16. Development of Onshore Fish Aquaculture Technology which is Expected the Load Mitigation to Coastal Environment

Syun-ichirou Oshima, Mao Houkin and Kentaro Hashiguchi

1. Preface

At present, the global population is growing exponentially. In addition, the Earth is a closed system with limited resources. Therefore, if the population continues to grow, it is easy to predict that the food problem will become more serious than ever before. We believe that one of the keys to solving this growing food problem is land-based fish farming. As food problems are also said to be water problems, the need for food production from marine living resources is expected to increase in the future.

However, climate change and other factors have led to a rise in sea temperatures, and the pollution of the oceans by chemical substances and man-made products (e.g. plastics) is becoming more serious. If these conditions continue, marine aquaculture will be forced into a difficult situation year after year. The ultimate goal of our research is the conservation of the marine environment, and we have been focusing on land-based aquaculture as a means to achieve this goal. In this section, we will discuss new methods for the treatment and prevention of infectious diseases, which are inevitably a problem in fish farming, including 1) research using nanoparticles, 2) research on fish stress, which is closely related to infectious diseases and fish growth, and 3) research on non-invasive methods of monitoring fish stress in real time. stress in fish, which is also closely related to infectious diseases and fish growth performance, and 3) a system for real-time, non-invasive detection of fish stress. In order to realize the establishment of a sustainable fish farming system, we believe that it is wise to learn from the saying “the future is already here” and to share the problems among many industries and bring together wisdom to solve various problems in the future.

2. The Food Situation as the Population Grows

The world’s population is currently estimated to be about 7.9 billion, which means that in 37 days, the Earth’s population is growing by about 8.6 million people, the equivalent of the size of the city of New York. At this rate of growth, the population could exceed 10 billion by 2050 (Figure 1). In this context, it is predicted that in the near future the global food problem will become more acute than ever before.

It is estimated that the demand for food in the world as a whole will be 1.7 times the current level in 2050. In developing countries where the population growth rate is high, it is predicted to be 2.7 times higher (Figure 2). Projections for food demand by category are also shown in Figure 3. Here, projections are given for livestock products, sugar crops, oilseeds and cereals for the period 2010-2050. Of particular interest is the growth in livestock products. Livestock products are projected to grow 1. 785 times faster than other foods compared to 2010. In other words, the demand for animal protein is shown to be particularly high. As the demand for food increases year by year in line with population growth, it is easy to predict that a stable supply of food will be required in the future.

Then, when we consider what the essence of the food problem is, we can see that it is also a problem of “water”. It is estimated that the water resources of the earth are about 1.4 billion cubic kilometres, of which 97. 5% is seawater. Freshwater makes up only 2.5% of the remainder, two-thirds of which is locked up as permafrost in the Arctic and Antarctic.

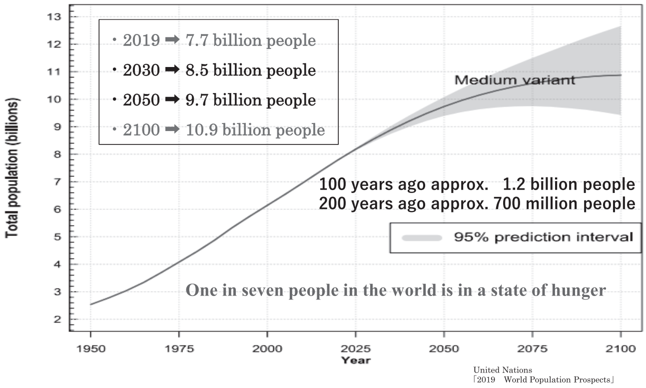
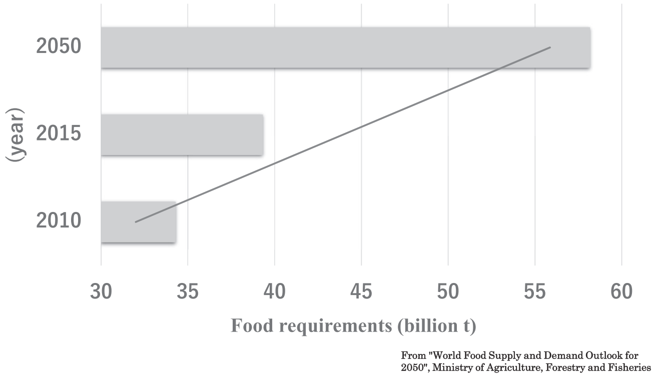


Figure 1 World population trends and projections



§ : 1.7 times the current level by 2050
§ : 2.7 times more in developing countries with high population growth rates

Figure 2 Food demand forecast

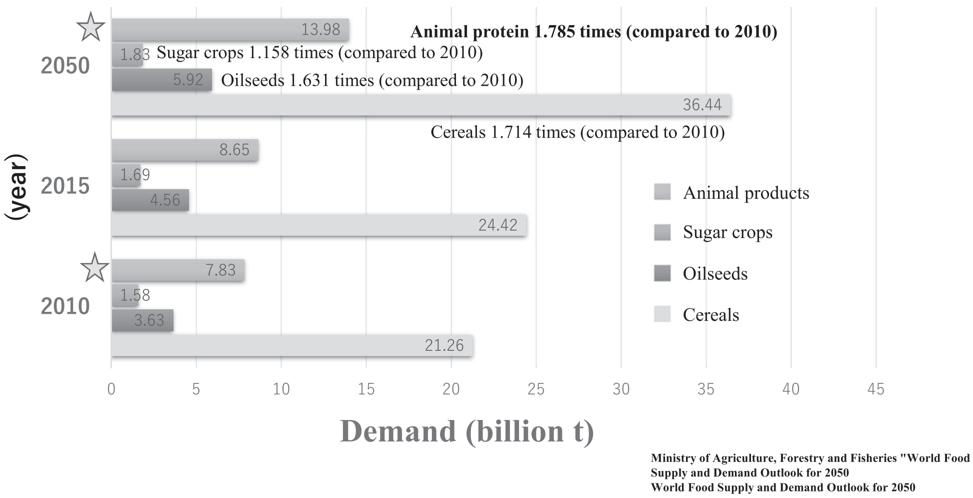


Figure 3 Forecast of food demand by category

The current situation is such that we are forced to produce food with limited freshwater resources. Professor Tony Allan of the University of London has proposed the concept of virtual water. It shows how much water would be needed to produce food in Japan if we did not import it. For example, it takes 3,600 litres of water to produce 1 kg of rice and 2 tons of water to produce 1 kg of soybeans. For meat, it takes about 4t of water for poultry, 6t for pork and 20t for beef to produce 1kg of each. As the population continues to grow, the question of how to recycle the limited water resources available in the future, or how to secure them, is a near-term issue.

3. The Need for Increased Aquaculture

As mentioned above, there is currently a limit to food production using the limited fresh water available on the planet. About 70% of the Earth's surface is covered by oceans, and it is easy to foresee that in the future we will need to produce food using marine living resources that are not associated with water (freshwater). In fact, as shown in Figure 4, fisheries and aquaculture production has increased more than fivefold since 1960, and this trend is likely to continue. However, most of the fish we farm are carnivorous, meaning that they grow by feeding on fish. Looking at the production to feed conversion efficiency of the animal protein we currently eat, it appears that fish require approximately 2kg of feed to gain 1kg of body weight, whereas cattle require more than 10kg of feed (1:Fisheries White Paper 2013). Thus, as an industrial animal, fish have a high advantage over other animals in feed conversion efficiency. However, there are many problems associated with marine resources, including problems with food resources and infectious diseases of fish. There are also serious problems associated with the use of the oceans, such as marine pollution and microplastics, which have become a serious problem in recent years. One possible solution to these problems could be to separate land-based aquaculture from the natural world.

As mentioned earlier, the world's fisheries and aquaculture production is undoubtedly increasing year by year, but most of the fish we eat are carnivorous. In recent years we have seen many articles about the poor catches of saury and sardines. The main ingredient used in fish feed is fishmeal, made from fish resources. Of course, fishmeal is not only used for fish feed, it is also used in other fields, and the price of fishmeal tends to

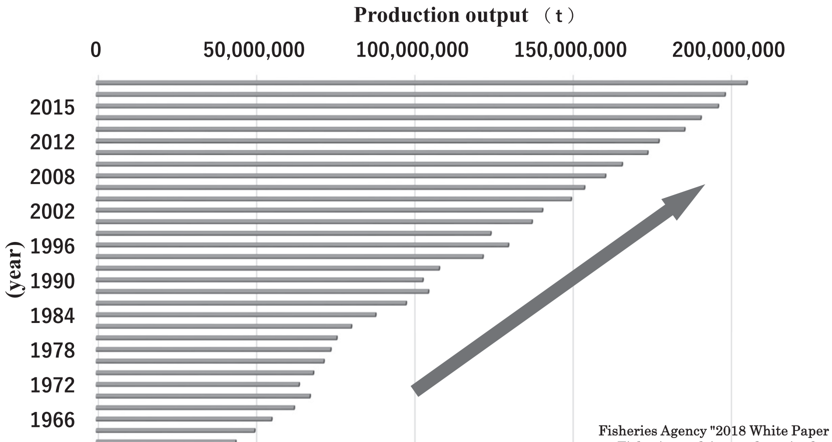


Figure 4 World fisheries and aquaculture production

rise year by year as production declines. The Fisheries Agency's "2017 White Paper on Fisheries and Aquaculture Production in the World The State of the World's Fishery Resources" shows that marine living resources are clearly on the decline, as shown by the "Trends in the Percentage of Resources at Biologically Sustainable Levels (Figure 5)", and that there is a limit to how much fishmeal can continue to be used as a feed resource in the future. There is a limit to how much fishmeal can be used as a feed resource in the future. There is another problem in aquaculture. Infectious diseases are one of them. Infectious diseases always occur in fish farming. Infectious diseases can be treated or prevented, with chemotherapeutic agents (e.g. antibiotics) being used for treatment. However, the extensive use of chemotherapeutic agents has led to the emergence of resistant strains of bacteria in the natural environment, a problem that is becoming more serious every year. The emergence of multidrug-resistant bacteria, which are resistant to multiple chemotherapeutic agents, has also been confirmed. On the other hand, various vaccines have been developed for the prevention of disease, but they are not effective if the pathogen changes. Vaccine prophylaxis is therefore effective against certain infectious diseases, but it is not a panacea.

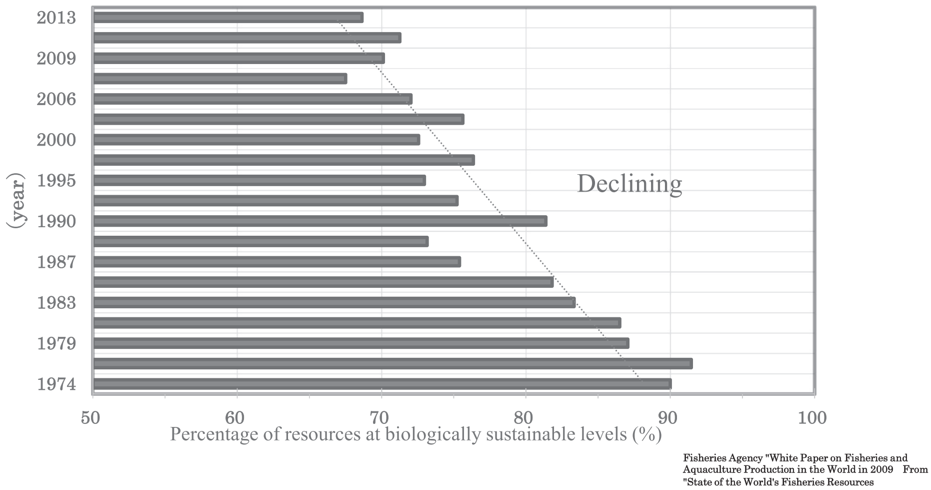


Figure 5 Sustainable level of bio-resource ratio

4. Infectious Disease Control Using Nanoparticles

In 2012, the amount of damage caused by fish diseases to farmed fish in Japan exceeded approximately 10 billion yen, and the amount of damage has remained almost the same every year (2: Nakanishi and Matsuura 2016).From 1980 to the present, damage caused by bacterial infections has had a significant impact in many parts of Japan. Therefore, the development of chemotherapeutic agents, such as antibiotics, and vaccines has been required as a preventive and therapeutic measure against the development of these infections in aquaculture sites throughout Japan, and has become an effective means of combating bacterial infections.

Antibiotics are produced by microorganisms such as actinomycetes and fungi and are effective in stopping the growth of microorganisms, and have long been used in the fisheries industry as a treatment for bacterial infections. However, the use of antibiotics has been drastically reduced due to the increase in drug-resistant bacteria and consumer concerns about the safety of antibiotic residues in fish (2:Nakanishi and Matsuura 2016).

To date, vaccines have been developed that have been shown to be effective in preventing various fish disease infections. Vaccines are a method of preventing infections by inducing the body's own defences against infection, by administering inactivated pathogens to fish in advance and artificially introducing the pathogens into the body to induce an immune response (3: Ichiro Shimamura 1999). However, while antibiotics are effective against a wide range of fish pathogens, vaccines are only effective against a maximum of four fish pathogens (a four-species combination is the limit) and are limited in the number of fish pathogens they are expected to prevent (2: Nakanishi and Matsuura 2016). As a result, it is difficult to use them against fish disease bacteria that infect many species of fish. Nanoparticles with a mechanism of action that physically disrupts bacterial cells have been identified as a potential candidate for the development of new fisheries drugs.

Nanoparticles have been used against a number of pathogenic micro-organisms in the medical and agricultural fields, and have been demonstrated to have antibacterial activity against a large number of bacterial species. In the past, silver nanoparticles have been reported to exhibit antibacterial activity against Escherichia coli (4:Xie Xiaobao et al. 2008). However, metal nanoparticles have also been reported to be toxic and there are concerns about their effects on living organisms (5:Carlson, C, SM Hussain et al. 2008).

Therefore, there is a need to develop nanoparticles that are safe while maintaining antimicrobial properties, and the compound we have focused on is cyanoacrylate, which is also used in the medical field. Cyanoacrylates include n-butyl cyanoacrylate and isobutyl cyanoacrylate. n-Butyl cyanoacrylate is used as an adhesive for medical applications, while isobutyl cyanoacrylate is used for industrial applications. Previous studies have reported that antimicrobial activity of n-butyl cyanoacrylate nanoparticles was observed against Alternaria spp, Aspergillus terrues, Cladosporium spp, Fusarium spp, Paecilomyces spp and Penicillium spp. and Penicillium spp. (6: Shoichi Shirotake 2015). The mechanism of antimicrobial action of cyanoacrylate nanoparticles is thought to be that they have a high affinity for the glycopeptides that make up the cell walls of bacteria, and by attaching to the cell walls, they induce distortion of cell wall synthesis, which leads to autolysis and lysis when the internal pressure of the bacteria cannot be maintained (7:Shoichi Shirotake 2008, 8: Kunio Chikami 2015).

Because of this mechanism of action, it is believed that the frequency of the appearance of resistant bacteria will be reduced because the antibacterial properties are shown by physical action, unlike the administration of antibiotics by chemical action.

Therefore, this study aims to clarify whether isobutyl cyanoacrylate nanoparticles can be an effective countermeasure against fish diseases of various bacterial infections that are a problem in the Japanese fishing industry. The aim of this study was to clarify whether isobutyl cyanoacrylate nanoparticles could be an effective tool to combat various bacterial infections that are a problem in the Japanese fishing industry. The antimicrobial activity of the nanoparticles was investigated against various fish disease bacteria that cause significant damage in aquaculture (Figure 6).

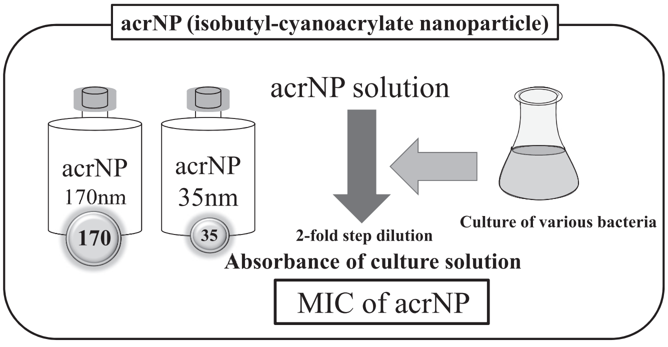


Figure 6 Determination of minimum inhibitory concentration (MIC)

Edwardsiella tarda, Photobacterium damselae subsp. *piscicida, Tenacibaculum maritimum*, and *Vibriosis maritimum*, which are the main causative agents of fish diseases in aquaculture. maritimum, *Vibrio alginolyticus, Aeromonas salmonicida, Lactococcus garvieae, Streptococcus iniae* and *Streptococcus iniae* and *Nocardia seriolae*, and the results showed that the antibacterial activity of acrNPs against *P*. damselae subsp. piscicida, *T*. maritimum, *V*. alginolyticus, *L*. garvieae, *S*. iniae and *N*. seriolae. The nanoparticles were also administered orally to saltwater yellowtail and freshwater rainbow trout to investigate their safety and feeding effects (Figure 7). No fish mortality was observed in yellowtail after 12 weeks of continuous feeding on the diets supplemented with acrNPs. The weight and length of fish in the diets supplemented with acrNP were significantly higher than those in the control diets. This suggests that the fish were fed the same amount of feed as in the control group, and the feedability was not significantly affected. Furthermore, experimental infection tests were conducted by immersion infection with *N*. seriolae in yellowtail and with *S*. iniae in rainbow trout.

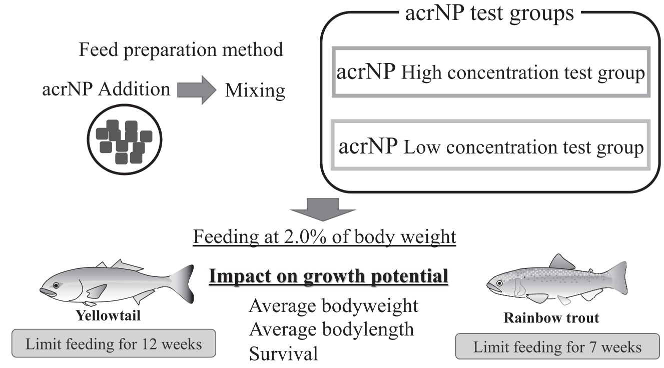


Figure 7 Oral administration Safety and feeding properties

In the prophylactic test (Figure 8), three test plots were set up: a high and a low concentration plot, an oral acrNP plot and a control plot. In the treatment study (Figure 9), there were five test plots: oral acrNP, intraperitoneal injection of acrNP, intramuscular injection of acrNP, injection of oxytetracycline hydrochloride (OTC) and control plots. In rainbow trout (Figure 10), there were four test plots: oral administration of acrNP, intraperitoneal injection of acrNP before immersion infection (pre-acrNP injection) and intraperitoneal injection of acrNP after immersion infection (post-acrNP injection).

In the experimental infection test, the incidence of N. seriolae in yellowtail and S. iniae in rainbow trout was suppressed in the zone where acrNP was orally administered continuously after immersion infection. These results suggest that the oral administration of acrNPs to the target fish species is effective in treating N.

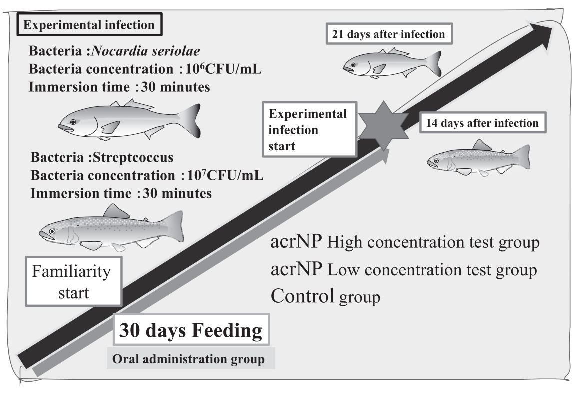


Figure 8 Preventive trials with acrNP in yellowtail or rainbow trout

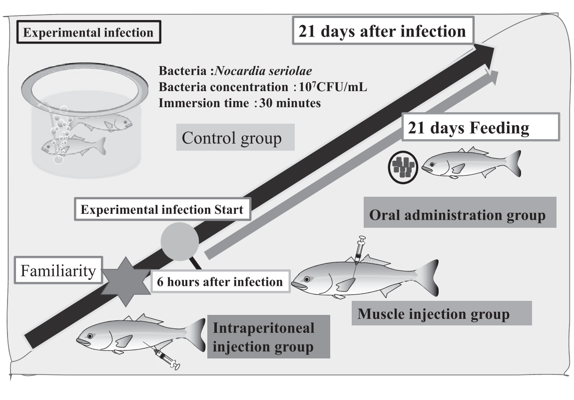


Figure 9 Treatment trials with acrNP in yellowtail

seriolae and S. iniae, and may be one of the potential drugs for fisheries. This suggests that the nanoparticles may be an effective tool against bacterial infections that may become a problem in aquaculture in the future.

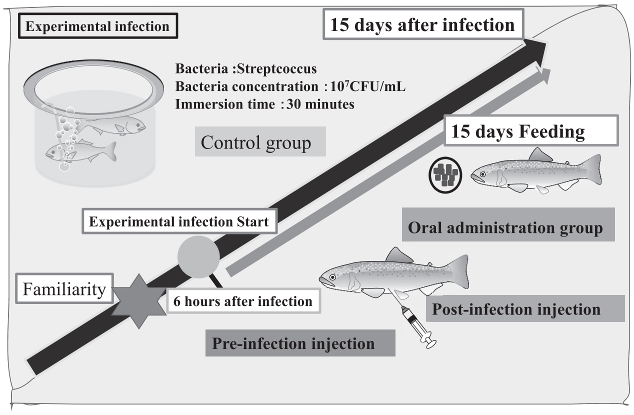


Figure 10 Treatment trials with acrNP in rainbow trout

5. Stress in Fish

Aquaculture production in Japan accounts for about 20% of the total fishery, with a production of 250,000 tonnes, of which 64% is yellowtail (yellowtail Seriola quinqueradiata, amberjack Seliora dumerili, sunfish Seliora lalandi) and 23% is sea bream Pagrus major (9: MAFF 2013). major (9: Ministry of Agriculture, Forestry and Fisheries 2013). In addition, about 60% of the total production of yellowtail and about 80% of red sea bream are farmed fish, making yellowtail and red sea bream the main marine cultured fish in Japan. Although the production value of flathead flounder, Paralichthys olivaceus, is lower than that of yellowtail and sea bream, it is popular as a high-class fish and is one of the most important fish species in the Japanese aquaculture industry. Thus, aquaculture has become an important industry with the increasing demand in the world every year.

One of the advantages of aquaculture is the possibility of stable production of safe, secure and high quality farmed fish, and traceability is becoming established in Japan to guarantee quality (10: Matsuzato 2010). In Japan, traceability is becoming established to guarantee quality (10: Matsuzato 2010). Another advantage of aquaculture is that it is easy to make a business plan because it is possible to produce fish based on an understanding of demand in advance, and it is also possible to cultivate fish efficiently and with high quality by selecting and breeding strong, high-quality strains (11: Fisheries Agency 2014). On the other hand, one of the problems in aquaculture is the occurrence of infectious diseases, such as bacterial diseases such as cysticercosis, nocardiosis, streptococcosis and gliding bacteriosis, viral diseases such as mahi-iridovirus disease and phosphocystis disease, and parasitic diseases such as elasmobranch disease and haddock disease. and parasitic diseases such as elasmobiosis and hydatidosis (12: Kawaguchi 2013; 13: Kimoto et al. 2013). Vaccinations are sometimes given to prevent infectious diseases, and drugs are sometimes administered to treat infectious diseases, but these procedures raise consumer concerns about the quality of farmed fish and increase costs for farmers because of the high price of vaccines and drugs. It is therefore desirable from the point of view of consumers and producers to avoid their use as much as possible. In addition, the incorrect administration of antibiotics can lead to the emergence of drug-resistant strains of bacteria, which can render the drugs ineffective (14: Dixon 1994). Therefore, there is a strong need to keep aquaculture fish as free from infectious diseases as possible.

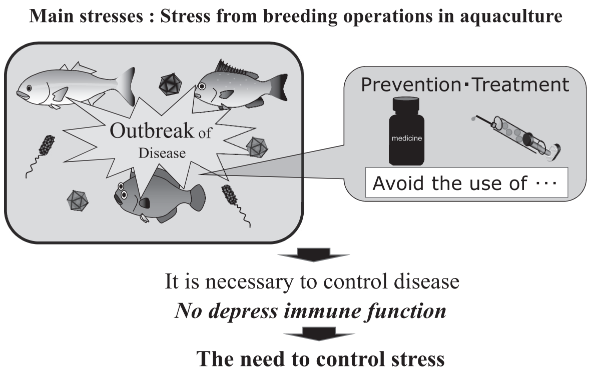


Figure 11 The relationship between infectious disease outbreaks and stress

Stress on the fish body has been reported to be involved in the occurrence of infectious diseases in aquaculture (15: Snieszko 1974; 16: Taksdal et al. 1998; 17:Ramsay et al. 2009) (Figure 11). Stress is broadly defined as any external stimulus that causes physiological changes in the organism (18: Murofushi 2005). On the other hand, stress in the narrower sense in fish can be defined as any phenomenon of non-specific biotic tension caused by external stimuli (19: Wakabayashi 2004), and can be defined as any negative phenomenon that affects the survival of the target fish. The causes of stress on farmed fish include chemical factors (e.g. water quality, pollution, feed quality and composition, excretion), physical factors (e.g. water temperature, light, sound, dissolved gases), biological factors (e.g. housing density, swimming space, microorganisms, parasites) and management factors (e. g. handling, transport, storage, feeding methods, disease treatment). (20: Sugita 2013) (Figure 12). The stress response can be divided into three phases: a warning phase, in which the individual shows a defensive response to stimuli such as hormone secretion; a resistance phase, in which the individual becomes more resistant due to the response in the warning phase; and an exhaustion phase, in which the individual is no longer able to withstand stimuli such as stunted growth or reduced immunity (19:Wakabayashi 2004; 21:Nardocci et al. 2014). When an individual is stressed, hormones such as cortisol are released as a primary response (22:Donaldson 1981). As a secondary response, these hormones are known to increase the concentration of glucose in the blood (23:Mazeaud and Mazeaud 1981). Glucose is a major source of energy for living organisms, and an increase in blood glucose concentration can prevent a decrease in blood glucose and blood pressure, thus immediately increasing physiological activity and ameliorating the stress state when an emergency situation is encountered (24: Suzuki et al. 2005; 18: Murobushi 2005). Blood glucose concentrations increase early after stimulation, but the degree of normalization and the degree of change after stimulation varies among fish species (25:Fanouraki et al. 2011; 26: Cockrem 2013). It has also been reported that the degree of change in blood glucose concentration varies with the type of stimulus, even in the same fish species (27: Acerete et al. 2004). Subsequently, it has been reported that the stress response has various effects on the organism and also affects physiology, growth, defence and reproduction in fishes, and most of these studies

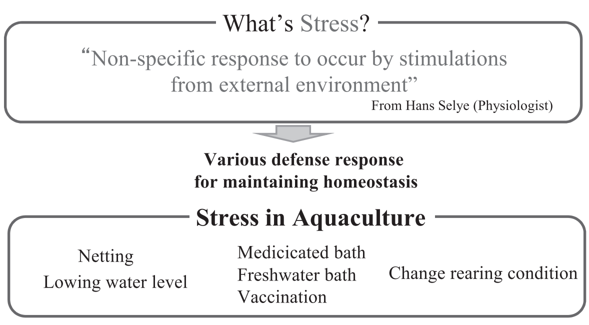


Figure 12 Stress caused by various operations in land-based aquaculture

have shown that stress has adverse effects on the organism. Therefore, it is desirable to breed farmed fish without stress, but it is difficult to breed fish without stress, and in order to further develop the aquaculture industry, it is necessary to study the methods to alleviate stress in the future.

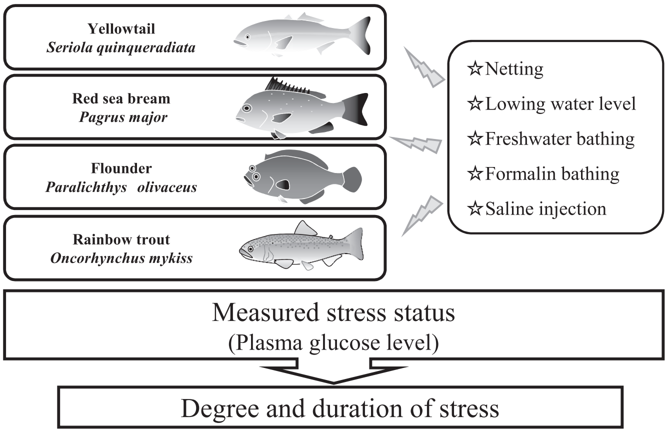
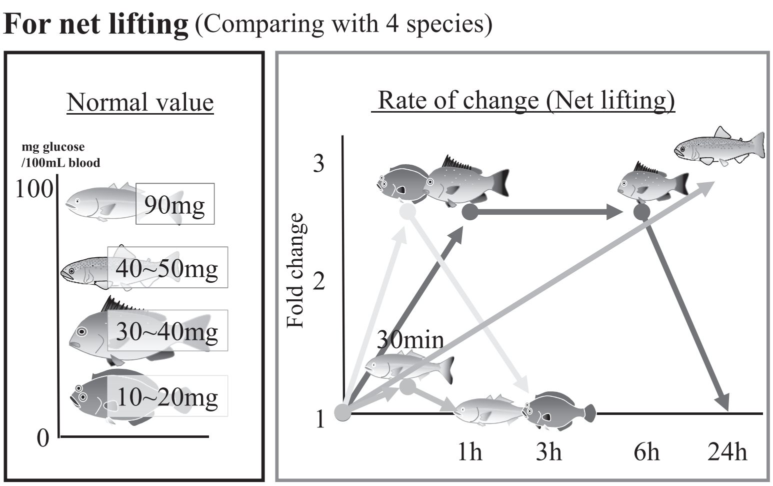


Figure 13 Degree and duration of stress by various operations in land-based aquaculture

Although the results of various studies have suggested that there is a close relationship between stress and the occurrence of infectious diseases, there have been few studies on the stress response in major aquaculture fish in Japan (28:Ishibashi et al. 2005; 29: Yokoyama et al. 2005). Therefore, it is unclear to what extent fish are stressed by the daily activities in aquaculture. In order to clarify the relationship between infectious diseases and stress in fishes, it is important to understand the level of stress to various stimuli. In this study, we investigated the effects of stress on yellowtail, red sea bream (*Plecoglossus altivelis*) and rainbow trout (*Oncorhynchus mykiss*), which are cultured all over the world (Figure 13). Comparison of blood glucose concentrations after the application of stress showed that yellowtail and flatfish did not respond to the stimuli but red sea bream did, and that yellowtail, red sea bream and rainbow trout did not respond to the stimuli with high glucose concentrations (Figure 14 ,15). In addition, the stimuli that increased the blood glucose concentration also differed among the fish species. The results of this study will contribute to the improvement of fish breeding methods and the prevention of infectious diseases, and to the further development of aquaculture.



Duration of stress varies according to fish species

Figure 14 Stress levels and duration after Net lifting in different fish species

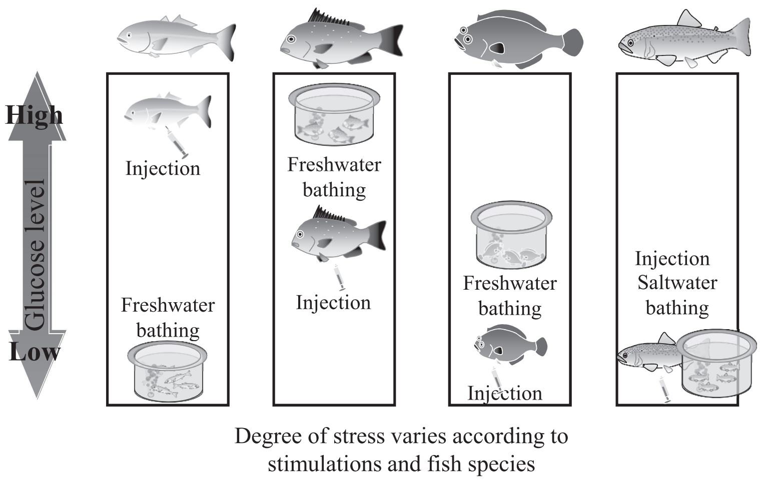


Figure 15 Stress levels and duration after various stress in different fish species

6. The Potential of Sensing Technology

Land-based aquaculture can control the rearing environment because it is not directly influenced by nature. This makes it possible to control growth potential and to produce a stable supply of fish of the desired size at the desired time. While land-based aquaculture has these advantages, the initial investment is large and running costs such as utilities are high. This is a disadvantage when compared to sea-based aquaculture.

If we can capture the biological information of the fish in real time, without damaging them, we can run the pumps and blowers 24 hours a day, 365 days a year, only when they are needed. We believe that it would be possible to run pumps and blowers 24 hours a day, 365 days a year, only when they are needed. We also constructed the system shown in Figure 16, thinking that it might contribute to the early detection of deterioration of water quality and outbreaks of infectious diseases. As a result, the system was able to detect the biological signals of fishes at a certain level. In addition, the correlation between the change of the glucose concentration in the blood, which is an index of stress, and the biological signal was examined, and it became clear that the relaxation state and the stress state of the fish could be understood as shown in Figure 17. It is hoped that further development of this system will increase the sensitivity of the system to biological signals. When this system is operational, it will be possible to build a stress-free breeding system for fish. The establishment of this system could also contribute to the further spread of land-based aquaculture.

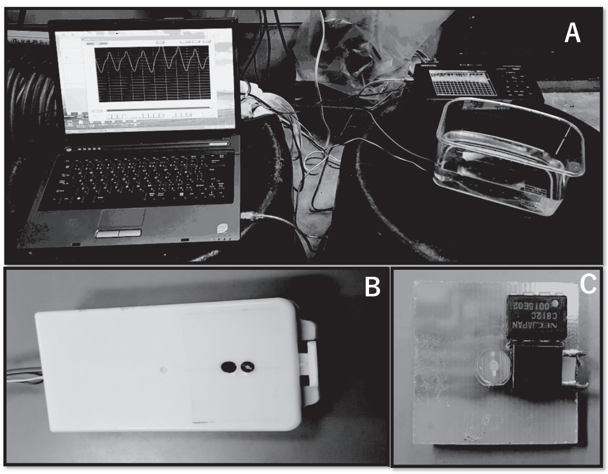


Figure 16 Fish bio-signal measuring system
A: General view B: Light emitting machine C: Inside the light emitter

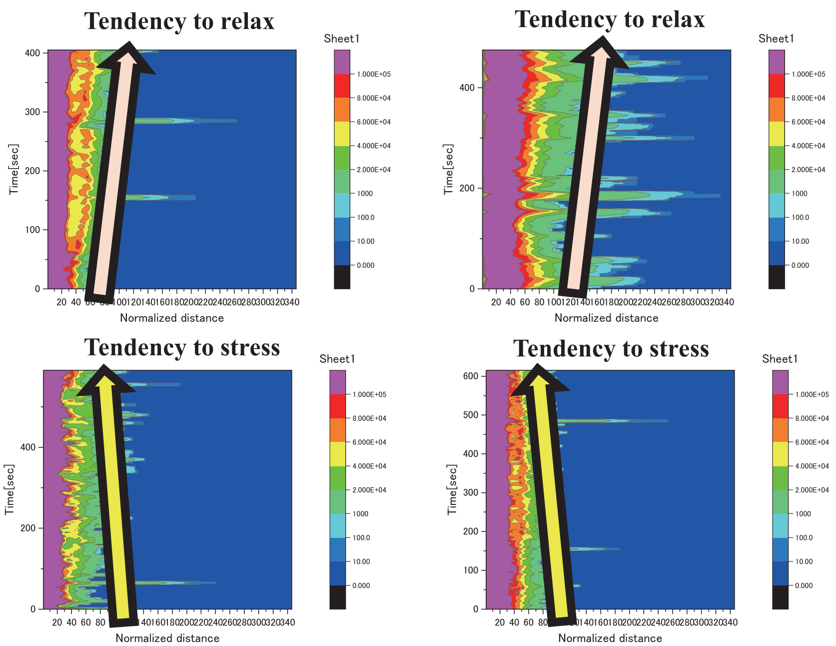


Figure 17 Detection of stress conditions in fish using a bio-signal measurement system

7. Towards the Year 2050

In the midst of climate change due to global warming and the resulting changes in the marine environment, as well as the increasing seriousness of marine pollution, there is a strong need to build a system in which all industries can work together to realize safe and stable food production and supply it in a sustainable manner, while working in harmony with humanity and the global environment.

In this issue, we talked about environmentally friendly nanoparticles for controlling infectious diseases for the construction of land-based aquaculture, and introduced our sensing efforts for the construction of a next-generation system for understanding the stress of fish and extracting their natural abilities for production (Figure 18). In order for this research to become a driving force for social action, it is necessary to connect people with diverse expertise.

It is the responsibility and mission of the adult generation to create a sustainable society for the future and to build a safe and secure society for our children and the generations to come. As we enter a new era, social systems are undergoing major changes. Acting solely on the goal of economic development has reached its limits, and human society is currently in a crisis situation on many fronts. In order to open up this era, it is essential to learn from the past and to make

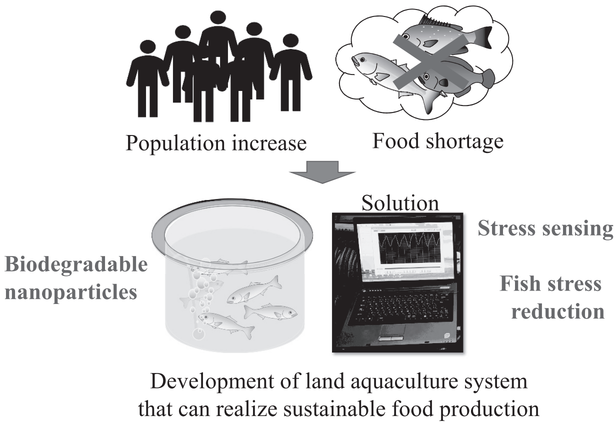


Figure 18 Establish next-generation aquaculture system

precise use of “science and technology”, including the social sciences.

Everything on earth, including human beings, is interconnected, and when discussing “society” and “science and technology”, it is necessary to consider the “optimum of the whole” rather than taking things in isolation. I believe that the new social system that needs to be created should be based on sustainable development that puts human beings’betterment of life first and foremost, and recognises the links between nature and people across generations. On the industrial side too, I believe that we need a future-oriented industrial philosophy with this kind of society in mind. In order to build a new social system, I believe that it is essential to have a comprehensive organisation and functions that go beyond the traditional framework to steadily realise this in the future.

Reference

１：水産庁 水産白書 (2013) 第 1 部 平成 25 年度 水産の動向, 第 1 章 特集 養殖業の持続的発展～, 第 2 節 養殖生産をめぐる課題, (1) 養殖業の経営.
２：中西照幸・松浦雄太 (2016) 魚類疾病の現状と課題. 日獣会誌, 69 27-35.
３：嶋村一郎 (1999) 水産用ワクチンについて. 徳島水研だより, 第 38 号.
４：Xie Xiaobao, Li Wenru, Zeng Haiyan, Ouyang Yousheng, Chen Yiben (2008) Study of Antimicrobial Activity and Mechanism of Silver Nanoparticles on *Escherichia coli. Cailiao Gongcheng* 10, 106-109.
５：Carlson, C, SM Hussain, et al. (2008) Unique cellular interaction of silver nanoparticles: size-dependent generation of reactive oxygen species. *Journal of Physical Chemistry*. 112 (43), 13608-19.
６：城武昇一. 優れた植物病害防除効果を有する菌類用抗菌剤. WO 2017033272 A1, 2015.
７：城武昇一. バンコマイシン耐性グラム陽性細菌用抗菌剤. WO2008126846 A1, 2008.
８：千頭邦夫. 抗菌剤および抗菌剤の製造方法. JP 2017-81852 A, 2015.
９：農林水産省 (2013) 平成 24 年漁業・養殖業生産統計, 31.
10：松里壽彦 (2010) 養殖　よくわかる！世界の養殖業, 緑書房, 東京, 14-20.
11：水産庁 (2014) 水産白書 平成 26 年版, 4-6.
12：河口真弓 (2013) 佐賀県玄海地区の魚介類養殖における魚病発生の変遷. 佐玄水振セ研報, 6, 125-128.
13：木本圭輔・福田 穣・吉岡左織 (2013) 海産魚介類の疾病診断と養殖衛生管理指導-1. 大分県農林水産研究指導センター水産研究部事業報告, 91-97.
14：Dixon, B. A. (1994) Antibiotic resistance of bacterial fish pathogens. *J. World Aquacult. Soc*., 25, 60-63.
15：Snieszko, S. F. (1974) The effects of environmental stress on outbreaks of infectious disease of fishes. *J. Fish Biol*., 6, 197-208.
16：Taksdal, T., A. Ramstad, K. Stangeland and B. H. Dannevig (1998) Induction of infectious pancreatic necrosis (IPN) in covertly infected Atlantic salmon, *Salmo salar* L., post-smolts by stress exposure, by infection of IPN virus (IPNV) and by cohabitation. *J. Fish Dis*., 21, 193-204.
17：Ramsay, J. M., V. Watral, C. B. Schreck and M. L. Kent (2009) Husbandry stress exacerbates mycrobacterial infections in adult zebrafish, *Danio rerio. J. Fish Dis*., 32, 931-941.
18：室伏きみ子 (2005) ストレスの生物学, オーム社, 東京, P.136.

19：若林久嗣 (2004) 魚病学概論 (室賀清邦・江草周三編), 恒生社厚生閣, 東京, 117-120.

20：杉田治男 (2013) 養殖の餌と水－陰の主役たち (杉田治男編), 恒生社厚生閣, 東京, 104-105.

21：Nardocci, G., C. Navarro, P. P. Cortés, M. Imarai, M. Montoya, B. Valenzuela, P. Jara, C. Acuña-Castillo and R. Fernández (2014) Neuroendocrine mechanisms for immune system regulation during stress in fish. *Fish Shellfish Immunol*., 40, 531-538.

22：Donaldson, E. M. (1981) The pituitary-interrenal axis as an indicator of stress in fish. In "*Stress and Fish*" (ed. by A. D. Pickering), Academic Press, London, 11-47.

23：Mazeaud, M. M. and F. Mazeaud (1981) Adrenergic responses to stress in fish. In "*Stress and Fish*" (ed. by A. D. Pickering), Academic Press, London, 49-75.

24：鈴木 譲・植松一眞・渡部終五・会田勝美 (2005) 魚類生理学の基礎 (会田勝美編), 恒生社厚生閣, 東京, 25-26.

25：Fanouraki, E., C. C. Mylonas, N. Papandroulakis and M. Pavlidis (2011) Species specificity in the magnitude and duration of the acute stress response in Mediterranean marine fish in culture. *Gen. Comp. Endocrinol*., 173, 313-322.

26：Cockrem, J. F. (2013) Individual variation in glucocorticoid stress responses in animals. *Gen. Comp*. *Endocrinol*., 181, 45-58.

27：Acerete, L., J. C. Balasch, E. Espinosa, A. Josa and L. Tort (2004) Physiological responses in Eurasian perch (*Perca fluviatilis* L.) subjected to stress by transport and handling. *Aquaculture*, 237, 167-178.

28：Ishibashi, Y., K. Inoue, H. Nakatsukasa, Y. Ishitani, S. Miyashita and O. Murata (2005) Ontogeny of tolerance to hypoxia and oxygen consumption of larval and juvenile red sea bream, *Pagrus major*. *Aquaculture*, 244, 331-340.

29：Yokoyama, S., S. Koshio, N. Takakura, K. Oshida, M. Ishikawa, F. J. Gallardo-Cigarroa and S. Teshima (2005) Dietary bovine lactoferrin enhances tolerance to high temperature stress in Japanese flounder *Paralichthys olivaceus*. *Aquaculture*, 249, 367-373.