論文の内容の要旨

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論文題目　Habitat use and behavior of *Lates japonicus* in Shimanto Estuary, an endangered endemic fish in Japan
（日本の絶滅危惧固有種アカメ *Lates japonicus* の四万十川河口域における生息場利用と行動）

Lates japonicus (Katayama & Taki, 1984), commonly known as the Japanese seabass or Akame in Japan, is a predatory species endemic of the estuaries and coastal waters on the Pacific coast of south-east Japan (Iwatsuki et al., 1993). This rare species is listed in the Red Book of endangered species of the Ministry of Environment of Japan as a Threatened IB species (correspondent to IUCN's Endangered, EN, category). *L. japonicus* is a game fish to reach sizes near 140 cm in total length (TL) and weight 39 kg (IGFA, 2016), often caught in the estuaries of big rivers in Kochi Prefecture.

One of the main areas where this species is targeted by catch-and-release sport anglers is Shimanto Estuary, Shikoku Island. Establishment of management plans in Shimanto Estuary and other areas for *L. japonicus* is necessary to conserve the remaining populations of this species while allowing a sustainable development of estuarine and coastal areas. Very few studies have been conducted on this species and little is known about its ecology. For establishing integrated management and conservation measures, information on the life history, habitat use and migrations of *L. japonicus* is necessary. This study aims to elucidate habitat use and behavior of this species at different life stages in Shimanto Estuary.

The microstructure and microchemistry of otoliths was used to reconstruct the life history of *L. japonicus* in Shimanto Estuary. To minimize the number of fishes sacrificed for their otoliths, frozen samples donated by local anglers and aquariums were also used. The Sr/Ca on the otoliths of juvenile fishes collected on seagrass beds, and in fishes cultured in brackish water tanks (salinity of 17-19) was used to identify threshold values for estuarine waters. Values over and below the thresholds of brackish water were allocated to seawater and freshwater respectively. The threshold values were used to analyze hatching areas and movements inside and outside the estuary at juvenile, young and adult life stages.

Results of the analysis of the otoliths suggested that most of the individuals hatched in the estuary, where brackish water was distributed. The levels of Sr/Ca in the otoliths of juvenile fishes caught in seagrass beds incremented towards the edge, and was linked with an increment in ambient salinity. Counting otolith's daily rings and relating the age of the fish with its TL, the increment was calculated to have started between the 11^{th} and 16^{th} day of life, at sizes near 5.3-7.9 mm TL. This increment in the ambient salinity water was linked with the recruitment to the seagrass beds where fishes were caught, located at higher salinity waters on the bottom of the estuary.

In the otoliths of young and adult fishes the levels of Sr/Ca in the otolith tended to decrease as fishes aged, which suggested a movement outside the seagrass beds and towards upper stream areas. However, Sr/Ca levels on the otoliths remained always within the range allocated for estuarine waters, which suggested that all analyzed samples had an estuarine life history, being born in the estuary and remaining on it without any long term residence shift to freshwater or coastal areas. The analyzed individuals can be considered as estuarine residents since they spent their whole life in estuarine brackish waters.

Otolith analysis can't discriminate short term movements to freshwater or coastal areas. To obtain habitat use and behavior information on a short scale, acoustic telemetry methodology was applied. Movements of *L. japonicus* in Shimanto Estuary were monitored using acoustic transmitters and receivers deployed in fixed arrays at selected points. The first tracking experiment was conducted from winter of 2014 to spring of 2015, using four 42.5 ± 0.5 cm TL aquarium-reared individuals. The fish belonged to a restocking project for *L. japonicus* in Shimanto Estuary conducted by the Akame Gakuyukan, a local aquarium in Shimanto City. Anaesthetized fishes were inserted with a Vemco V9P transmitter in their intra-peritoneal cavity, and their movements tracked with nine acoustic receivers.

The results of this tracking study showed that 100% of the individuals survived during the first month, and at least 75% of them did for six months after releasing. Individuals remained in the estuary in proximity to the seagrass beds located in the small streams on both sides of the main stream of the estuary. Diurnal movements between residences following daylight were detected for two fishes, and those following the tides in another one. It suggested that young *L. japonicus* selected habitats strongly linked to the presence of seagrass beds. The great survival rate and estuarine fidelity of the released individuals showed that stock enhancement of *L. japonicus* releasing wild-captured aquarium-reared fishes might be a successful measure to increase the number of *L. japonicus* in Shimanto Estuary.

To analyze the behavior and habitat use of wild adult *L. japonicus*, a second tracking experiment was conducted between mid-June and early October of 2016, covering the whole spawning season of *L. japonicus*, estimated between late June and August. Five individuals between 69 and 92 cm TL were captured in Shimanto Estuary and tagged externally using a Vemco V13P acoustic transmitter attached to a modified dart tag. The individuals were tracked with nine acoustic receivers deployed in fixed stations along the estuary, seven of which were retrieved on the 13th -15th of October of 2016.

Apparently, some of the adults of *L. japonicus* tracked concentrated in the upper reaches of the estuary, close to the limit of the incursion of the salt wedge. These individuals remained in the upper estuary during the whole tracking period except two fishes, which migrated downstream to the estuary mouth. This migration towards the estuary mouth observed for two different individuals occurred in July for one fish and in August for the other, and was severely influenced by lunar phase. It seems that this downstream migration is related to spawning, since a very similar behavior has been observed in the related species *L. calcarifer* (Moore, 1980; Moore, 1982; Davis, 1986; Garcia, 1992).

The migration towards the estuary mouth started during full moon periods. The two individuals disappeared from the range of the receivers during the following last quarter moon. The disappearances continued for three consecutive nights. One individual returned to the estuary and again disappeared for another three consecutive nights during the same last quarter moon period, whilst the other did it only once. After returning to the coverage, one individual remained in Shimanto Estuary, while the other left and was found inhabiting a nearby estuary located 30 km south of Shimanto River along the coastline.

Reports on the spawning migration from freshwater to the estuary mouth and nearby coastal waters of *L. calcarifer* supports that the migration in *L. japonicus* might be for spawning. In Shimanto Estuary, not all of adult *L. japonicus* spawn every year, even when they are potentially capable according to size, and they don't do it all at once. Spawning is synchronized with last quarter moon periods, occurring several times during the spawning season. Spawning grounds are not identified but very close to the estuary mouth. After spawning, spawned individuals may re-enter Shimanto Estuary or move to nearby estuarine systems.

In order to investigate the habitat use of adults of *L. japonicus* into more detail, the data collected with an animal-borne digital still-camera logger (DSL) attached to an 89 cm TL wild *L. japonicus* in summer of 2009 was analyzed. The analysis of the images recorded during the six hours of experiment revealed adults of *L. japonicus* forming shoals. The experiment individuals rapidly found a shoal of *L. japonicus* after released, which suggests that individuals *L. japonicus* form groups frequently. The shoals were detected in July on the upper reaches of the estuary, and consisted of individuals of apparently similar sizes to the released fish.

This study revealed the great importance that estuarine waters have for this species at all life stages. In Shimanto Estuary, all of their life cycle seems to be strongly linked to the brackish estuarine waters. Spawning may have occurred close to the estuary mouth and larvae hatched out in the estuary. Juveniles were found in estuarine seagrass nurseries, and young fishes shifted residences in areas close to seagrass beds. Adult fishes were found in the upper reaches of the salt wedge, and some of them remained for many years inside the estuary. Two tracked adult individuals, migrated to lower reaches of the estuary and disappeared for three days. This migration probably relates to spawning.

The importance that residences located in the estuary have for *Lates japonicus* at many life stages is conspicuous. To protect the remaining populations of this species in Shimanto Estuary and create comprehensive management actions, all the estuary must be taken into account. Restocking programs might provide a solution to increase the stock of this species in the estuarine waters. However, it is imperative to protect key habitats, especially seagrass beds, necessary for the survival of several life stages of *L. japonicus*.