# F-3 The study for methods and measures of invasive alien species risk assessment

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#### 1. Introduction

Invasive alien species (IAS) is one of the main factors threatening biodiversity world wide in the present day1). The ecological impacts caused by IAS are very serious because they can increase population by themselves and can make irreversible change on the natural fauna once they become established in the new field. It is demanded all over the world in the Convention on Biological Diversity to control and eradicate the IAS in each country in order to conserve natural ecosystems.

The Japanese Government enacted a new law "Invasive Alien Species Act" in 2005 for controlling the IAS ecological impacts. In this law all the alien species specified as invasive are restricted to be introduced, reared and released. So, it will be urgent matter to establish clear criteria and guideline for assessment of ecological risks caused by IAS.

On the other hand, little attention has been paid, even in the new law, on the alien parasite invasion, which will cause most serious ecological damages2). Actually, only disease against human and domestic animals have been restricted in Japan though a lot of animals and plants are imported as pet animals and as biological agents together with many unknown micro organisms. So, the status of parasite invasion and their ecological risks should be assessed urgently.

And we must give it the highest priority to conserve endemic and isolated ecosystems like as the South-west islands and Ogasawara Islands against IAS.

## 2. Research Objective

In the present study we aim to develop methods for IAS risk assessment. And we will put emphasis on investigation for parasite invasion problem. Furthermore, we will construct control systems against IAS at the Okinawa and Amami Islands where considered as "most important nature preserves" (Fig.1).

In the sub-theme (1): "Establishment of ecological risk assessment for IAS", we select some serious IAS species and analyze their ecological properties. Accumulating information about ecological impacts and expansion of distribution of the IAS, we distill the ecological factors for risk assessment. We test computer simulation for estimate what ecological properties will drive biological invasion. And we establish methods for data-map estimating expansion of distribution of IAS

In the sub-theme (2): "Research for ecological risks of parasitic alien species", we collect sample of alien parasites attached with imported reptiles and insects, and make database of them. We investigate ecological impacts of the alien parasites against the native species. And we clarify co-evolutional relationships between host-parasites in order to grasp evolutionarily significant unit (ESU) of host-parasite interactions.

In the sub-theme (3): "Study for IAS impacts and eradication measures in Okinawa and Amami Islands", we investigate the status of biological invasion of mongoose, cats and other exotic animals in the Islands. We develop effective measures for control the IAS and also construct social system for driving the IAS controls.



Fig.1 Outline of this study project

3.Study contents and results

(1) Establishment of ecological risk assessment for IAS

For developing risk assessment for plant IAS, four approaches were used. First, we estimated the area dominated by alien plant species in the floodplains of 123 Japanese rivers under the administration of the Ministry of Land, Infrastructure and Transport. 87 of 444 alien species found in the floodplains were dominant in at least one plant community. Furthermore, 18 of 87 dominant alien species made up about 90% of the total cover of alien vegetation. These species were shown to have strong adverse effects on biodiversity and ecosystem functions. 87 dominant alien species included mostly agricultural weeds or revegetation species in Copositae, Leguminosae or Gramineae from north America. Using CART (classification and regression tree), we developed the model to identify non-dominant and dominant alien species based on the immigration route, origin, life form, family, plant height, seed width, seed weight and flowering period. 81.4% of correct

classicification rate was achieved in this model. Next, we investigated dormancy/germination traits of dominant alien species. Germination experiments for 29 alien species revealed four types of dormancy/germination traits. Different dominant alien species had different types of the traits, suggesting that different processes facilitate population expansion. Third, in order to develop a predictive model for the population expansion of an invasive alien grass, *Eragrostis curvula*, in river floodplains, the effects of micro topography and grain size of the substrate on the establishment of *E. curvula* were studied. Both factors showed significant effects. The results of vegetation survey also showed the overlap distribution of *E. curvula* and endemic riparian plants. Because they had similar habitat requirements, most of areas dominated by endemic riparian plants were lost and the vegetation dominated by *E. curvula* between 1996 and 2000-2002. The selective removal of *E. curvula* will be needed for conservation of riparian habitats. Finally, a database of alien vascular plants in Japan is being developed that currently includes information on taxonomical and ecological traits and cultural aspects, e.g. utilization or attempts to eliminate the species. As basic examples, database of fifteen invasive alien species were constructed.

For developing risk assessment for mammalian IAS, we investigated ecological properties, expansion of distribution and ecological impacts of the raccoon. The invasive alien raccoon has already naturalized throughout Japan. The raccoon has opportunistic and omnivorous feeding habits and competes for getting food resources with native species such as the red fox and the raccoon dog. It also became infected with leptospire and new parasites, and we need to care about the spread of infection diseases not only to human but also to wild animals. We ran successive trapping in Nopporo Forest Park and captured more than 80% of adult raccoons, but we also identified the immigration of new individuals. The management of the dispersal is the key to the raccoon eradication. It became clear by the habitat analysis that invasive alien raccoon have a preference for human residential area and waterside. We also established the species discrimination method by DNA analysis of the carnivore fees and found out that the American mink preyed on amphibian animals, crustaceans and fishes mainly. The existence of the dangers of the expansion of the muskrat.

To establish a firm basis for the risk assessment of exotic reptiles and amphibians, we conducted following five case studies on the biological properties and interactions with indigenous biota of exotic reptiles and amphibians currently naturalized in Japan: (1) genetic properties of a freshwater turtle population in the Hokuriku District that consists of individuals exhibiting mosaic distribution of character states usually unique to the native species, *Chinemys reevesii*, or exotic species, *Mauremys mutica*; (2) feeding ecology of a feral population of the predatory freshwater turtle, *Pelodiscus sinensis*, in the northern part of Okinawajima; (3) recent range extension and parasite fauna of an originally Southeast Asian tree-frog, *Polypedates leucomystax*, within the Ryukyu Archipelago; (4) effect of poison of the exotic toad, *Bufo marinus*, upon the native amphibian-eating snakes of the Ryukyus: and (5) status of feral populations in southeastern Kyushu of *Japalura polygonata*, an agamid lizard originally endemic to the Ryukyu Archipelago and Taiwan, and in Ishigakijima (southern Ryukyus) of *Iguana iguana*, a large-bodied Neotropical lizard. Results of these studies indicate that: (1) the turtle population in problem has been formed through the recent introgression from a few individuals of *M. mutica* toward a large population of *C. reevesii*; (2) on

Okinawajima, *P. sinensis* preys upon a high variety of native animals with substantial divergence in prey items between sexes; (3) the tree-frog has recently been rapidly extending its range onto Kumejima (southwestern extremity of the central Ryukyus) and islands adjacent to Miyakojima (southern Ryukyus), and the Miyako populations show a high frequency of occurrence a parasite that may belong to an undescribed species never reported from native amphibians of the Ryukyus; (4) the damage from the toad poison to native amphibian-eating snakes largely correlates with the duration of their isolation from the toad as estimated from broadly prevailing paleogeographical hypothesis; and (5) *Japalura polygonata* and *Iguiana iguana* already occur as feral established populations in one small part of southeastern Kyushu and Ishigakijima, respectively.

As a case study for IAS risk assessment for freshwater fish, we investigated the present status of biological invasion of centrarchid fishes. Our electrophoresis analysis of largemouth bass in Lake Biwa revealed the recent mass introduction of the Florida subspecies, suggested by the dramatic genomic change from the initially colonizing population of the Northern subspecies to that dominated by the hybrids between the Northern and Florida largemouth basses. This suggests heterosys between these two subspecies of largemouth bass. The genetic approach was also used for investigation on the geographical distribution patterns of mtDNA haplotypes of the smallmouth bass, using the samples from most of the water bodies suffering from its invasion. The result showed that the proportion of the major two haplotypes was almost equal for most of the populations located in north-eastern Japan whereas either of the two tended to predominate in the western Japan, suggesting the difference in the process of its intentional introductions. We also accumulated the data of ecological impacts by the three centrarchid fishes (largemouth and small-mouth basses and bluegill) by means of inquiries to local specialists, and revealed their serious ecological impacts on a lot of endangered freshwater animals including fish, crustaceans, and insects.

For establishing risk assessment of insect IAS, we investigated mainly two species, the European bumblebees imported for pollination of tomato crops and the exotic stag beetles imported as pet animals. The recent naturalization of introduced bumblebee, *Bombus terrestris*, in Japan is expected to give strong impact on native bumblebee community. In this study, we investigate the present status of naturalization of *B. terrestris* and the ecological features of the species. *Bombus terrestris* was found form 46% of all monitoring sites and was one of dominant species in study area. An overwintering queen of this species was found under soil in a deciduous forest. From the result of principal component analysis for land uses and vegetation around the monitoring sites, naturalization areas of *B. terrestris* were dominated by agricultural land uses. For naturalization of this species, both open environments for nesting habitat and forests for hibernaculum are needed. Two native species of bumblebees, *B. hypocrita* sapporoensis and *B. pseudobaicalensis*, also use this combination of environments and, thus, these two species, especially the former, were considered as potential competitor of *B. terrestris*.

In order to settle "most important stag beetle preserves", we accumulated information of collecting sites of the native beetles and estimated their geographical distribution combined with temperature data map. Simultaneously we got information about sites of pet-shops selling the exotic beetles. We estimated the geographical area where the exotic species can be naturalized based on their growth zero temperature, as well. Furthermore, we constructed ESU map for the native species based on mitochondria DNA phylogeny. Analyzing these data maps comprehensively, we decide the

area where there is precious and endemic ESU population and there are shops, and furthermore the exotic species can live. The area was each of the South-west Islands. We settled this area as "most important stag beetle preserves". We studied cross experiments for assessing the cross-risk between exotic and Japanese native populations. The study revealed that there was little reproductive isolation between the South-east Asian and Japanese strains of *Dorcus titanus* which is one of the major pet species in Japan, even though DNA data suggested over 1.5 million years isolation between the strains.

Simulation study on biological invasion was conducted in two ways: 1) the evolutionary cause of vulnerability of insular communities, and 2) the relationship between the duration of food web evolution and the vulnerability to biological invasion. Communities on oceanic islands, which evolved without suffering invasion, are considered vulnerable to biological invasion. As a result of simulation, communities without invasion evolved to have an unstable structure, in that a small number of plant species supported a large number of animal species, and each species had small biomass. Consequently, collapses of community caused by biological invasion were more frequently occurred in such communities. According to available data for real insular communities, some are suggested to have reached the endangered state predicted by this model. Next, the relationship between the duration of food web evolution and the vulnerability to biological invasion was investigated. Communities without evolution consisted of animal species with a limited number of prey species and producer species with small intrinsic growth rate. Such communities were vulnerable to invasion of powerful omnivore but resistible to plant invasion. However, with time, communities evolved to have a structure that a small number of producer species supported a large number of animal species. Such communities were vulnerable to plant invasion. Therefore, the vulnerability of community to plant invasion increased with time. The result of this study strongly suggested that a food web changes with time, and consequently, the vulnerability to invasion changes with time.

Habitat suitability prediction of invasive species was made to help risk assessment. The map was to be incorporated into the invasive species database at the website of the National Institute for Environmental Science. A map of the northern Japan was created for raccoon established in Japan, based on altitude, vegetation index, and night-light, and the census data of the Hokkaido Prefecture. The resulting map was proved reliable to predict the area suitable for invasive racoon establishment.

#### (2) Research for ecological risks of parasitic alien species

For assessment of the infectious disease risk from imported reptiles, we investigated the prevalence of *Cryptosporidium* infection in captive lizards and the pathogenicity for Japanese native lizards of *Cryptosporidium* arising from imported species. We found *Cryptosporidiosis* was related to the cause of death in 28 captive lizards of 5 imported species. The average rate of infection was 11.0% for lizards in exhibition facilities and 66.7% for lizards privately owned, but was extremely low in wild native lizards. Infection tests showed that many species of wild native lizards were susceptible to *Cryptosporidium*, but particularly *Giniurosaurus kroiwae splendens*, for which infection was fatal. Then, *Cryptosporidium* arising from imported Leopard gecko that was the exoticism was inoculated to the snake, and the pathogenicity to the snake was examined. It was clarified that the corn-snake is susceptible to *Cryptosporidium*. We investigated the prevalence of

*Cryptosporidium* infection in native gecko in Okinawa Prefecture, and revealed 3%(1/33) infection rate. It will be needed to investigate the origin and routs of *Cryptosporidium* infection in native gecko. The fatal herpes virus infectious disease of imported emerald tree monitor and cobalt tree monitor was found. This virus induced necrotizing stomatitis (mouth rot).

We found deadly intranuclear coccidiosis in a colony of terrestrial tortoise. Furthermore, Chytridiomycosis was discovered with several imported frog species, which was the first case in Asia. We have constructed an inspection system network for monitoring of spread of Chytridiomycosis.

We have also found ticks on exotic reptiles imported as pet animals. The ticks were identified to be *Ambryomma* and *Aponoma* species by morphological traits, however their mtDNA haplotypes have never been known. Furthermore, the ticks possessed Boreria bacteria those DNA haplotypes also have been unknown.

As the parasites of stag beetles have been little investigated, it is very difficult to estimate the ecological risks caused by the invasion of parasites. However, we exactly found and listed many species of mites attached with commercial exotic individuals. We have also investigated genetic variation of an endoparasitic mite, Coleopterophagous berlesei, which is one of the most common mites living on the surface of stag beetles. We collected the mite from individuals of the native and exotic D. titanus, and analyzed sequence variation of mtDNA extracted from them. Same as mtDNA of D. titanus, we are constructing phylogenetic tree of mtDNA haplotypes in the mite. The tree indicates genetic divergence among the mite strains. And furthermore, associations of the host stag beetles and the parasitic mites phylogenies shows that the mites are considered to be diverged into strains specific to host strains. Such host specificities indicate that associations between the host and the parasite transcend speciation events and are therefore relatively old. We re-classified the mite species based on these DNA data combined with detailed morphological data. And we described four new species; Canestrinia spectanda, Coleopterophagus berlesei, Sandropheraamarae and Noemiphera izabelae. We revealed also that there are differences in ecological potential between the exotic and Japanese populations of the mite species. These investigations suggest that the importation and commercialization of stag beetles will disturb not only the evolutionary process of stag beetles but also the co-evolutionary associations between the stag beetles and their parasites, which will cause unexpected influence on the native stag beetle populations.

## (3) Study for IAS impacts and eradication measures in Okinawa and Amami Islands

We accumulated the ecological information of mongoose that is the most serious IAS in the Okinawa and Amami Islands for developing effective control systems against the species. Detailed examination for the dynamics of distribution of mongoose and native species revealed that a lot of endemic species had decreased according to expansion of distribution of mongoose. Computer simulation tests suggested that it will need long time to eradicate the mongoose populations under the present status by the control operations of mongoose. We developed fences to prevent mongooses from escaping and we found an effective type. We also examined a type of the bait station using in New Zealand for control mongoose.

Among invasive species of the Yanbaru area, in which endemic species and endangered species are highest on the Okinawa Island, feral cats sings decreased in the forest road, while the

mongoose sings increased with expanding distribution area according both information of capturing and witnessing, recently. For raising the capture efficiency of the mongoose in the Yanbaru area, we analyzed trapping data, effects on the rare species by predation of the mongoose and infection disease. One of effective trapping method should be line trappings along logging loads in the Yanbaru area, in which mongoose density is low compare with grid trappings because of efficiency. Both the evidences of predation on native reptiles and amphibians including insects and birds by mongoose and decrease of amphibian population seem to be serious condition in the Yanbaru area. It became clear that the mongoose could become a vector of infectious disease, i.e. zoonosis, including nematode and tapeworm, and the Japanese encephalitis virus.

In order to clarify the status of understanding of IAS problem between local residents in the Okinawa and Amami Islands, we conducted a questionnaire survey in main cities and town. The survey revealed that many people are aware of IAS, and the degree of costs for control of the IAS. This result suggests that cooperation between government and residents will drive the control of IAS in the Island. The Trump type teaching material "Mr. Pinch" was Developped. The model class was executed in two high schools. "Mr. Pinch" is a valuable teaching material which understands the alien species problem.

### 4. Discussion

It is very difficult to eradicate IAS perfectly, once it has succeeded in naturalization. We have ever constructed database of IAS and spread information of IAS among people through the Global Environmental Research Program (F-3; 2001 - 2003). Then the new law "Invasive Alien Species Act" was enacted at June, 2005.

For enforcement of this law, scientific base-data is being needed for practical risk assessment for IAS. However, IAS is so variable in ecological and genetical characteristics both within and between species that it is impossible to assess their ecological risk only depending on a standard guideline. We must pay attention to that ecological risks of IAS will change by temporal and spatial environmental dynamics. So, we must construct not only a basic screening method for assess the characteristics common to IAS, but also a peculiar assessment method for prediction of IAS.

In sub-theme (1), we have accumulated practical data aiming to establish ecological risk assessment system. We have tried to construct a primary screening method in plant and animal IAS through analysis of ecological traits of IAS and environmental factors. Such a trial will result in establishment of basic IAS screening methods.

On the other hand we have found many examples of exceptional impacts caused by IAS. For example, we indicated alien toads cause not only predatory impact against the native species but also toxic impact against the native predator. And we found that an alien reptile species could expand its distribution over common northern limit of the habitat. For stag beetles, it was thought that reproductive isolation mechanism evolved regardless of genetic distance between local populations so that they can cross with each other even if far isolated. All these results are exception cases over our usual prediction. Accumulation of such exception data will result in a peculiar assessment method for prediction of exception of IAS impact.

We have also tried computer simulation for prediction of IAS characteristics. Although we

have so far indicated only common sense of IAS by the computer model, we should extract conditions under which "exception" of IAS will occur.

In sub-theme (2), we have studied parasite invasion about which we have little information though it is one of the most serious factors of IAS impacts. In the present study we have found many new-type parasite attached with reptiles and insects imported from abroad, which suggests that there is a great biodiversity in parasites.

Furthermore we have found that there are ESUs in parasite populations and also in host-parasite co-evolutionary associations. We think it will be important to conserve such a biodiversity in parasites for our co-existing with parasites.

In sub-theme (3), we aim to establish a system for controlling IAS at the Okinawa and Amami Islands where biodiversity is peculiarly high but simultaneously biodiversity is being destructed by rapid developments and IAS expansions. It is thought that establishment of IAS control-system in such an urgent area will be important case-study for considering a measure against IAS in Japan.

The results of the present study for ecological information of mongoose and for developing fence against their expansion will contribute to eradication of the species.

And we have promoted education acts to people in the Okinawa and Amami Islands, aiming to realize IAS control system united with inhabitants and government. Results of the questionnaire for surveying awareness against IAS in inhabitants will be important basic data for considering systems that bear closely on local communities.

As the construction units of global biodiversity are localities of species and populations, conservation of local ecosystems results in conservation of global biodiversity. So, it must be most important issue to conserve the South-West Islands of Japan for global biodiversity conservation.

From the present study, we have so far gained a lot of useful information for establishment of IAS risk assessment system, but also a lot of difficulties of prediction of IAS risks. We can say that conservation of localities of gene, species, and biological interaction must be ultimate theory for control of IAS. We will apply the results of this study project to Invasive Alien Species Act of the Environmental Miistry.

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