Scientific evidence and questions identified by the Hanasaki Program

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Introduction

A joint research project, the Hanasaki Program, conducted by Nemuro City and Sakhalin Research Institute of Fisheries and Oceanography (SakhNIRO), is in its fifth year. Although the program is small, it has a large scientific scope and has identified several important pieces of scientific evidence and posed new scientific questions. Thus, we would like to present them as potential items for the Okhotsk Sea component of the new PICES integrative science program on Forecasting and Understanding Trends, Uncertainty and Responses of North Pacific Ecosystems (FUTURE).

What is the Hanasaki Program?

The Hanasaki Program is a program dedicated to the study of Hanasaki crab, *Paralithodes brevipes*, which is distributed mainly around the Nemuro Peninsula, once called the Hanasaki Peninsula, in the waters around Hokkaido, Japan. Hanasaki crab is the symbolic sea food of Nemuro City because of its brilliant red color when boiled, and its vivid taste. However, the catch of this crab has been very low in spite of stock management and enhancement efforts. Moreover, the Nemuro market, among other Hokkaido fisheries markets in recent years, has been supplied with Hanasaki crab landings as well as by illegal catches from the Okhotsk Sea and nearby waters.

The Hanasaki Program is addressing the following questions which have a broad scientific scope covering biology, ecology, oceanography, ecosystem modeling, fisheries management, culturing technology, and taste quality.

Why does stock not recover in spite of stock management efforts? Is it due to:
- Illegal catch?
  Action: identify the unit of stock management.
- Overestimation of abundance resulting in too large of an allowable catch?
  Action: review the stock assessment method and develop a tagging method.
- Abundance of settling larvae?
  Action: conduct a joint larval survey.

2) How can the culturing method be improved?
   - How can the mass mortality in seed production be avoided?
     Action: conduct a comparative parallel test on possible alternative measures.

3) How can the value of stock in the market be realized and enhanced?
   - When is the best taste season and where is the best place to find the best tasting crab?
     Action: monitor the changes in taste through the seasons and between sites by conducting organoleptic tests.
   - Is the molting phase linked to taste?
     Action: examine changes in the umami relating chemical components through the molting process.

The five following themes were developed to address these questions, and four years after the program was implemented, the following results have been obtained by the Nemuro and SakhNIRO teams.

Major Achievements of Research

Theme 1. Study on the dynamics of the *P. brevipes* fishery and population

Objectives and research items

- To identify hidden problems in stock management practice through an overview of fishery operations and catch dynamics;
- To review if the appropriate level of allowable catch has been set or not;
- To develop tags to be retained through multiple moltings for the estimation of stock abundance, mortality and emigration/immigration;
- To elucidate the effects of the environment on stock dynamics through investigation of seasonal changes in water temperature of the fishing grounds;

- To know the status of local populations of *P. brevipes* distributed in the Okhotsk Sea and adjacent waters by looking at:
  1) *Features of the fishery and catch efficiency of crab pots*;
  2) *Improvement of stock assessment and development of tagging methods*;
  3) *Oceanographic conditions and structures*;
  4) *Status of local populations*.

Results

1) *Features of the fishery and catch efficiency of crab pots*

Catch efficiency of crab pots

<u>Nemuro</u>

Results of past surveys showed that:

- The attracting range of crab pots was estimated to be between 9 and 12 m; the maximum number of crabs in a pot was estimated to be around 5 individuals; and the duration of pot immersion to maximize crab catch was about 3 days;
- Selectivity of pot cover nets by crabs indicated no significant difference in carapace size composition for meshes of 30, 60, and 120 mm (Fig. 1).

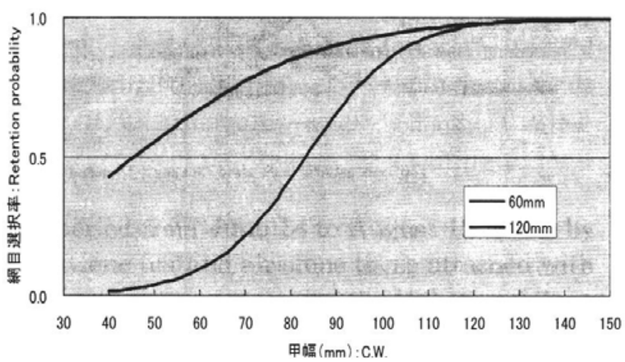

Fig. 1 Mesh selectivity curve of Hanasaki crab pots of 60 and 120 mm mesh using 30 mm mesh catch as reference. Horizontal axis is carapace width.

<u>SakhNIRO</u>

Diving results showed that:

- *P. brevipes* in crab pots stuck to the inside of the side net, or at the outside of the crab pots, attached to the bottom of the pot, or stuck outside or close to the net wall, crowding together in two or three tight rows, as if waiting in the turn to enter the pot;
- Within a distance of 10 m from the pots, groups of 2 to 3 individuals hid under brown algae, at the bottom of protruding rocks, or in the cracks of rocks;
- At a distance of 15 to 20 m, crabs were distributed as single individuals;
- Beyond 20 m from the pots, no crabs were observed;
- Just after molting, *P. brevipes* showed high feeding activity at the crab pots and, at night, moved actively to form local swarms; the pot catch in the survey area showed sudden jumps at various points.

2) *Improvement of stock assessment and development of tagging methods*

Improvement of stock assessment methods

<u>Nemuro</u>

- A review of the present method for *P. brevipes* stock assessment indicated a large uncertainty resulting from peculiarities in catch efficiency of the crab pots. The major source of uncertainty causing an overestimation of stock was the individual number-based catch–density relation. In other words, stock estimation by the CPUE–density relation at around a saturation level of gear capacity can cause a larger uncertainty and possible overestimation of stock density, especially when the size dependency of the gear saturation level is ignored in the CPUE–density relation.

Development of tagging methods

<u>Nemuro</u>

- Tagging was one of the most efficient methods for field surveys of population dynamics. However, in the case of crustaceans, tags had a tendency to drop off with the old carapace at the time of molting, and the duration of the tagging survey was limited to a short interval between molting. The straight-type anchor tag (Photo 1) and marking by making an excision on the side of the carapace (Photo 2) were tested, and confirmed that tag retention after the fifth molting was a highly practicable method for *P. brevipes*.

Photo 1 Straight anchor tag: (top to bottom) putting on the tag, just after tagging, after second molting, and after fourth molting.

Photo 2 Excision of the side of the carapace: (top to bottom) side of carapace before excision, just after excision, after first molting, and after fourth molting.

- The inner membrane taken from a chip excised from the side of the carapace for marking has proven to be useful for DNA analysis, and the possibility of a tag mark with DNA information for individual identification is being developed.

3) *Oceanographic conditions and structures*

Nemuro

- Water temperature of fishing grounds near the bottom was monitored for 3 years. The very special nature of seasonal changes of oceanic conditions there (not changes in vertical structure but changes in horizontal shore–offshore structures) was clarified. These changes appear to continue to offshore oceanic conditions such as the Coastal Oyashio and the East Hokkaido Warm Current;
- Seasonal variations of the East Hokkaido Coastal Current were investigated. Results showed that the outflow of Okhotsk Sea Water does not influence the East Hokkaido Coastal Current Water. The water would have been greatly modified before it reached the sea to the east off Hokkaido. The results support the conclusion of DNA analysis on the genetic difference between the Sakhalin-Shiretoko populaton and the Kuril-Nemuro-Erimo population.

(For details, please refer to the paper by Nagata in these proceedings.)

SakhNIRO

- Downwelling was observed and measured in the aggregation areas of *P.brevipes* larvae and adults;
- From the spatial distribution of temperature and salinity, a pair of cyclonic and anti-cyclonic eddies were identified in the South Kuril Strait which showed that the current field is favorable for retention of planktonic larvae;
- The Princeton Oceanographic Model (POM) approach applied to *P. brevipes* larval distribution in the study area was confirmed by field surveys, except in shallow waters around Nemuro Strait and its vicinities.

4) *Status of local populations*

SakhNIRO

- Aggregations of *P. brevipes* in the coastal waters of the South Kuril Islands were in a good state. Survey results on planktonic larvae indicated a high level of reproduction in the region;
- However, in the 2005 and 2006 surveys, many females with unfertilized eggs were sampled;
- Diving surveys in 2004 determined the habitat of *P. brevipes* and population densities in the embayments around the islands of Shikotan, Yuriy, Zeleniy, and Polonskiy;
- A survey on the distribution of *P. brevipes* in the coastal area from Cape Odyan to the eastern boundary of the northern Okhotsk Sea was performed. Results showed that the spatial distribution of *P. brevipes* is uneven. The crab is confined specifically to the shoal area, and along the broken coastline there are several isolated spots with dense congregations of *P. brevipes* where high catches of commercial individuals have been obtained (MagadanNIRO);
- A survey of *P. brevipes* on the eastern Kamchatka shelf was performed. As there is no demand for *P. brevipes* on the domestic market, this stock is not exploited by commercial fisheries. In some areas, small-sized crabs are dominant (KamchatNIRO);
- Surveys of the *P. brevipes* population along the eastern coast of Terpeniya Peninsula, on Sakhalin Island, identified stable abundant colonies of this crab. Molting was observed mainly from the last 10 days of July to the first 10 days of August. Fecundity averaged 40,000 eggs for crabs with average carapace width of 104 mm (SakhNIRO);

Future study

1) *Features of the fishery and catch efficiency of crab pots*

Catch efficiency of crab pots

- Determine the saturation point of crab pots under different carapace size composition, and the relation between the pot escapement rate and size of crab.

2) *Improvement of stock assessment and development of tagging methods*

Improvement of stock assessment

- Improve methods of obtaining fishery data, especially on fishing locations and size composition of catch.

Development of tagging methods

- Implement field tests and practical applications.

3) *Oceanographic conditions and structures*

Nemuro

- Determine the relationship between seasonal changes in bottom temperature of fishing grounds and catches;
- Increase efforts to obtain direct current measurement data;
- Develop CTD and hydro-chemical data archives.

SakhNIRO

- Understand the possible causes of downwelling (by examining the possibility of a secondary flow by residual currents in the vertical section), and the current structure;
- Understand the formation mechanism of eddies in the South Kuril Strait (including the possibility of tidal residual currents) and their seasonal variability;
- Perform seasonal and interannual water property monitoring by remote sensing and *in situ* measurements.

4) *Status of local populations*

- Understand the genetic relationship between the Sakhalin population and the population at the northern coast of the Okhotsk Sea; the relationship between the distribution area and the area of circulation formed by the East Sakhalin Current and offshore countercurrent during the warm period of the year; the spatial difference in size composition within the distribution area and distribution of recruitment;
- Understand the genetic relationship between the eastern Kamchatka and Sakhalin populations; the relationship between the distribution area and the area of circulation formed by the Northern Okhotsk Current and its countercurrent; the spatial difference in size composition within the distribution area and distribution of recruitment.

Theme 2. Study of ecology and population structure of *P. brevipes*

Objectives and research items

- To elucidate the independency and mutual interchange among local populations, through DNA analyses, for identifying a strategy for stock management and stock recovery;
- To elucidate the ecology of crab;
- To develop an ecosystem model on larval transport and survival processes by looking at:
  1) *Genetic diversity and population structure*;
  2) *Transport and dispersion of planktonic larvae*;
  3) *Field survey of planktonic larvae*;
  4) *Ecological study*.

Results

1) *Genetic diversity and population structure*

Nemuro

- Novel DNA markers (eight microsatellite DNA and mtDNA AT-rich regions) were used to assess the genetic variability and population structure of *P. brevipes* (Fig. 2);
- Both microsatellite DNA and mtDNA analyses suggested that the populations of Sakhalin-Shiretoko, Kamchatka, and South Kuril-Nemuro-Erimo are genetically different;

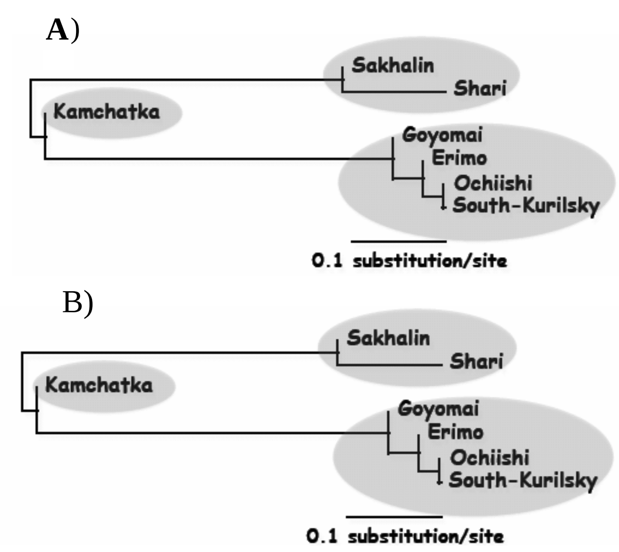

Fig. 2 Genetic relationships among Hanasaki crab (*P. brevipes*) populations inferred from (A) micro-satellite and (B) mitochondrial DNA data.

- The population on the coast of Nemuro Peninsula had high genetic variability and is clearing the critical level of effective population size (Ne) of 500 for keeping genetic variability of the population and preventing extinction;
- Artificial seeds produced by Nemuro City had relatively high genetic variability compared to the natural population;
- A new DNA typing method for future tag-and-release surveys was established without harming the individual and commercial value of the crab;
- Groups of *P. brevipes* in the Nemuro Peninsula–east of Cape Erimo area and in the South Kuril waters had minimum genetic differences and probably belong to the same population. Greater differences were obtained between the Sakhalin and northwest coast of Shiretoko Peninsula populations and Kamchatka populations (Fig. 3).

2) *Transport and dispersion of planktonic larvae*

<u>Nemuro</u>

Results of numerical simulations (POM) clarify the following:

- Planktonic larvae of Sakhalin origin reach the coast of Hokkaido Island to at least north of the Shiretoko Peninsula;
- Seasonal variability of the East Sakhalin Current and Soya Current plays an important role in the settlement of planktonic Sakhalin-originating larvae;
- The recruitment of crab must be influenced by interannual variability of oceanic conditions, especially surface currents and sea surface temperature around the Nemuro coast and Okhotsk Sea coast of Japan;
- The South Kuril waters around Malaya Kurilskaya Gryada and Nemuro Bay were identified as the most possible settling sites for larvae;
- An anti-cyclonic eddy in the South Kuril Strait may play an important role in the retention and accumulation of larvae.

3) *Field survey of planktonic larvae*

<u>Nemuro</u>

- Stations where planktonic larvae were sampled were mainly on the south side of the Nemuro Peninsula (Fig. 4, Table 1); the number of larvae sampled were considerably fewer than those sampled in the waters of the South Kuril Strait by SakhNIRO;
- In the 2007 and 2008 surveys, no *P. brevipes* larvae were sampled from Nemuro Strait;
- *P. brevipes* larvae appeared early, suggesting early shifting of the hatch-out time due to the effects of regional warming (Fig. 5).

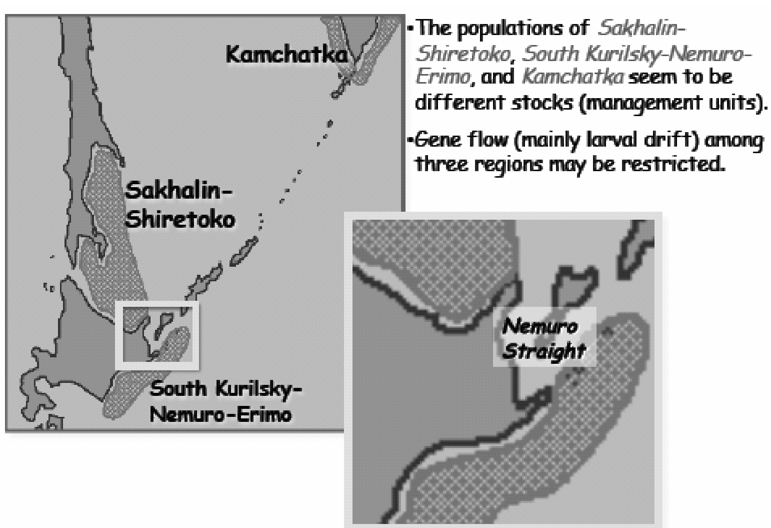

Fig. 3 Genetic population structure of Hanasaki crab (*P. brevipes*; cross-hatched areas) estimated by DNA analyses.

Table 1 Results of field surveys of planktonic larvae in 1984, 1985, 2006 and 2007 for stations off Nemuro Peninsula (total number of individuals sampled).

	1984				**1985**				**2006**				**2007**			
	Z1	**Z2**	**Z3**	**G**	**Z1**	**Z2**	**Z3**	**G**	**Z1**	**Z2**	**Z3**	**G**	**Z1**	**Z2**	**Z3**	**G**
May 1–10	–	–	–	–	35	–	–	–	16	36	–	–	–	1	1	–
May 10–20	10	–	–	–	2	6	–	–	–	–	–	–	–	–	–	–
May 20–31	–	–	–	–	–	37	86	–	–	–	–	–	–	–	52	–
June 1–10	–	–	–	–	–	–	15	–	–	–	4	1	–	–	–	1
June 10–20	–	1	4	–	–	–	–	4	–	–	–	–	–	–	–	–
June 20–30	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–
July 1–10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
July 10–20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Subtotal	10	1	5	–	37	43	101	4	16	36	4	1	–	1	53	1
Total	16				185				57				55			

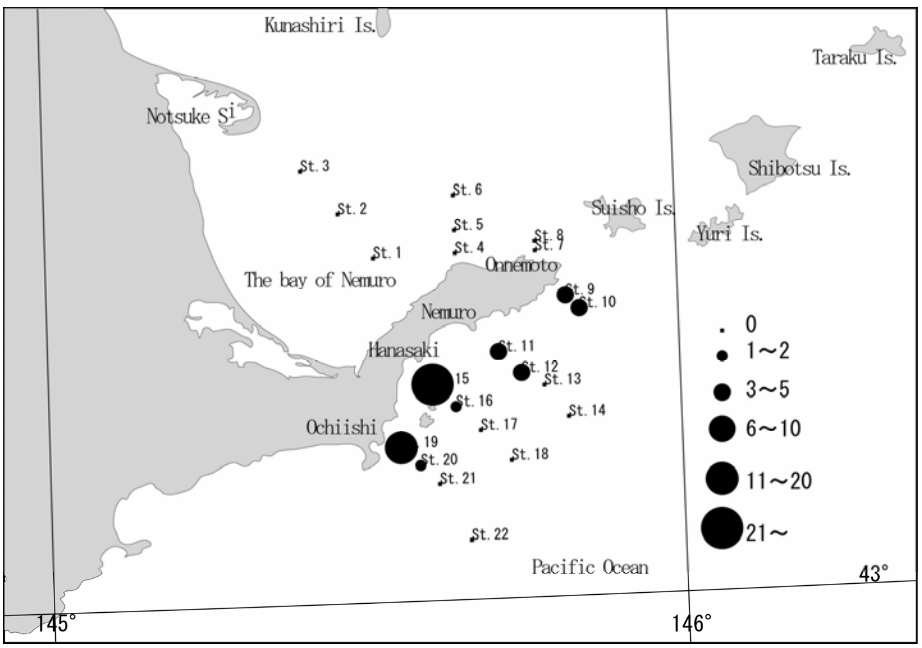

Fig. 4 Geographical distribution of sampled *P. brevipes* larvae in the last 10 days of May 2007 off Nemuro Peninsula.

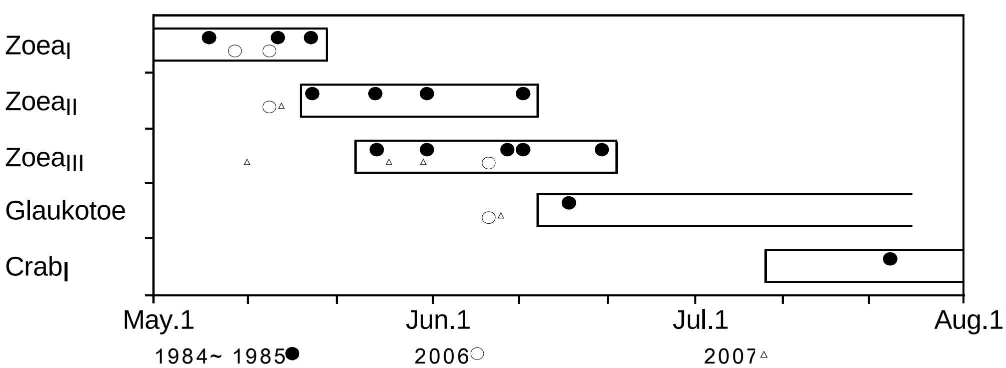

Fig. 5 Comparison of appearance times of *P. brevipes* larvae at different developmental stages, for surveys conducted in 1984, 1985, 2006 and 2007.

SakhNIRO

- May–June distribution density of planktonic larvae of *P. brevipes* in the waters around the South Kuril Islands was high and varied from 10s to 100s of individuals/m^2.

4) *Ecological study*

Nemuro

- From previous research conducted by Torisawa *et al.* (1999), the growth curve was re-examined to overcome the difference between early growth obtained through rearing individuals (Kittaka and Onoda, 2002) and that obtained through examination of wild samples (Abe and Koike, 1982; Sasaki and Yoshida, 1999). By synthesizing past results, a new age–carapace width–length relation was proposed (Torisawa, 2005);
- Molting of *P. brevipes* was observed during culturing studies;
- Results of laboratory measurements on the phototaxis swimming speed of larvae were introduced as an important parameter for larval transport model experiments.

SakhNIRO

- The diet of male king crab (*Paralithodes camtschaticus*), Hanasaki crab (also known as red spiny crab) (*Paralithodes brevipes*) and golden king crab (*Liathodes aequispinus*) near the South Kuril Islands were compared; Sea urchins of genus *Strongylocentrotus* prevailed in the diet of these three crabs; 90.8% for *L. aequispinus*, 84.1% for *P. brevipes*, and 53.8% for *P. camtschaticus*; High stomach fullness of *P. brevipes* was recorded only near the Malaya Kurilskaya Gryada between 19 and 48 m depth.

Future study

1) *Genetic diversity and population structure*
   - Study the genetic diversity and population structure of *P. brevipes* in the waters of northern and eastern parts of the Okhotsk Sea, Kuril Islands, Kamchatka and Bering Sea (Fig. 6).

2) *Transport and dispersion of planktonic larvae*
   - Using the POM approach to validate the model and examine the factors controlling distribution of the larvae, a biological oceanographic database on the habitat of *P. brevipes* larvae is needed (Fig. 7):
     – A GIS-oriented approach should be used;
     – Because of a lack of initial data in the region, including a deficiency of chemical materials, new specific and task-oriented field/database/model studies will be needed in the coming 1–2 years;
     – Intergovernmental efforts would be productive and helpful for the joint development of a biological oceanographic database, its implementation and scientific publication;
   - Problems to resolve:
     – Obtain Russian and Japanese governmental approvals for joint research;
     – Establish a task team;
     – Look for funding;

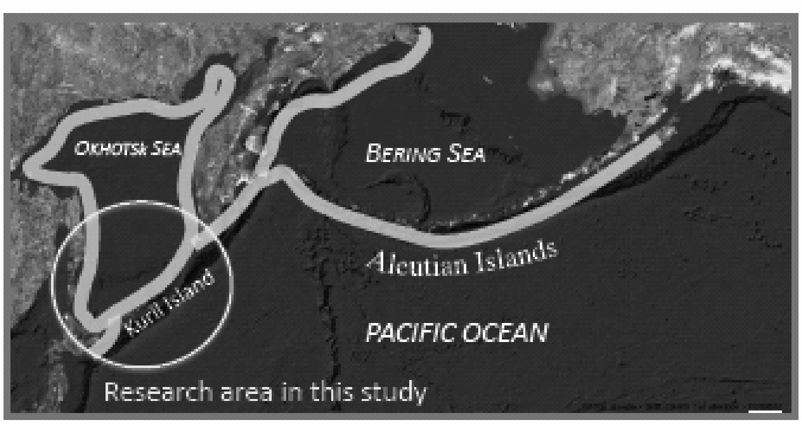

Fig. 6 Area of this study (circled) and possible distribution (thick grey lines) of *P. brevipes*.

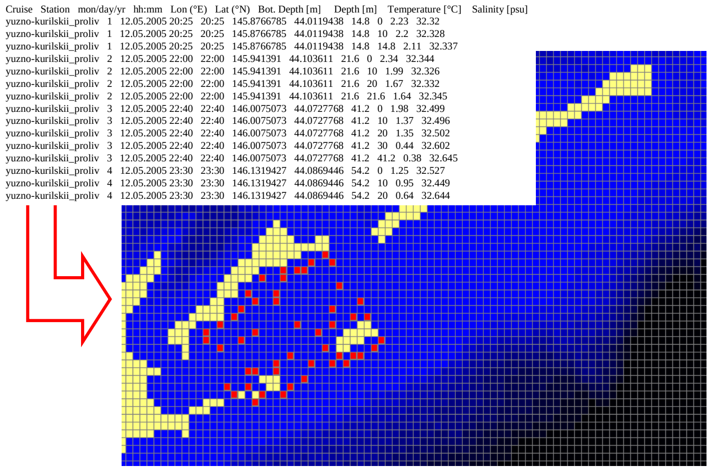

Fig. 7 Example page of a biological oceanographic database.

- In order to identify the hatching-out sites and settling sites of *P. brevipes* larvae from the results of field larval surveys, a modeling study on larval transport in the South Kuril waters has been implemented by a local physical oceanography model. An example of output bottom concentration of *P. brevipes* larvae (without other zooplankton) is shown in Figure 8;
- In this example, *P. brevipes* larvae are settling in the southern part of Nemuro Strait, while no individuals were sampled during field surveys by the Nemuro team. Further improvements need to be made to the model and field survey.

3) *Field survey of planktonic larvae*
   - Propose a joint international Japan–Russia expedition for a larval survey in the southern Okhotsk Sea and its vicinities to be executed by one or two research vessels in May–June, 2008, or later. The proposed sampling plan is shown below (Fig. 9).

4) *Ecological study*
   - Conduct ecological and muscle physiology studies on molting;
   - Perform rearing tests on recovery from molting.

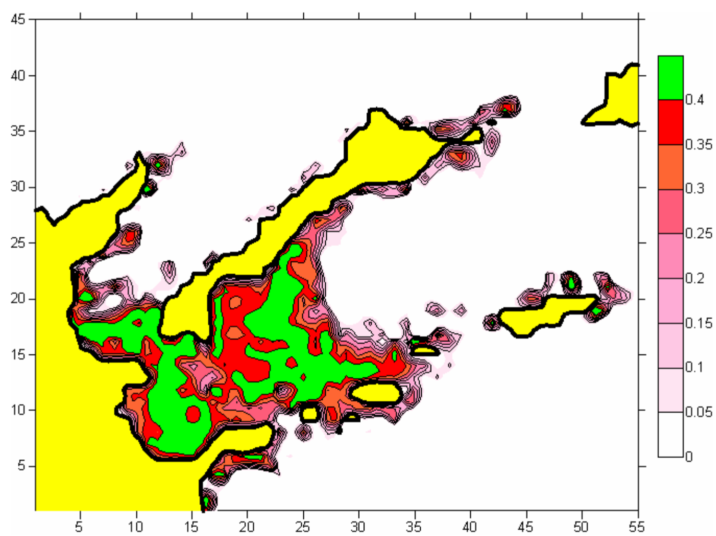

Fig. 8 Example of output on larvae distribution; *x* and *y* axes are model coordinates and the color pattern is larval density of the water column (individuals per m^2).

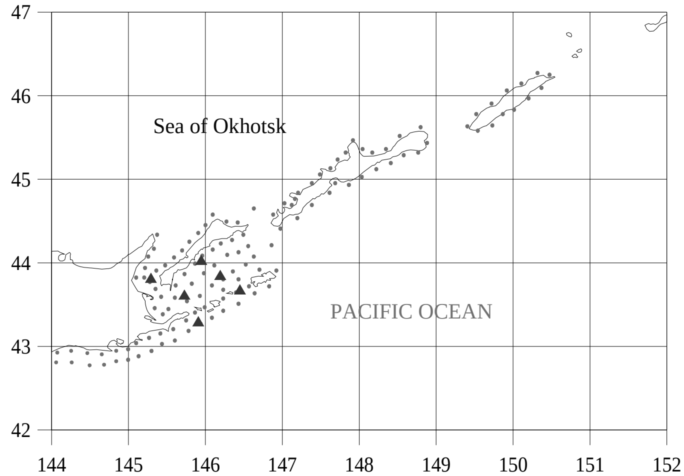

Fig. 9 Proposed larvae sampling plan. Grey dots represent net sampling stations and triangles denote mooring stations for current, temperature, salinity and sea-level measurements, with larval collectors.

Theme 3. Improvement of culturing technology for *P. brevipes*

Objectives and research items

- To improve the practicability of culturing technology by improving survival rate and economical efficiency through:
  1) *Improvement of seeding technology*;
  2) *Improvement of intermediate culturing technology*;
  3) *Development of a stock enhancement technology system*.

Results

1) *Improvement of seeding technology*
   - The cause of mass mortality of *P. brevipes* larvae at the glaukotoe stage was examined.

2) *Improvement of intermediate culturing technology*
   - A test of two batches of seeds using females with eggs at early hatch-out dates and females with late hatch-out dates, reared under different water temperatures, was implemented successfully.

3) *Development of a stock enhancement technology system*
   - A "Manual for seed production and seed release" was compiled as summary results.

Future study

1) *Improvement of P. brevipes seeding technology*
   - Develop seed releasing technology.

2) *Improvement of intermediate culturing technology*
   - Develop an artificial settlement base.

3) *Development of a stock enhancement technology system*
   - Develop stock assessment capabilities for identifying a strategy for stock enhancement.

Theme 4. Study on preservation and improvement of taste quality of *P. brevipes*

Objectives and research items

- To develop technology to preserve and improve taste quality of *P. brevipes* through analysis of chemical components governing the quality of taste, and to develop an index to make objective grading possible by:
  1) *Developing indices of freshness/liveliness*;
  2) *Investigating the geographical/seasonal change of taste components*;
  3) *Studying methods to improve taste quality*.

Results

1) *Development of indices of freshness/liveliness*
   - Energy Charge was an effective index for determining the liveliness of *P. brevipes*.

2) *Geographical/seasonal change of taste components*
   - Taste differences were found between male and females, but no systematic geographic differences in taste were detected.

3) *Methods to improve taste quality*
   - Rearing in higher salinity water increased the taurine level in *P. brevipes*, which suggests a possible method for improving taste;
   - The recovery process after molting indicated that the increased water content ratio and taste composition were dramatically recovered in accordance with hardening of the carapace;
   - Panelists composed from staff of the fishermen's cooperative evaluated the taste quality of *P. brevipes* during different stages of recovery from molting by organoleptic tests. Crab at one stage before complete recovery were graded relatively higher than at other stages;
   - The best cooking method and best time for flavour was boiled crab when it was still warm;
   - Results from chemical analyses of taste components indicated a degradation in part of the taste components by freezing or boiling;
   - A "Manual for preservation and improvement of taste quality of *P. brevipes*" was compiled as summary results.

Future study/activity

To publish a best procedure for cooking and tasting P. brevipes *for consumers*.

Theme 5. Study on the design of the *P. brevipes* fishery system for recovering and sustaining its resources

Objectives and research items

- To compare cost/performance of stock recovery measures;
- To identify a most suitable stock management method;
- To design a fishery system for establishment of a sustainable *P. brevipes* fishery.

Results

A "Manual for selecting stock recovery measures" was compiled as summary results.

Future study

- To improve stock assessments in selecting stock recovery measures;
- To arrange *P. brevipes* total catch information and exchange.

Final Remarks and Acknowledgements

The authors expect that the FUTURE Program of PICES will provide an opportunity to extend our small scientific collaboration into an international one. The scientific results reached in the Hanasaki Program are planned for publication in SakhNIRO proceedings in 2009.

The authors would like to express thanks to the scientists who contributed their efforts and results in the Hanasaki Program, obtaining new information on the ecology, biology, and habitat of *P. brevipes*, the Promoting Committee for the Nemuro City Hanasaki Program and SakhNIRO. We recognize the importance of widening scientific collaboration on *P. brevipes* issues around the Okhotsk Sea and its vicinities in the near future.

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