

Development of a method to understand the current distribution and abundance of endangered aquatic insects living in Satochi (Rural Settlements) in the Ryukyu Archipelago based on environmental DNA and formulation of their conservation units

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[Abstract]

Key Words: Ryukyu Archipelago, Satochi (Rural Settlements), Aquatic Insects, Endangered species, DNA library, Environmental DNA, ESU (Evolutionary Significant Unit)

In this research, a library of mitochondrial DNA data on rare aquatic insects in the Ryukyu Archipelago was constructed, and the genetic information obtained was used for their taxonomic revision and formulation of conservation management units, as well as for food habits analysis of alien species and for considering reinforcement to wild populations and reintroduction. We also developed a primer set for species-specific environmental DNA detection based on the DNA library, collected basic data on the relationship between population size and DNA copy number, as well as the relationship between water quality and DNA degradation rate, and established optimal sample collection methods. Using environmental DNA, we estimated the current distribution and abundance of endangered species and, based on scientific evidence, supported the regional extinction of the national endangered species of wild fauna and flora in Japan such as the diving beetle, *Cybister limbatus*, and the aquatic bug, *Laccotrephes grossus*, with a high degree of certainty. We also tried to develop a non-invasive DNA collection method using environmental DNA and a method for detecting intraspecific variation.

Accumulate of Distribution and In-situ information on the endangered water insects living in the Satoyama on the Amami-Ryukyu region, with apply for conservation actions.

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[Abstract]

Key Words: Reason for the reduction of water insects in the Amami-Ryukyu region, Neonichochinoid, Neonicotinoide-based agrochemical, Invasive alien species, New face of endangered species, Creation of using heavy equipment.

- 1) Current situation of water insects in the Ryukyu archipelago are quickly changing worse, especially on the endangered species shows serious decline and rather common species recently shows rapid declines.
- 2) Paddy field in this area, now some of islands are completely missing it and remaining islands also sharply declining.
- 3) Neonicotinoide-based agrochemical commonly appeared on the usual paddy field and with very few species and numbers of water insects, contrarily, non-agrochemical paddy field with rich fauna and a numbers of water insects still survived.
- 4) Artificial plastic ponds effective for conservation for water insects in the Ryukyu archipelago too.
- 5) Water flora control method on the habitat of critical endangered odonatan species in Amami-Ohshima island, that effective for keep habitat of their reproduction; Creation of conservation ponds are very effective, when before pond making, it is important that make screening for the situation of agrochemical and invasive alien species.
- 6) During study for the endangered water insects, we study environmental factor at the same time, it is clear that main reason of declination are a) disappearance of environment of swampy area, b) Impacts of Neonicotinoide-based agrochemical , c) severe drought (recent occur frequently), d) Invasive alien fishes and water plants.

We should use these knowledges and making conservational plan for the endangered species.

Development of ex-situ conservation methods for endangered aquatic insects living in Satochi (Rural Settlements) in the Ryukyu Archipelago, Japan

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[Abstract]

Key Words: ex-situ conservation method, Aquatic insects, Ryukyu

In this study, we developed ex-situ conservation techniques for endangered aquatic insects of the Ryukyu Archipelago, and examined the effects of population restoration using captive bred individuals as a reinforcement to wild populations or for use in reintroduction. For the development of ex-situ conservation techniques, we used two model species, the diving beetle, *Cybister limbatus*, and the aquatic bug, *Laccotrephes grossus*, because they are large species and are distributed only in the the Ryukyu Archipelago in Japan. We determined their growth process from egg to adult and the number of eggs laid by females. We also found that the most important prey for the larval stage of *Cybister limbatus* is dragonfly nymph, and that the optimal water temperature for spawning is 25° C or higher. In addition, a rearing apparatus that does not require water changes for the larval stage was created, enabling simple rearing. For *Laccotrephes grossus*, the optimal water temperature for the larval stage was investigated, and 28° C was found to be optimal.

Assessment of the Impact of Invasive Alien Species on endangered aquatic insects living in Satochi (Rural Settlements) in the Ryukyu Archipelago and Development of Countermeasure Methods

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[Abstract]

Key Words: Amphibians; Aquatic insects; Invasive species; Predation, Ryukyu

The diet of the nonnative American Bullfrog *Lithobates catesbeianus* occurring in Ogimi Village of Okinawajima, Ryukyu Archipelago, is investigated. Seventy two of 89 frogs (nine adults, five subadults, and 58 juveniles) captured had food items in its stomach. We identified a total of 64 taxa from 253 food items. Our analyses show that (1) the diet consists mainly of terrestrial prey, (2) mollusks and vertebrates are the essential prey groups for adults and subadults, and (3) odonates, mollusks, and arachnids are the main prey for juveniles. Such dietary habits are quite different from those of several conspecific nonnative populations in mainland Japan and other regions. Part of this is most likely related to the absence of the frog's favorite prey, nonnative American crayfish *Procambarus clarkii*, in the study area. We also confirm the predation of a poisonous newt *Cynops ensicauda popei* (Salamandridae) and several aquatic insects by this frog and present some implications for these results.

[References]

- 1) Nakamura, Y., Tominaga, A. 2021. Diet of the American bullfrog *Lithobates catesbeianus* naturalized on Okinawajima, Ryukyu Archipelago, Japan. *Current Herpetology*, 40 (1) 40-53.
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Development of community-based in-situ conservation and habitat restoration methods for endangered aquatic insects living in Satochi (Rural Settlements) in the Ryukyu Archipelago and Development of Countermeasure Methods

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[Abstract]

Key Words: in-situ conservation, habitat restoration, Aquatic insects, Ryukyu

In this study, the following contents were implemented: 1) Development of methods to utilize sedimentation ponds as biotopes for rare aquatic insects, which are unique to the Amami and Ryukyu Regions; 2) Development of methods to utilize fallow rice fields as biotopes; 3) Examination of pesticide (insecticide) effects; 4) Implementation of community-based in-habitat conservation and habitat restoration techniques, and in particular, publicizing the potential for maintaining and improving the multifunctionality of agricultural land through the use of biotopes for sedimentation ponds and fallow rice paddies.