onward. The prevalence of infection and mean abundance of parasitic larvae per host fluctuated each year. High survival rate of larvae in summer 2009, which was caused by lower water temperature than in other years, was considered to be the factor of the increase in *N. tapetis* larval numbers from October 2009 to March 2010.

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Shellfish production and new approaches in Turkey

Serpil Serdar*

Faculty of Fisheries, Ege University, Bornova-Izmir, Turkey
*Corresponding author: serpil.serdar@ege.edu.tr, serdarserpil@yahoo.com

Turkey is a large, roughly rectangular peninsular country bridging Europe and Asia. This peninsula is surrounded by the Mediterranean Sea (south part), the Black Sea (north part) and the Aegean Sea (west part). The Sea of Marmara, the Bosphorus and the Dardanelles which are the Turkish Straits demarcate the boundary between Thrace and Anatolia. Turkey has 8333 km of coastline with different ecological properties, so it is one of the best countries having suitable conditions for aquaculture and fisheries. Fishery production consists of marine fisheries (56%), marine and freshwater aquaculture (26%), inland fisheries (11%) and other marine species fisheries (7%) such as crustaceans and molluscs. Aquaculture is a relatively new industry in Turkey. While the total fisheries production has remained stable over the last decade, aquaculture production has been increasing rapidly. Rainbow trout (*Oncorhynchus mykiss*), seabass (*Dicentrarchus labrax*), gilthead seabream, (*Sparus aurata*) and carp (*Cyprinus carpio*) are the main aquacultured fish species. Shellfish production is mainly from capture fisheries except mussel production in a few amounts within a short period. An economical species are *Venus gallina, Ruditapes decussatus, Mytilus galloprovincialis, Ostrea edulis, Venus verrucosa, Donax trunculus,* and *Chlamys glabra*. Moreover *Ruditapes philippinarum* was recorded from Turkish waters in 2003. This species is collected irregularly from Çardak Lagoon-Çanakkale, and the catch statistics is combined with that of *Ruditapes decussatus*. Republic of Turkey Ministry of Agriculture and Rural Affairs arranges some legislations and guidelines for safety and sustainable bivalve production in Turkey. Also regulation and control cover from harvest to process (process techniques), as well as transport and marketing. Totally, 32 regions are monitored regularly for bivalve production, and 8 regions have been investigating in order to start bivalve production in Turkish coastline. There are 12 commercial companies with a depuration plant that export shellfish. Recently, several enterprises have started to culture mussel and also some official permission has been completed for the production especially along the coast of the Marmara Sea. Clam and oyster cultures are expected to be accelerated in Turkey.

Variations of glycogen, protein and lipid contents depend on size of *Ruditapes decussatus* in Bostanli Seashore Izmir Bay, Aegean Sea, Turkey

Serpil Serdar*, Hazal Pulat and Gulcin Gungor

Faculty of Fisheries, Ege University, Bornova-Izmer, Turkey
*Corresponding author: serpil.serdar@ege.edu.tr, serdarserpil@yahoo.com

Biochemical composition of bivalves is important factor for understanding physiological activities such as feeding, growth and reproduction. Energy is stored prior to gametogenesis in the form of glycogen, lipid and protein. Furthermore, environmental factors particularly food availability play a dominant role in the level of biochemical components. The aim of this study was to compare biochemical contents of the Carpet shell clam, *Ruditapes decussatus*, among different sizes. Carpet shell clam samples were collected monthly between May 2013 and April 2014 from Bostanli seashore, Izmir Bay in Turkey. The samples were divided into three size groups; Group I from 20.0 to 25.0 mm, Group II from 30.0 mm to 33.0 mm and Group III from 45.0 mm to 51.0 mm. At the same time, temperature, salinity, chlorophyll a, total particulate matter were measured each month. Temperature ranged between 11.0°C (in December) and 26.0°C (in June and August) during the study. Salinity was measured between 35.68‰ and 37.44‰ in the study area. Total particulate matter and chlorophyll a concentration changed from 1.7 mg/l to 8.4 mg/l and from 0.21 µg/l to 19.68 µg/l, respectively. The maximum glycogen value was determined in all groups in September: 18.36% (Group I), 18.86% (Group II) and 20.81% (Group III). Protein and lipid contents in Group I had the highest value in May 71.66% and 8.21%. The maximum lipid level in Group II and Group III was found to be 6.74% and 5.98% in April. The highest protein content in Group II and Group III was 61.67% in April and 63.01% in March, respectively. The results of this study showed that the size and glycogen ratio increased synchronously, whereas protein and lipid contents decreased to relatively larger extents within sampling period. It is thought that this situation is closely related to not only water temperature and food availability but also reproduction and spawning period.

Use of *Pyropia* spheroplasts as a live food substitute for culturing Manila clam, *Ruditapes philippinarum*

A.M. Shahabuddin[1], Mohammad Nakib Dad Khan[1], Novi Arisman[1], Debasish Saha[1], Takao Yoshimatsu[1*] and Toshiyoshi Araki[2]

[1]Graduate School of Bioresources, Mie University, Tsu, Mie, Japan; [2]Mie University Iga Community-based Research Institute, Iga, Mie, Japan.
[*]Corresponding author: takaoyos@bio.mie-u.ac.jp

An experiment was conducted to assess the *Pyropia* spheroplasts as a live food substitute for culturing Manila clam in indoor flow through system. *Pyropia yezoensis* (red algae) is one of the most important aquaculture species that contains a higher percentage of protein, as well as minerals and vitamins. Spheroplast is a cell from which cell wall is removed by enzymatic treatments. Three different kinds of cell wall degrading enzymes (i.e., agarose, β-1-3 mannan agar and β-1-3 xylanase) were used to breakdown the cell wall. Four diets (PS inclusion levels of 1%, 2% and 3% body weight basis, and live diatom *Chaetoceros calcitrans*) were fed to the three replicate groups of clams for 9 weeks. Significantly higher survival was obtained in the PS diets than the diatom group ($P < 0.05$). Manila clam cultured with PS level 2% diet showed higher carcass weight (soft tissue) compared to the other PS diets and was not different than diatom ($P > 0.05$). Results of the experiment indicated that PS is a good candidate for a substitute to live diatom for culturing Manila clam.

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Main ecological processes for the determination of *Ruditapes philippinarum* population size on an extensive intertidal sandflat in Ariake Sound, Kyushu, Japan

Seiji Takeuchi[1a*] and Akio Tamaki[1]

Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki, Japan;
[a]Present address: Japan Agency for Marine-Earth Science and Technology, Kanagawa, Japan
*Corresponding author: stakeuchi@jamstec.go.jp

For the sustainable use of fishery resources under a proper ecosystem management program, it is necessary to understand the role of main ecological processes in community organization in which the target species population is involved. These include predation, competition, adaptation and limitation by habitat carrying capacity. In the present study, by combining field monitoring with field and laboratory experiments, we attempted to extract several ecological processes that can primarily determine the population size of the Manila clam, *Ruditapes philippinarum*, on an extensive intertidal sandflat in Ariake Sound, western Kyushu. In 2004, the macrobenthic community on the sandflat was mainly composed of five phytoplankton-feeders, two powerful bioturbating shrimps, *Nihonotrypaea japonica* and *Upogebia major*, and three veneroid clams, *Ruditapes philippinarum*, *Mactra veneriformis* and *Meretrix lusoria*. The results of the field survey suggested that the five species competed for space, with superiorities exerted by shrimps to clams through the former's intense bioturbating activities and by one or two species of clams to the other in specific tide zones, and for food to the limit of the carrying capacity provided by the sound. The two shrimp species populations, which predominated over the high- and mid-tide zone, considerably declined almost to the extirpation during 2004 to 2008. This event was followed by the higher recruitment and biomass increment of the three clam species populations than before, most probably owing to competitive release from the shrimp. Thereafter, only *Me. lusoria* and *Ma. veneriformis* were successful in establishing their adult populations in the newly vacated habitat (i.e., with no shrimps). The sediments there were more unstable than that of the low-tide zone where *R. philippinarum* population persisted. We hypothesized that some high burrowing ability of juvenile clams is required for their persistence in the unstable sediment, especially in the wintertime. The results of the field and laboratory experiments suggested that burrowing ability can be one key trait for success/failure in the population establishment of clam species in the newly vacated habitat. These findings could be used as a basis for the ecosystem-based management of Manila clam populations in well-mixed shallow estuaries.

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Age determination of asari *Ruditapes philippinarum* using growth rings on shell surface and section in Mutsu Bay, northern Japan

Daisuke Sugiura[1,2*] and Naohisa Kikuya[2]

[1]Fisheries Research Institute, Aomori Prefectural Industrial Technology Center, Hiranai, Aomori, Japan; [2]The Headquarters, Aomori Prefectural Industrial Technology Center, Kuroishi, Aomori, Japan
*Corresponding author: daisuke_sugiura@aomori-itc.or.jp

Asari has scarcely been caught for commercial aim in Mutsu Bay, northern Japan. Hence, productivity of asari in the bay is largely unknown. In the recent summers, Mutsu Bay has sometimes encountered higher water temperatures than observed in the past. To enhance fisheries production in such environmental condition, the species tolerant for the warm water should be efficiently used. As the first step for stock assessment of asari in Mutsu Bay, we explored the age characteristics on its shell surface and section. The specimens were collected between April 2014 and March 2015 from two locales in Mutsu Bay: Ashizaki Bay and Noheji. First, we observed the shell outer surface and found dark-olive colored bands accompanied by notches. Second, the shell was embedded in epoxy resin, and then sectioned into ca. 300 µm thick along its axis of maximum growth. When the thin section was observed under a light microscope, translucent lines started from umbo and passed through middle layer of shell. Each line finally reached a notch on shell outer surface. Thus, the dark-olive colored bands with notches on shell surface were assumed to be "outer growth rings". However, seasonal changes in the structures of the ventral margins of shell sections suggested that the translucent line was mainly formed in August (summer) and also in December (winter). Some translucent lines on shell sections branched off in middle layer of shell and reached nearby the shell outer surface. We used only the ventral one of those as "inner growth rings" to avoid overestimating age. The mean shell lengths (SLs) at the first, second and third growth rings were estimated to be 10, 22, 30 mm based on the outer growth ring and to be 14, 27, 32 mm based on the inner one, respectively. The observed maximum ages were 7 years old based on the outer growth ring and 5 years old based on the inner one. The results suggest asari reach SL 30 mm (commercial size) in around 3 years in Mutsu Bay, although further evidence should be collected to establish the criterion of outer and inner "growth rings" as age characteristics.

Abundance of planktonic larvae of the asari clam *Ruditapes philippinarum* around fishing grounds in Mikawa Bay

Nobuo Kuroda[1*], Shunji Okamoto[2], Takaharu Matsumura[1] and Masami Hamaguchi[3]

[1]Marine Resources Research Center, Aichi Fisheries Research Institute, Minamichita, Aichi, Japan; [2]Fishery Division, Aichi Prefectural Government, Nagoya, Aichi, Japan; [3]National Research Institute of Fisheries and Environment of Inland Sea, Hatsukaichi, Hiroshima, Japan
[*]Corresponding author: nobuo_kuroda@pref.aichi.lg.jp

Densities of planktonic larvae of the asari clam were monitored by fluorescent antibody method in Mikawa Bay, which has the largest asari clam resource in Japan, from 1999 to 2008. Monitoring stations were set in 4 areas near Isshiki tidal flat, Fukue Bay, Toyogawa river mouth and Yahagi river mouth; the exact location of the stations differed from year to year. Former 2 sites are the main fishing grounds in Mikawa Bay and latter 2 sites are known as high level juvenile occurring areas. The larvae occurred between spring and autumn every year with varying densities from 10^2 to 10^4 individuals per m^3. The occurrence peaks were found in different time from year to year and area to area. The years and areas with apparently two occurrence peaks in spring and autumn were not common. The D-shaped stage larvae occurred at density of 10^3 individuals per m^3 on the average, whereas full grown stage larvae at 10^2, and thus the survival rate during planktonic stage was estimated to be about 10%. Significant correlation in D-shaped larvae densities was not found among stations except for between Isshiki tidal flat and the neighboring Yahagi river mouth. This fact indicates that the spawning periods of the clam are different among different habitats in Mikawa Bay. It was considered that the high level of juvenile occurrence in Toyogawa river mouth is attributable to the high survival rate after settling rather than high larval supply because abundance of the full grown stage larvae in this area were not particularly higher than in other areas.

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Population status of Manila clam, *Ruditapes philippinarum,* and physical environmental conditions in the Natori River estuary in northeastern Japan after the Great Tsunami

Kinuko Ito*, Hirofumi Yoshida, Yoshiaki Morioka, Ayu Katayama, Daisuke Takahashi, Michihiro Shoji, Koichi Sasaki and Satoshi Katayama

Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan
*Corresponding author: itokn@bios.tohoku.ac.jp

The Great East Japan Earthquake and tsunami in March, 2011, resulted in ground subsidence, deposition of rubble and mud. Severe morphological changes occurred in the Natori River estuary in northeastern Japan, damaging bivalve fishing grounds. To clarify the population status of the manila clam, *Ruditapes philippinarum*, and physical environmental conditions, surveys have been proceeding regularly since the tsunami. Population density was estimated based on a quadrat method. Water temperature and salinity were measured in the field whenever sampling was conducted. To obtain accurate diurnal changes in salinity, data-loggers have been installed since 2011. Bottom sediments are also collected and analyzed at regular intervals. Two years after the tsunami, the manila clam population in the Natori River recovered temporarily: many juveniles were collected during the period from autumn, 2012, to the spring of 2013. However, a marked reduction in the population was recorded after heavy rainfall in the summer of 2013. Manila clam recruitment has not yet occurred successfully since the tsunami. It is suggested that low salinity has induced the damage to the manila clam population. The data logger recorded salinity as low as 0 psu during flood tides. Heavy rain and dam discharge to relieve high reservoir levels have induced longer periods of fresh water detention in this part of the river. Topographical changes, especially the formation of sand spit intrusions into the river, have reduced the width of the river mouth. The destruction of a training wall at the river mouth appears to be the cause of sedimentation. In contrast, the abundance of another clam species, *Nuttallia olivacea*, has returned to approximately the same level as before the tsunami. This bivalve is a euryhaline species, which inhabited the same region in the Natori River estuary as *R. philippinarum* before the tsunami.

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Dynamics of the sea spider parasitism on asari in Matsukawaura Lagoon, Fukushima, Japan

Takeshi Tomiyama[1*], Takashi Iwasaki[2], Kunihiro Wakui[2], Katsumasa Yamada[3], Masanori Tamaoki[4] and Katsumi Miyazaki[5]

[1]Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima, Japan; [2]Fukushima Prefectural Fisheries Office, Iwaki, Japan; [3]Seikai National Fisheries Research Institute, Fisheries Research Agency, Taira, Nagasaki, Japan; [4]Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan; [5]Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Shirahama, Wakayama, Japan
[*]Corresponding author: tomiyama@hiroshima-u.ac.jp

The parasitic sea spider *Nymphonella tapetis* is recognized as one of the harmful organisms for Manila clam (asari) *Ruditapes philippinarum* in Japan. In 2007, sudden outbreak of *N. tapatis* occurred and probably caused mass mortality of Manila clam in Tokyo Bay. Then, only three locations in Japan (Tokyo, Aichi, and Fukushima) have been found as the habitat of *N. tapatis* during 2007 - 2011. In Matsukawaura Lagoon, Fukushima, *N. tapatis* was first found in 2009 from wild Manila clam, and thereafter rapidly spread into almost entire lagoon. On 11 March 2011, a huge tsunami caused by the Tohoku-Pacific Earthquake hit the lagoon and greatly altered its environment and landscape. After that incident, the prevalence of *N. tapatis* greatly reduced from 2011 - 2012, and finally *N. tapatis* disappeared from the lagoon in 2013 whereas the Manila clam density has greatly increased. This result implies that *N. tapatis* population may collapse when environmental condition greatly changes despite host clams densely inhabit.

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Life history and distribution of a sea spider, *Nymphonella tapetis*, parasitic on the Manila clam, *Ruditapes philippinarum*, in Tokyo Bay, Japan

Katsumasa Yamada[1*], Katsumi Miyazaki[2], Seinen Chow[3], Toshihiro Yamamoto[4], Takeshi Tomiyama[5], Tomoyoshi Yoshinaga[6], Yoshifumi Miyama[7], Masanori Tamaoki[8] and Mitsuharu Toba[9]

[1]Seikai National Fisheries Research Institute, Fisheries Research Agency, Taira Nagasaki, Japan; [2]Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Shirahama, Wakayama, Japan; [3]National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama, Kanagawa, Japan; [4]National Research Institute of Aquaculture, Fisheries Research Agency, Yokosuka, Kanagawa, Japan; [5]Graduate School of Biosphere Science, Hiroshima University, Kagamiyama, Higashi-Hiroshima, Japan; [6]Graduate School of Agricultural and Life Sciences, University of Tokyo, Bunkyo, Tokyo, Japan; [7]Tokyo Bay Fisheries Laboratory, Chiba Prefectural Fisheries Research Center, Futtsu, Chiba, Japan; [8]National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan; [9]Tokyo University of Marine Science and Technology, Shinagawa, Tokyo, Japan.
[*]Corresponding author: k.yamada@affrc.go.jp

Nymphonella tapetis is a sea spider that is endoparasitic on bivalves including the commercially important Manila clam *Ruditapes philippinarum*. In 2007, it showed a sudden outbreak in a narrow area of *Tokyo Bay* and brought serious fishery damage. Because the occurrence of this parasitic sea spider from the shallow coastal areas of Japan had scarcely been reported, knowledge on its fundamental ecological characteristics was limited. Under such circumstances, we first established the monitoring methods for quantitative capture, length measurement and identification of developmental stages. In order to reveal the life history and distribution pattern, monthly monitoring using these methods was conducted on the Manila clams and parasitic sea spiders in Banzu and Futtsu tidal flat of Tokyo Bay from 2010 to 2011. The seasonal occurrence pattern of the parasitic sea spider showed a clear tendency, i.e., higher in summer and lower in other seasons. Based on the monthly changes in the composition of growth stages of the parasite, its primary reproductive season was considered to be in early summer. On the other hand, low recruitment of larvae in hosts was observed almost throughout the year, except for May. We suggest that this year-round continuous recruitment may contribute to a massive increase of the parasite densities in Tokyo Bay and the other regions (e.g., Mikawa Bay).

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Distribution, morphology and mode of life in the sea spider *Nymphonella* spp. (Arthropoda, Pycnogonida): potential harmful endoparasitism on asari outside Japan

Katsumi Miyazaki[1*], Takeshi Tomiyama[2], Katsumasa Yamada[3] and Masanori Tamaoki[4]

[1]Seto Marine Biological Laboratory, Field Science Education and Research Center, Kyoto University, Japan; [2]Graduate School of Biosphere Science, Hiroshima University, Japan; [3]Seikai National Fisheries Research Institute, Fisheries Research Agency, Japan; [4]Center for Environmental Biology and Ecosystem Studies, National Institute for Environmental Studies, Japan
[*]Corresponding author: miyazaki.katsumi.7e@kyoto-u.ac.jp

Sea spiders or pycnogonids are a distinct monophyletic group of exclusively marine arthropods, consisting of about 1,300 described species (ca. 160 species are recorded from Japan's waters). The taxonomic position of sea spiders has been controversial for a long period, but most recent phylogenetic analyses support their class status in the subphylum Chelicerata. Although the information on their mode of life is still insufficient, some species show a close association with soft-bodied invertebrates such as sponges, cnidarians, annelids and molluscs including apparent cases of parasitism. *Nymphonella* is a curious form of sea spider genus composed of the following three (or two) species: *N. tapetis* Ohshima, 1927 recorded from Japan, *N. lambertensis* Stock, 1959 from southern Africa, and *N. lecalvezi* Guille and Soyer, 1967 (often synonymized to *N. tapetis*) from the Mediterranean and Namibia. The adult morphology is very similar among these species, but the parasitism is known only in Japanese *N. tapetis*, which caused serious fishery damage on asari in recent years. In this species, larvae show endoparasitism on bivalves whereas adults live freely on or just under the surface of sandy bottoms. Although the mode of life in other species is not yet fully studied, the sharing of some distinct apomorphies such as the markedly modified second head appendages (palps) and first walking legs raises a possibility of endoparasitism on bivalves, and thus a potential harmful nature of *Nymphonella* species outside Japan. Accordingly, we would like to point out the possibility of prospective fishery damage on asari and other commercial bivalves by means of the endoparasitic *Nymphonella* larvae especially in the eastern Asian and the Mediterranean coastal countries where the aquaculture of bivalves is highly active.

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Larval and juvenile dynamics of the Manila clam *Ruditapes philippinarum* in northeastern Japan

Hirokazu Abe[1*], Masami Hamaguchi[2], Naoto Kajihara[2], Yuichi Taniai[3], Akio Oshino[3], Takashi Iwasaki[4], Toshihiro Wada[4] and Takashi Kamiyama[1]

[1]Tohoku National Fisheries Research Institute, Fisheries Research Agency, Shiogama, Miyagi, Japan; [2]National Research Institute of Fisheries and Environment of Inland Sea, Hatsukaichi, Hiroshima, Japan; [3]Miyagi Prefecture Fisheries Technology Institute, Ishinomaki, Miyagi, Japan; [4]Soma Branch, Fukushima Prefectural Fisheries Experimental Station, Soma, Fukushima, Japan
[*]Corresponding author: hirokazuabe@affrc.go.jp

Investigations of the larval manila clam *Ruditapes philippinarum* were conducted weekly or fortnightly in Matsushima Bay from 2012 to 2014 and Mangoku-ura Inlet from 2013 to 2014. Monthly field investigations of the juveniles were conducted at 13 stations in Matsukawa-ura Lagoon from June 2011 to December 2014. Results of larval investigations showed considerable short-term variability of larval densities from June to October. The peak of larval density was observed usually from mid-July to early-August and the second peak occasionally occurred in September. The highest larval density observed in the present study was comparable to that of other major clam habitats in Japan. Therefore, reproductive potential of the clam populations in Matsushima Bay and Mangoku-ura Inlet was considered to be high. Recruitment of juvenile clam (>1 mm SL) was observed in Matsukawa-ura Inlet in December 2011 and from August to February in 2012 and 2013. Early juveniles grew slowly with decreasing their density in winter season and their growth rate rose sharply from spring. Mortality of juvenile clam was observed in summer in 2012 and 2013. Although mortality was not observed in 2014, the growth of juveniles was very slow from August to December. The juveniles which settled in previous year reached almost 20 mm in December 2014. Larval supply and the recruitment of the clam seemed to be basically favorable in the clam habitats in northeastern Japan. However, substantial annual variability in juvenile survival in summer would be critical for the dynamics of the clam populations in northeastern Japan.

Condition index and reproduction of the Manila clam *Ruditapes philippinarum* in suspended and bottom culture

Toshie Matsumoto*, Yuka Ishihi, Masahiko Awaji and Junya Higano

National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan
*Corresponding author: mtosie@affrc.go.jp

Bivalve suspended culture offers a number of advantages over bottom culture. In this study, we compared the condition index and gametogenesis of the Manila clam *Ruditapes philippinarum* between suspend and bottom culture. We suspended the clam in a plastic container with gravel at a depth of 2 m from a raft in Gokasho Bay, Mie and cultured the clam in a mesh bag containing gravel placed in a tidal flat adjacent to the raft. Samples from each culture were collected monthly from September 2012 to May 2013. The soft tissues were removed from their shells and fixed for histology in Davidson fluid. The tissue samples were embedded in paraffin wax and 5 µm sections were stained with hematoxylin and eosin. The prepared microscope slides were examined to determine sex and stage of reproductive development. Clam reproductive maturity was categorized into six stages: undifferentiated, early developing, late developing, ripe, spawning, and spent. At the start of the study, in September 2012 when clams reached shell length of 15.9 mm (mean, n = 15), most individuals were in a gonadal resting period. There was an increase of the condition index from February to May 2013 in suspended culture, while the index of clams on bottom culture reached the highest value in March and decreased in April and May 2013. Spawning on bottom culture appears to have occurred by May 2013 as suggested by the presence of spent individuals in the May sample. However, spawning in suspended culture appeared to continue from March to May 2013. By May 2013, the majority of samples was in the ripe and spawning stages. It appears from these observations that the spawning period was extended in suspended culture.

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Report of a novel flagellated parasite in the Manila clam *Ruditapes philippinarum* on the west coast of Korea

Kyung-Il Park*

Department of Aquatic Life Medicine, Kunsan National University, Korea
*Corresponding author: pk1469@hanmail.net

The Manila clam *Ruditapes philippinarum* is endemic to the Yellow Sea of Korea and China and commercially important in this region. Mass mortality of the Manila clam has occurred since mid 1990s, and the protozoan parasite *Perkinsus olseni* has been regarded as one of the causative agents for the mortality due to its rapid infection and severe pathogenicity. In the present study, I report an unidentified protozoan parasite (MPX) in the Manila clam collected from west coast of Korea. Because MPX was morphologically similar to *P. olseni* and co-parasitizing Manila clam with *P. olseni*, MPX was isolated and diagnostic methods for *P. olseni* were applied to distinguish MPX from *P. olseni*. When trophozoite of MPX were incubated in Ray's fluid thioglycollate medium (RFTM) for 2 weeks and staining with Lugol's iodine, cell enlargement and dark brown spheres were observed as like *P. olseni* but lysed with 2M NaOH, suggesting that cell-wall composition of MPX is different from *P. olseni*. The zoospore of MPX observed by SEM averaged 2.38 μm in body length with 6.03 μm of anterior flagellum and 4.37 μm of posterior flagellum. Mastigonemes were found on the anterior flagellum with bilateral array. In PCR diagnosis, MPX was found to spread in the Manila clams collected from the south and west coasts of Korea but not found in the clams from east coast. Clams injected with MPX trophozoites showed 50% of mortality within a week, suggesting that MPX has high pathogenecity in the Manila clam.

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Geographical distribution of mitochondrial COII haplotypes in the brackish water clam, *Corbicula japonica* (Yamato-shijimi), around the Japanese archipelago

Mitsuya Yamada[1*], Ryo Ishibashi[2], Kei Toyoda[1], Kouichi Kawamura[1] and Akira Komaru[1]

[1]Graduate School of Bioresources, Mie University, Tsu, Mie, Japan; [2]Aquatic Life Conservation Research Center, Research Institute of Environment, Agriculture and Fisheries, Neyagawa, Osaka, Japan

[*]Corresponding author: hemigramms26@hotmail.co.jp

Genetic structure of the brackish water clam, *Corbicula japonica*, around the Japanese archipelago and adjacent areas were investigated using partial mitochondrial COII (990 bp) gene sequences. 142 haplotypes were detected among 283 individuals collected from 52 sites (260 individuals) in Japan, one site (eight individuals) in Sakhalin Island and three sites (15 individuals) in South Korea. Both of neighbor-joining and maximum likelihood phylogenetic trees revealed the presence of two major groups (Group I and II). Group I was monophyletic, and comprising five monophyletic lineages (Lineages A-E) of *C. japonica*, while Group II including *Corbicula* sp. was paraphyletic. Mean pairwise genetic distance between Group I and II was 1.9%, and they were conspicuously distinct in shell coloration. Lineages A-C were recognized in Japan and Sakhalin Island, while Lineages D, E and *Corbicula* sp. were found in the Korean peninsula. Lineage A was dominant in Japan. Nested clade analysis (NCA) revealed three sublineages in Lineage A, which were distributed in Pacific Ocean, northern and southern regions of Japan Sea, respectively. In sublineages, ancestral haplotype was commonly observed throughout their entire distribution, with 110 haplotypes unique to single localities in Japan and Sakhalin Island. Mismatch distribution of Lineage A showed the population expansion in the near past. Lineage B was restricted to a single locality on the western coast of Kyushu. Lineage C was mainly observed at the areas between Sendai and Tokyo Bays. Lineages D and E were distributed in the eastern and southern coasts of the Korean peninsula, respectively. Geographical distribution of these lineages appears to have been influenced by major ocean currents around the Japanese archipelago. The ubiquity of ancestral haplotypes and the pattern of mismatch distribution indicate that early stages of range formation of *C. japonica* were characterized by rapid dispersal influenced by ocean currents. In contrast, the presence of many haplotypes specific to single locality suggests that current gene flow among extant populations is rather limited.

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Effect of different artificial sea waters on survival and growth of adult Manila clam, *Ruditapes philippinarum*

Mohammad Nakib Dad Khan, A.M. Shahabuddin, Novi Arisman, Debasish Saha, Toshiyoshi Araki and Takao Yoshimatsu*

Graduate School of Bioresources, Mie University, Tsu, Mie, Japan; [2]Iga Community-based Research Institute, Mie University, Iga, Mie, Japan
*Corresponding author: takaoyos@bio.mie-u.ac.jp

We investigated the influence of water-types (T_1: Sea water, T_2: Distilled water with 3% NaCl, T_3: Deionized water with 3% NaCl, T_4: Deionized water containing 3% natural sea salt and T_5: Tap water mixed with 3% NaCl) on survival of adult Manila clam, *R. philippinarum* in a twenty-day experiment. The adult clams were reared in plastic mesh cages at 6 individuals per cage with three replicates. One diet (PS: *Pyropia* spheroplasts) was fed to the test clams two times a day (10:00 and 15:00) at the rate of 0.25% body weight per day in a closed water system. Survival and growth of shell size were taken into consideration for evaluating dietary performance. A significantly ($P < 0.05$) higher survival was obtained in T_1 followed by T_4, T_5, T_3 and T_2, indicating the superiority of natural sea water over other forms of water in spite of having the same basic nutritional value and exhibiting the deficiencies of certain salts considered to be indispensable for clams` life and survival.

Method for detection of colored sand using image analysis and its application to monitoring sediment movement on a tidal flat

Aiko Kurosawa*, Takumi Okabe, Yusuke Aoki and Shigeru Kato

Toyohashi University of Technology, Toyohashi, Aichi, Japan
*Corresponding author: a-kurosawa@umi.ace.tut.ac.jp

Field investigation of sediment movement in estuarine area is necessary to elucidate not only the sediment dynamics and tidal-flat geomorphology but relation between estuarine morphology and ecological environment. In coastal areas, there are sand transport studies and observations by use of colored and/or fluorescent sand as a tracer. For detecting the colored tracer from sediment samples, human visual inspection has been conventionally used, thereby a huge cost is required to the tracer survey. Frequent and multipoint tracer surveys to understand coastal and estuarine morphodynamics need a low-cost method for detection having satisfactory the accuracy. In this study, we developed a method of colored sand detection using computer-assisted digital image analysis technique and human visual inspection. These digital images of sediment samples were taken by flatbed scanner from papers which were pasted the sand. The colored sand detection was carried out by image analysis. In the final stage of this procedure, check of trimmed-image outputs as the results of image analysis by human visual inspection was employed to eliminate the erroneous detection and to increase its accuracy. This method was time-saving in the tracer detection from a large number of the samples, therefore it was suitable for the multipoint tracer survey. In addition, we applied this method to field observation in a tidal flat of the Toyo River estuarine (Rokujo-gata), Aichi, Japan. This observation was carried out for 9 months, from September 14, 2012 to June 18, 2013 to grasp characteristics of sediment movement in the tidal flat. The sediment samples were collected at 18 points within a spatial coverage extended 1 km alongshore and 800 m cross-shore direction on the tidal flat and once a month during the observation period. The analysis results from the 156 samples using the method indicated that colored sand tracers were detected from samples of the primary observation in the range of up to distance of about 500 m from the location of the tracer injection. After 6 months passed, the tracer had been distributed throughout the survey area. Therefore, the sediment in the tidal flat was estimated to move widely but to remain in the tidal flat.

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Four-year investigations into asari clam and molluscan community in tidal flats after the 2011 Tohoku earthquake

Kenji Okoshi*, Wataru Shinohara and Saki Shimizu

Laboratory of Marine Ecology, Department of Environmental Science, Graduate School of Science, Toho University, Chiba, Japan
*Corresponding author: kenji.okoshi@env.sci.toho-u.ac.jp

The 2011 Tohoku earthquake and tsunami caused a lot of damage to the Tohoku region including marine life and fisheries industry. Bivalves like *Mya arenaria oonogai* normally distributed in deep and asari clam *Ruditapes philippinarum* were dug out to surface by liquefaction and by strong wave action of massive tsunami. The “tsunami break” was observed almost all shell surface of asari clam collected in May 2011. The earthquake caused sudden land subsidence of about 1m in maximum where intertidal zone became subtidal zone. Spat of oyster *Crassostrea gigas* was observed to attach to new hard substances after summer 2011. *Littorina brevicula* and *Batillaria cumingi* were found at shores that were land before the earthquake. What creatures advanced to new environment and disappeared within four years after the tsunami? The result suggests that sort of disturbance was different by place. The species richness decreased significantly at Matsukawa-ura Inlet, Fukushima, in comparison to other two sites investigated in this study. Larval recruitment of asari clam was found every year after the tsunami, and they are surviving and growing. Reed field were rushed by the tsunami, and mollusks that depend on reed also disappeared. Juveniles of deep burrowing bivalve *Macoma contacrata* have not developed after the earthquake. In Matsushima, Miyagi, juveniles and adults of mollusks were found just after the tsunami at the same place. The community structures have not changed since the earthquake. *Musculista senhousia* dominated in the place of subsidence in Mangoku-ura Inlet after the earthquake. From the result of nMDS analysis, molluscan community gradually changed in Mangoku-ura Inlet. Dominant species changed from species live in sandy bottom (asari clam) to those live in muddy bottom (*M. incongrua* and *C. angustus*). We have to clarify not only short-term effect which may explain an initial recovery of intertidal and subtidal animals but also a long-term effect which may explain continuous changes in population with land subsidence.

Effect of alien predator *Euspira fortunei* on the distribution and abundance of asari clam after the 2011 Tohoku earthquake and tsunami

Masahiro Suzuki* and Kenji Okoshi

Laboratory of Marine Ecology, Department of Environmental Science, Graduate School of Science, Toho University, Chiba, Japan
*Corresponding author: 7615001s@nc.toho-u.ac.jp

On 11 March 2011, the great earthquake occurred in Japan. It caused huge damage to the asari clam *Ruditapes philippinarum* along the Pacific coast of northern Japan. The alien naticid gastropod *Euspira fortune*, which was introduced with imported asari clams from China and Korea, prey on asari clam and other mollusks, has been found living after the earthquake. The purpose of this study is to examine the distribution and abundance of asari clam and *E. fortunei* after the earthquake. Quantitative and qualitative samplings were conducted in the tidal flats at Mangoku-ura Inlet and Matsushima Bay in Miyagi Prefecture and Matsukawa-ura Inlet in Fukushima Prefecture from May 2011 to November 2014. The size and weight were measured in all individuals collected. Population density of *E. fortunei* dramatically decreased after the earthquake but many juveniles have collected in 2012. However, it has been decreased since 2013. Over 500 dead shells including about 150 shells with drill hole were collected from a quadrat of Matsukawa-ura Inlet in July 2011. *E. fortunei* ate at least 15 species of mollusks and diets of the snail were heavily dominated by the infaunal bivalves mainly of Asari clam. Therefore, *E. fortunei* has given multiple effects on asari clam stocks after the earthquake.

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Asari clam lived in new intertidal flat after subsidence of the 2011 Tohoku earthquake

Teppei Uemura* and Kenji Okoshi

Laboratory of Marine Ecology, Department of Environmental Science, Graduate School of Science, Toho University, Chiba, Japan
*Corresponding author: 6614005u@nc.toho-u.ac.jp

Mangoku-ura Inlet in Miyagi Prefecture, northern Japan had a great catch of asari clam and had great fields of shell gathering. Our study area "Ohama" in Mangoku-ura Inlet was one of the famous fields of shell gathering. The earthquake on March 11, 2011 caused land subsidence, and the land has sunk by about 78 cm at this location where the intertidal zone became subtidal zone. After that event, the mussel *Musculista senhousia* has increased dramatically and formed mats over the sediment surface. The presence of these mats has altered the benthic habitat. The mats have led to siltation of the sediment and have resulted in hypoxia of water just above the seabed. The mussel mats have reduced the densities of asari clam, which suggested that Ohama has become unsuitable for habitation of asari clam. Parts of the land became intertidal zone. In May 2013, habitat of asari clam has been confirmed in the new tidal flats where the land sank after the earthquake. It indicated asari clam changed their habitat to the new tidal flats. This examination proceeded by quadrat method to find creatures and measured distance from the shoreline. Finally, what creatures advanced to the new environment, focusing on asari clams inhabit conditions and growth condition were discussed. It was found asari clam lives in landside up to 8 m from the shoreline at greater ebb. Although the sexual maturation and reproduction was also observed, almost all the asari clams in new tidal flat had rounded shell morphology, indicating that growth was bad. In addition, the spats (shell length: 1 - 2 mm) have been collected through the year and few middle-sized individuals (shell length: 10 - 20 mm). The recruitment and growth of asari clams were not constant by 2014. These suggested the new environment was not suitable for clam growth and survival. Since 2015, the number of spats and middle-sized individuals has increased. It suggested this new tidal flat is changing continuously.

Development of in situ measurement of clearance rate of the Manila clam under suspended culture conditions

Satoshi Watanabe*, Natsuki Hasegawa and Junya Higano

National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan
*Corresponding author: swat@affrc.go.jp

Due to drastically declining production of the Manila clam (*Ruditapes philippinarum*) fishery caused by environmental degradation and other anthropogenic effects, suspended culture of the Manila clam has recently been receiving much attention in Japan. Research institutes are trying to improve the efficiency of suspended culture to promote commercialization of this technique. Bivalve suspended cultures are commonly operated on sessile or epibenthic species, such as oysters and scallops that do not require sediments. In the case of the bottom burrowing Manila clam, however, the suspended culture is done in a container (e.g. plastic container or net cage) with substrate (e.g. sand, gravel or pumice) hanging in the water column. Manila clams in suspended culture thus face environmental conditions quite different from those in their natural habitat. In this study, a method for in situ water clearance measurement was developed for monitoring physiological conditions of the Manila clam under suspended culture conditions. The device consisted of a semi-translucent plastic chamber (55 L capacity) with two smaller plastic boxes attached to both lateral sides. The smaller boxes were equipped with optical chlorophyll/turbidity loggers (Compact-CLW, JFE Advantech Co. Ltd.), and the inside of each box was painted a non-reflective matte black. One of the boxes had a submersible water pump, which pumped water out at a rate of 240 L/hr to the water surface via a tube, drawing in water from the other smaller box though the main chamber containing Manila clams. Preliminary test runs with (600 pieces of Manila clams, approx. 3 cm shell length) and without Mania clams in the chamber were carried out at a depth of 2 m, suspended from a pier at the National Research Institute of Aquaculture in Mie, Japan. Inflow and outflow chlorophyll *a* (chl *a*) concentrations were simultaneously measured every 15 minutes (i.e. complete water replacement in the chamber occurred in about 15 minutes). Water clearance rate (CR) was calculated as CR = F [(C_i - C_0) / C_0], where F is flow rate, C_i is inflow chl *a* concentration and C_o is outflow chl *a* concentration. The mean C_i and C_o did not differ significantly without Manila clams ($p = 0.24$). The mean C_o (2.13 μg/L) was significantly lower than the mean C_i (3.04 μg/L) with Manila clams ($p < 0.001$). With Manila clams, the CR greatly fluctuated ranging from 0 to 616 mL/hr/ind, and the mean CR (178.1 ± 103.7 mL/hr/ind) was 10 to 20 times lower than previously reported values measured for similar sized clams at similar temperatures in the laboratory (i.e. in beakers). The CR changed gradually, and it seemed all the clams in the chamber were acting similarly. However, the pattern in the CR change was irregular and did not correlate with tidal height, water temperature or time of the day. The CR was positively correlated with C_i ($p < 0.001$).

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Growth and food environment of the Manila clam in suspended culture

Yuka Ishihi*, Satoshi Watanabe, Toshie Matsumoto and Junya Higano

National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan
*Corresponding author: ishihi@fra.affrc.go.jp

Suspended culture of the Manila clam (*Ruditapes philippinarum*) has recently been developed due to drastic decline of the fishery production. In this study, we compered the growth of the clam between suspend culture in a plastic container in the water column and bottom culture in a mesh bag in a tidal flat. We also determined the relationship between the growth of the clam and food environment by analyzing chlorophyll *a* level and carbon and nitrogen stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of the clam and particulate organic matter in water column and sediment. We conducted an experimental suspended culture of the clam at rafts in Gokasho Bay and Ounoura Bay in Mie from September 2012 to May 2013 and from March to September 2013. We suspended the clam in a plastic container with gravel at a depth of 2 m from the rafts, and cultured the clam in a mesh bag containing gravel placed in a tidal flat adjacent to the rafts. We measured the size, total wet weight and stable isotope ratios of the clam every one to four weeks, and also monitored the chlorophyll *a* level continuously using a logger. The fastest growth was observed in the suspended culture in Gokasho Bay, where the clam grew from 12.8 mm shell length (0.3 g) to 34.5 mm (8.2 g) in 8 months. The growth was faster in suspended culture than bottom culture by 2 to 4 times in shell length and 2 to 6 times in wet weight. The maximum mean growth rate was 0.26 mm/day (0.055 g/day) in suspended culture, and there was a significant positive correlation between the mean growth rate and chlorophyll *a* level. The stable isotopic signature indicated that the clam with faster growth mainly assimilated organic matter in the water column rather than those in the sediment.

Relationship between abundance of planktonic larvae and benthic juveniles of asari *Ruditapes philippinarum* in eastern Hokkaido, Japan

Natsuki Hasegawa[1*], Toshihiro Onituska[2], Sayaka Ito[2] and Hiroya Abe[3]

[1]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [2]Hokkaido National Fisheries Research Institute, Fisheries Research Agency, Kushiro, Hokkaido, Japan; [3]Graduate School of Environmental Science, Hokkaido University, Sapporo, Hokkaido, Japan
[*]Corresponding author: hasena@fra.affrc.go.jp

The larval recruitment process has been focused as a key aspect for understanding the population dynamics of asari and improving stock enhancement strategies of this symbolic fishery species in coastal areas of Japan. In southern parts of Japan, such as Honshu and Kyusyu islands, asari usually spawn intermittently from spring to autumn, and thus multiple cohorts occur in the same year. In eastern Hokkaido contrastingly, asari show a single synchronized spawning event during summer because of a slow maturation process associated with low-temperature water from the cold Oyashio current. Additionally, they are distributed in semi-closed estuaries with minor larval exchanges among one another. Therefore, the population structures are simple, and this makes it suitable to trace each population and evaluate the dynamics during the early life stages including planktonic larvae and benthic juveniles. A research (2012 - 2015) on the planktonic larvae and benthic juveniles of asari in Akkeshi area consisting of estuary and bay, which is one of the main asari production area in eastern Hokkaido, showed that abundance and the appearance frequency of planktonic larvae did not have a clear relationship with the magnitude of recruitment of benthic juveniles to the intertidal fishing grounds in the estuary. Moreover, high level of settlement of benthic juveniles was observed at the fishing grounds only in 2012, wherein planktonic larvae were collected only once during the 7 sampling days only in the bay area. These results showed that a large number of the planktonic larvae in the estuary drifted to the bay and then succeeded in returning to the benthic habitats in the estuary only in 2012. This may explain the highly variable year class strength of asari in eastern Hokkaido.

This study was supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan (Coastal Ecosystem Complex Project).

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Energy budget and neuroenzymatic activity in asari, *Ruditapes philippinarum*, following short-term exposure to the toxic alga, *Heterocapsa circularisquama*: implications for reproductive success

Leila Basti[1*], Satoshi Nagai[2], Susumu Segawa[3] and Yuji Tanaka[1]
[1]Tokyo University of Marine Science and Technology, Minato, Tokyo, Japan; [2]National Research Institute of Fisheries Science, Fisheries Research Agency, Yokohama, Kanagawa, Japan; [3]Faculty of Bio-Industry, Tokyo University of Agriculture, Abashiri, Hokkaido, Japan
[*]Corresponding author: lbasti1@kaiyodai.ac.jp

The Manila (asari) clam, *Ruditapes philippinarum*, is an important fishery resource in Japan; however, several factors have contributed to the decline of its commercial fishery production over the past two decades. Reclamation of tidal flats, overfishing, hypoxic coastal waters and diminished food supply as a result of eutrophication, heavy metal pollution, outbreaks of flood events, strong winds, the introduction of alien species including predators, and inadequate fishery management practices have all been identified as playing a major or a synergistic role in the decrease of Manila clam stocks. Pathogens, mainly bacterial and protozoans are also responsible for mass mortalities or the reduction of fitness of Manila clams so should do harmful algal blooms (HAB). Among the HAB occurring in Japan, *Heterocapsa circularisquama* is by far the most toxic to Manila clams, and to shellfish in general. Mass mortalities caused by this HAB are well-documented, recurrent, and geographically-expanding events around Japan. In spite of the relative decrease in the frequency and intensity of its blooms after 2000, *H. circularisquama* is highly lethal and/or detrimental even at low cell-density, and has been going through a revival period since 2008. The mortalities of Manila clams in fishing grounds have been reported when *H. circularisquama* blooms at several hundred cells per ml. Nonetheless, the impacts of background exposures to *H. circularisquama* on Manila clams, and bivalves in general, have so far been overlooked. Therefore, we assessed the energy budget and neuroenzymatic activity of adult Manila clams following short-term exposures to several realistic cell densities of *H. circularisquama*, at two incubation temperatures which are lower than the ones at which *H. circularisquama* causes the reported mortality in Asari. The Energy budget (J h^{-1} g^{-1}) was significantly affected following a 2-h exposure to 50 and 5 cells ml^{-1} *H. circularisquama* at 15 and 20°C, respectively. The Acetylcholinesterase activity (nmol min^{-1} mg total $protein^{-1}$) was also decreased following 3 – 48 h of exposure to 5 cells ml^{-1}. The results of this study demonstrate that even short exposures to low cell densities of *H. circularisquama* could affect the key neuroenzymatic activity acetylcholine and the energetic performance of Manila clams without necessarily causing mortalities. These findings imply that *H. circularisquama* may play a role not only in the survivability of Manila clam fishing grounds but also on the reproductive success of Manila clam in Japan. The management and restoration of sustainable fishing grounds of Manila clam would reply on proper understanding of the environmental factors that contribute the most to stock depletion, which should include HAB as background negative effectors.

Evaluating the growth and mortality of transplanted asari *Ruditapes philippinarum* juveniles in the Matsusaka region, Mie prefecture, Japan

Ryogen Nambu[1*], Yoshimi Fujioka[2], Natsuki Hasegawa[2], Tomoaki Kudo[3] and Hisami Kuwahara[1]

[1]National Research Institute of Fisheries Engineering, Fisheries Research Agency, Kamisu, Ibaraki, Japan; [2]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [3]Kaiyo Engineering Co., Ltd., Tokyo, Japan
[*]Corresponding author: cjapo@affrc.go.jp

Asari juveniles settle in estuary and often die out due to freshwater discharges, resulting in failed recruitment into commercial size (> 20 mm) in the Matsunase area Mie, Japan. In this study, we examined the growth and mortality of asari juveniles transplanted to two subtidal areas (Matsunase area: M1, Miwatari area: M2) with environments different from those in the nearby estuary areas to simultaneously monitor physical environments (i.e., wave-current flow, salinity, temperature, dissolved oxygen and turbidity) above the bottom sediment. Initial density of transplanted asari juveniles was approximately 1200 ind/m^2 in both areas on 31 May 2013. The density of transplanted asari juveniles at M1 drastically decreased during the first two months to 35 ind/m^2 on 8 July and then to 2 ind/m^2 on 19 August 2013. In contrast, the density at M2 was 534 ind/m^2 on 8 July, 262 ind/m^2 on 10 September and 120 ind/m^2 on 2 October 2013. We also examined the characteristics of mortality of transplanted asari juveniles in relation to physical environments, using generalized liner model (GLM). A best-fitted model selected the maximum wave-current velocity during the survey period as the positive explanatory variable for the juvenile growth although the velocity at M1 tended to be higher than at M2 that had higher juvenile survival than at M1. Thus, growth and survival are in trade-off relationship, and in order to maximize the efficiency of transplantation, a habitat with lower wave current velocity such as M2 should be selected.

This study was supported in part by the Ministry of Agriculture, Forestry, and Fisheries of Japan.

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Risk assessment on the mortality of asari clam under oxygen deficiency

Junya Higano[1*] and Tomomi Mizuno[2]
[1]National Research Institute of Aquaculture, Fisheries Research Agency, Minamiise, Mie, Japan; [2]Mie Prefecture Fisheries Research Institute, Shima, Mie, Japan
[*]Corresponding author: higa@affrc.go.jp

Oxygen deficiency has often occurred in major habitats of asari clam in Japan, such as Tokyo Bay, Mikawa Bay, Ise Bay, and Isahaya Bay. It has been considered that prolonged oxygen deficiency brought about the mass mortality of the clam. On the other hands, most bivalves can switch to anaerobiosis to maintain their metabolism and survive several days in anoxic conditions. Asari clam also has tolerance for anoxic and hypoxic conditions through the anaerobic biosis. Since the impact of anoxia on the clam population is poorly understood, it is necessary to quantitatively assess the risk of oxygen deficiency for survival of the clam. In order to evaluate the effect of low oxygen concentration on the survival of asari clam, exposure experiments at different oxygen concentrations (1.0 mg/l, 0.5 mg/l, 0 mg/l and 0mg/l with 10 mg-S/l sodium sulfide) were conducted in 700 ml volume containers. Ten clams were placed in each container and water temperature was set at 20, 25 and 30°C. The results showed that mortality was higher at lower oxygen levels and with the presence of sulfide. We obtained the following linear regression curves to express the median lethal time (LT_{50}, h) as a function of water temperature (*WT*, °C). Oxygen concentration of 1.0 mg/l: $LT_{50} = -17.7\ WT + 582.7$ ($R^2 = 1$), 0.5 mg/l: $LT_{50} = -15.7\ WT + 523.9$ ($R^2 = 1$), Anoxic: $LT_{50} = -9.4\ WT + 318.4$ ($R^2 = 0.98$), Anoxic + Na_2S: $LT_{50} = -3.2\ WT + 129.2$ ($R^2 = 0.72$). Particularly, the mortality rate (*Mo*, %) in the anoxic condition was expressed as a multiple regression equation with *WT* and duration of anoxia (*D*). $Mo = 26.3\ WT + 2.6\ D - 828.6$. We further conducted experiments for evaluating the long-term influence of experienced anoxia on the survival of the clams. Asari clams were exposed to anoxic water for 24, 48, and 72 h at 20 and 25°C. After the exposure, the clams were placed in oxygen-saturated water, and the survival of the clams was subsequently monitored for two weeks. The mortality rate in two weeks (*Mo'*) was expressed as the following equation. $Mo' = 6.0\ WT + 1.9\ D - 167.6$. The results implied that anoxia caused not only acute but more seriously prolonged mortality. The equations would be useful for predicting the mortality in combination with the in situ measurement of oxygen deficiency.