EFFECT OF SEASONAL CHANGES AND RIVER STRUCTURES ON THE BEHAVIORAL PATTERNS OF THE HYBRID GIANT SALAMANDER IN THE KAMO RIVER, JAPAN

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Abstract.—The introduction of Chinese Giant Salamanders (Andrias davidianus) has been a problem in various regions in Japan. Hybridization between A. davidianus and native Japanese Giant Salamanders (A. japonicus) has jeopardized survival of the latter species. Conservation of A. japonicus requires implementation of measures that prevent further interbreeding. The lack of knowledge of the behavior of Andrias spp., however, impedes development of appropriate conservation strategies. We used biotelemetry to examine seasonal variations in behavioral patterns of seven hybrid giant salamanders in the Kamo River, Kyoto, Japan. We successfully tracked five individuals for one year. Hybrids occupied habitats within 50 m downstream of vertical river structures significantly more frequently during the pre-breeding (May-August) and post-breeding (September-December) seasons than during nonbreeding months (January-April; $\chi^2 = 124.7$, df = 4, P < 0.001). Home range (Kernel Density Estimations; KDE) tended to be larger in September-December (95% KDE: 208.3 ± 70.7 m; 50% KDE: 57.2 ± 34.7 m) but smaller in January-April (95% KDE: 108.1 ± 65.3 m; 50% KDE: 22.7 ± 8.6 m). Water level and season had no significant effects on either distance or direction of movement. Movement was, however, affected by weirs installed to mitigate flooding affects; hybrids traveled upstream over only two of 11 of these structures. We propose leaving intact the river structures in the lower reach and conducting intensive captures at breeding sites and below the river structures during the breeding season to identify and remove introduced or hybrid individuals to limit potential hybridization.

Key Words .- biotelemetry; introduced species; reproductive movement; river ecosystem

INTRODUCTION

The impact of invasive alien species on native species through competitive exclusion, niche displacement, hybridization, introgression, and predation has been widely studied (Mooney and Cleland 2001; Falaschi et al. 2020). When reproductive isolation between invasive and native species is incomplete, there is a risk of native species facing extinction because of the invasion of nonnative species (Allendorf et al. 2001). For example, the high reproductive rate of the invasive subspecies of the fish the Rosy Bitterling (Rhodeus ocellatus) and the fertility and superior fitness of the hybrids have led to the extinction of the native subspecies (Kawamura et al. 2009).

The Japanese Giant Salamander (*Andrias japonicus*) is one of the largest amphibians in the world and is endemic to the western parts of the Honshu, Shikoku, and Kyushu islands of Japan (Kobara 1985). *Andrias japonicus* inhabits various flowing waters, ranging from relatively large rivers to small tributary streams, and plays an important ecological role as a top predator in river ecosystems (Okada et al. 2008). This species is listed as Near Threatened in the International Union for Conservation of Nature (IUCN) Red List (Kaneko and Matsui 2004) and is protected as a Special Natural Monument of Japan.

Chinese Giant Salamanders (A. davidianus) were introduced into Japan in the 1970s (Yoshikawa 2011). Populations of this species were established and subsequent interbreeding with native A.



FIGURE 1. A hybrid Japanese and Chinese giant salamander (*Andrias japonicus* \times *Andrias davidianus*) photographed 29 June 2017. (Photographed by Koshiro Hara).

japonicus created conservation concerns. Hybrid giant salamanders (Fig. 1) have been found in the Kinki, Chugoku, and Tokai regions (Matsui 2017; Sankei Shimbun. 2018. Kuni-sitei no tokubetutennen-kinenbutu ga chugoku-shu ni, Oosansyouuo kouzatu-syu hakken, Okayama. Available no from https://www.sankei.com/article/20180127-TG7VXF2EYNMQVGCZHZY2A6QVHM/ [Accessed 4 July 2023]; Mainichi Newspapers Co. 2023. Tokubetu-tennen-kinenbutu Oosansyouuo, Kouzatu-syu kennai de hatu-kakunin, Seto Jagahoragawa, Aichi. Available from https:// mainichi.jp/articles/20230628/ddl/k23/040/107000c [Accessed 4 July 2023]). Matsui (2017) suggests that the population inhabiting the Kamo River of Kyoto is likely sustained through the breeding of hybrids; whereas, native A. japonicus is on the verge of extinction. The invasion of hybrid giant salamanders into other rivers and continued interbreeding with A. japonicus are profound threats to the conservation of this species. Countermeasures, including the effective capture of hybrid individuals based on the behavior and life cycle of Andrias spp. and their hybrids, are required to minimize further interbreeding of the two species. The movements of giant salamanders in the wild, however, are not well understood.

The breeding seasons of both *A. japonicus* and *A. davidianus* occur between August and September (Taguchi 2009; Liang and Wu 2010). As the breeding season approaches, sexually mature individuals actively search for suitable spawning sites, and *A. japonicus* tends to migrate upstream before the breeding season and downstream thereafter (Taguchi 2009). Additional studies are needed to obtain more detailed information on their movement

during their breeding period, including the effects of anthropogenic and environmental factors.

One possible factor affecting the movement of giant salamanders is the presence of artificial river structures, including dams and weirs. These river structures potentially disrupt the continuity of rivers and have a major impact on the environment (Pringle 2003). Wofford et al. (2005) reported that both natural and artificial barriers decreased the genetic diversity of the Coastal Cutthroat Trout (Oncorhynchus clarki), resulting in increased genetic differences among populations. In contrast, river barriers could minimize the expansion of interbreeding between native and non-native species (e.g., salmonids; Fausch et al. 2006). These river structures also impede the upstream migration of A. japonicus (Taguchi and Natuhara 2009; Takahashi et al. 2016), however, possibly because A. japonicus is unable to climb dams > 80 cm tall without a gently sloped structure over the dam or a detour on the riverbank (Taguchi and Natuhara 2009). Wakabayashi et al. (1976) reported that some A. japonicus individuals that initially stayed below a weir would continue upstream migrations to breeding sites when the salamanders were manually translocated above the obstruction. Thus, physical barriers potentially prevent hybrid giant salamanders from migrating upstream.

In addition, changes in river flow or water levels due to rainfall potentially affect the movement of hybrid giant salamanders. In the Kamo River, hybrid giant salamanders are found often on riversides following heavy rains (pers. obs.), suggesting that they use a terrestrial detour along the riverside to avoid river structures when the water level increases. In contrast, in the Izushi River in Hyogo Prefecture, many A. japonicus occupied the lower reaches of a river after a typhoon in 2004 (Ikuno. 9 Jun 2005. Toyooka Izushi-gawa no tyuuryuu 20 km, Oosansyouuo 200-piki, Ken-kakunin, Taihuu de ryuusyutu ka. The Kobe Shimbun.). Tochimoto et al. (2007) reported that they were washed downstream by flash floods caused by typhoons. Hybrid giant salamanders may, therefore, move downstream as water levels increase.

In the Kamo River, nests and eggs of hybrids have been found in the upstream reaches that flow through mountains, but neither nests nor eggs have been discovered in the lower reaches that flow through urban areas (pers. obs.), even though many hybrids have been found in these lower reaches. Therefore, investigations of behavioral patterns are important to increase our understanding of movement behaviors and how this information can be used to reduce hybridization. If the upstream movement is hindered by river structures, capturing individuals in the lower reaches would likely be less important than capturing them at breeding sites. In contrast, if washed downstream because of increased water flow associated with flooding events, there is the possibility of them entering another river. We tracked hybrid giant salamanders throughout a year using biotelemetry in the lower reaches of the Kamo River to quantified the effects of: (1) season; (2) river structure; and (3) changes in water level on movement patterns and home range size.

MATERIALS AND METHODS

Animals for experiment and field survey.—We used landing nets to capture seven adult hybrid giant salamanders (H1-H7) in the lower reaches of the Kamo River between 15 May and 5 June 2017. We used microsatellite markers to identify individuals as hybrids (Yoshikawa et al. 2011, 2012) and body size (> 80 cm in total length) to determine sexual maturity (Table1). We could not confidently determine sex by external observation; however, we did determine the sex of three individuals (H1, H3, and H4) by examination of gonads via dissection after the tracking survey. We surgically implanted radio transmitters (mass: 44.9-46.6 g; 0.9-1.4% of body mass; Lotek Wireless Inc., Newmarket, Ontario, Canada) into the abdominal cavities according to the procedure described by Tabata et al. (2018). Longterm captivity potentially affects seasonal behavior, including movement; therefore, we implanted transmitters within 24 h of capture and released telemetered salamanders at the capture points within 48 h. We assumed that the surgical incisions of the hybrid giant salamanders healed within one or two months after surgery (Tabata et al. 2018).

The section of the river we studied flows through an urban area. The river in this area is relatively straight and approximately 50 m wide. The substrate consisted mainly of sand and vegetation, such as reeds, which grow along the riverbanks. Weirs, constructed to control river velocity, occurred at regular intervals (100–200 m; Fig. 2). The weirs consist of vertical features, descending from upstream to downstream, with heights of 54.3–84.3 cm. The weirs, riverbeds around them, and the riverbanks on both sides were reinforced with concrete. We identified each weir in the study area with numbers (T0–T12 from the downstream side) and defined the area between weirs



FIGURE 2. A weir in the Kamo River, Kyoto, Japan. The structure is made of concrete, has a height of 54.3–84.3 cm, and is used to reduce the effects of flooding. (Photographed by Koshiro Hara).

as a single section.

We tracked seven telemetered hybrid giant salamanders using a radio receiver (FT-290mkII, Yaesu Musen Co., Ltd., Tokyo, Japan) and a fourelement Yagi antenna. Giant salamanders are nocturnal; consequently, we conducted our surveys after sunset every day for three weeks after release, and about once every 3 d thereafter. We walked along both sides of the river with a four-element Yagi antenna facing the antenna perpendicular to the river flow and recorded the locations on the riverbanks using a GPS device (eTrex 20 J, Garmin Ltd., Schaffhausen, Switzerland) with the strongest signals received from the radio transmitters. Zheng and Wang (2010) reported that reintroduced A. davidianus traversed 200-400 m of a stream reach within 3-10 d, on average. Therefore, we searched for the signal in a maximum of five sections, including the section where the signal was last received in previous surveys and two upstream and downstream sections. If we did not hear a signal at any of the five sections from both riversides, we recorded the attempt as no signal.

Data analysis.—We converted the latitude and longitude data obtained using the GPS device into plane coordinates (Japan Plane Rectangular CS VI, EPSG: 2448) using XYBL TOOL 4 (https:// www.n-survey.com/xybl/). We then projected the locations of the tagged animals onto the midline of each river section, which was defined by connecting the midpoints of two consecutive weirs. When we received signals from the transmitters on both sides of the river, we assumed that the position of the tagged salamander was at the intersection of the lines between the received locations on both sides and the river midline. When only one location was recorded on either side of the river, we considered the position of the salamander to be at the intersection point between a line perpendicular to the river midline from the received location and the river midline. We determined the error in estimating positions of this method by searching transmitters hidden on land and in water as 1.15 ± 0.82 m and 4.72 ± 4.30 m (average \pm standard deviation), respectively.

To quantify the space use of the tracked salamanders, we calculated 95% and 50% Kernel Density Estimations (KDE) of the positions of each salamander as home range and core area, respectively, using the ks (Duong 2007) package in R ver.3.4 (R Core Team 2017). Taguchi (2009) reported that A. japonicus breeds from late August to early September, therefore we estimated home ranges and core areas during four seasons: May-August 2017 (pre-breeding; year of release), September-December 2017 (post-breeding), January-April 2018 (nonbreeding), and May-August 2018 (pre-breeding; year after release). We selected the bandwidth using the plug-in method of Sheather and Jones (1991) and assumed that it was the same for all individuals and seasons.

To examine the effects of river structure, we investigated whether individuals were positioned upstream within sections between weirs and we determined which specific weirs they had traversed. We focused on whether the tracked salamanders were positioned within 50 m downstream of the weir and tested the differences in the ratio between the seasons. We used Generalized Linear Mixed Modeling (GLMM) based on Logistic Regression, which included seasons as explanatory variables and individual IDs as random effects.

We analyzed the effects of water level and season on individual movements using Linear Mixed Modeling (LMM). We evaluated the movement distance as the difference in position over 3 d, calculated as the difference in consecutive positions obtained in the surveys once every 3 d. When the interval of the tracking survey was not 3 d, owing to unfavorable weather and tracking conditions, we calculated the movement distance every 3 d, assuming that the daily movement distance was constant. Before incorporating the response variable into the model, we logarithmically normalized the movement distance data and represented the upstream and downstream movements as positive and negative values, respectively. We considered the effects of water level and season as explanatory variables, whereas those of individual IDs were considered as random effects. For the water level, we adopted the maximum values of water level between consecutive tracking surveys at the Kitayama Ohashi Bridge nearest to our study area available from Kyoto Prefecture River Flood Information (http://chisuibousai.pref.kyoto.jp/main. html?fnm=openMap&no=2&no 2=0). In addition to the LMM, we tested the effects of water level and season on the direction of movement using a binomial GLMM that incorporated binary data (upstream or downstream movement) as the response variable.

We used the lme4 (Bates et al. 2015) package in R for all LMM and GLMM analyses. When the variance of the random effect was negligible, we excluded it from the model and used simple Linear Modeling (LM; essentially the same as Analysis of Variance) or Generalized Linear Modeling (GLM). We used *F*-tests for LM and the Likelihood Ratio Test (LRT) for the GLM. We also tested pairwise differences among four levels of seasons via Tukey's post-hoc comparisons using the multcomp (Hothorn et al. 2008) package when the seasonal effect was significant. For statistical hypothesis testing, we used $\alpha = 0.05$.

RESULTS

We successfully tracked five hybrid giant salamanders for one year following their capture, tagging, and release (Fig. 3). Three of these five individuals (H1, H2, and H4) remained near the upstream areas of the released sections and moved downstream after November 2017 (Fig. 3). Between May and June 2018, H2 and H4 moved upstream over the weir. H3 moved upstream over the weir between June and August 2017 and then gradually moved downstream. H5 moved upstream over the weir when the water level rose in October 2017, and returned to the section where it was released (Fig. 3). We assumed that this individual either died, or that the transmitter was expelled at the end of April 2018, because the estimated position did not change during May 2018 and the received sound reached a maximum when the antenna pointed to the soil along the shore. The estimated positions of the remaining two individuals (H6 and H7) did not change from June 2017 and from August 2017, respectively, and we found their bones underground 8 November 2017. We excluded all data from subsequent analyses after the possible expulsion of the transmitters or death of these salamanders. We directly observed two individuals (H1: 29 June 2017; H2: 8 November

				Home range (95% KDE; m)			Core area (50% KDE; m)				
ID	Total length (cm)	Body weight (g)	Tracking period	M-A 2017	S-D 2017	J-A 2018	M-A 2018	M-A 2017	S-D 2017	J-A 2018	M-A 2018
H1	85.5	3,270	17 May 2017–31 July 2018	45.8	238.7	38.1	46.7	18.3	33.9	15.2	21.7
H2	81.5	3,190	17 May 2017–27 June 2018	63.3	313.1	141.3	125.9	27.0	33.9	30.5	20.1
Н3	87.2	5,000	5 June 2017–31 August 2018	212.7	189.8	193.4	370.7	62.6	57.7	31.5	52.0
H4	93.3	5,040	7 June 2017–30 July 2018	141.3	128.4	119.9	197.8	35.8	43.7	12.8	43.5
Н5	90.6	4,130	7 June 2017–25 April 2018	173.4	171.3	47.8		20.7	116.7	23.5	
H6	84.9	3,220	17 May 2017–8 June 2017	110.0				32.6			
H7	81.4	3,220	7 June 2017– 3 August 2017	127.4				41.6			-
Ave. (SD)				124.9 (58.6)	208.3 (70.7)	108.1 (65.3)	185.3 (138.2)	34.1 (15.0)	57.2 (34.7)	22.7 (8.6)	34.3 (15.9)

TABLE 1. Summary of Kernel Density Estimations (KDE) for home range and core area of hybrid Japanese and Chinese giant sala-
manders (*Andrias japonicus × Andrias davidianus*). Time periods of home range estimates are M-A = May-August, S-D = September-
December, and J-A = January-April. Abbreviations are ID = identification, H1-H7 = hybrids 1–7, and Ave. = average.



FIGURE 3. (Upper panel) Movement of hybrid Japanese and Chinese giant salamanders (*Andrias japonicus* × *Andrias davidianus*) along the Kamo River, Kyoto, Japan. Horizontal lines indicate the locations of drop structures (weirs; T0-T12). White, light gray, and gray background colors indicate pre-breeding, post-breeding, and non-breeding seasons, respectively. (Lower Panel) the change in water level of the river.

2017) during tracking and confirmed that there was no surgical dehiscence, and that the transmitters were not expelled. At the end of tracking, we recaptured three individuals (H1, H3, and H4) and confirmed that the surgical incisions were fully closed, although the antenna of the transmitter was partly exposed from the closed surgical incision in H1. Upon dissection, we found a transmitter in only one (H1) of the three salamanders, where it adhered to adipose tissue.

The size of home ranges varied widely among individuals but tended to be large during the postbreeding season in September-December (95% KDE: 208.3 ± 70.7 m; 50% KDE: 57.2 \pm 34.7 m) and small during the non-breeding season in January-April $(95\% \text{ KDE: } 108.1 \pm 65.3 \text{ m}; 50\% \text{ KDE: } 22.7 \pm 8.6 \text{ m};$ Table 1). The use of space within 50 m downstream from weirs varied significantly by season (LRT, $\chi^2 =$ 124.7, df = 4, P < 0.001); the individuals used this area significantly less frequently during the nonbreeding season than during the other seasons (Z= 8.287 - 10.23, all Ps < 0.001; Fig. 4). Upstream and downstream movements over weirs occurred 11 and 21 times, respectively. Upstream movement occurred across only two weirs (T7 and T8), whereas downstream movement occurred across 11 weirs (T1-T11; Fig. 3).

The maximum distances of upstream and downstream movements per 3 d were 260.6 m and 386.3 m, respectively, although 50.9% of upstream

and downstream movements were 10 m or less (346 of 680 movements; Fig. 5). Because both the LMM for movement distance and the GLMM for movement direction indicated that the random-effect variances were close to zero, we tested the models as LM and GLM, respectively. In both models, the water level (LM: $F_{1,691} = 1.59$, P = 0.208; GLM: $\chi^2 = 0.39$, df = 1, P = 0.533) or season (LM: $F_{3,691} = 0.180$, P = 0.910; GLM: $\chi^2 = 0.63$, df = 3, P = 0.891) had no significant effects. When the water level increased, owing to typhoons or heavy rains, only one individual (H4: 5–9 July 2018) moved over 130 m downstream, and two individuals moved upstream over a weir under similar conditions (H3: 7 August 2017; H5: 22 October 2017).

DISCUSSION

We succeeded in tracking hybrid giant salamanders throughout the year, although two individuals died within 3 mo of their release. Tabata et al. (2018) confirmed that hybrid giant salamanders in captivity can survive for at least 3 mo after surgery. We assume that one of our telemetered salamanders (H6) died as a result of its burial underground associated with riparian work using heavy machinery carried out in the release section. We found the transmitter and part of the skeleton underground at the location of the riparian civil engineering work. This observation



FIGURE 4. Differences in the probability of observing hybrid Japanese and Chinese giant salamanders (*Andrias japonicus* × *Andrias davidianus*) \leq 50 m of weirs along the Kamo River, Kyoto, Japan, among seasons (bold bars, boxes, and whiskers indicate median values, 50% quartiles, and ranges, respectively). Different characters above the boxes indicate significant differences.



FIGURE 5. Histogram of movement distance per 3 d of hybrid Japanese and Chinese giant salamanders (*Andrias japonicus* \times *Andrias davidianus*) along the Kamo River, Kyoto, Japan. Positive and negative values indicate upstream movement and downstream movement, respectively.

suggests that riparian work could be a threat to Japanese Giant Salamanders. The cause of death for the other individual (H7) is unknown, but we found the transmitter and some bones under plants along the riverbank. We cannot exclude the effects of surgery, but fighting among males is another possible factor associated with mortality of our study animals. The population density of hybrid giant salamanders is very high, and injured individuals are common in the study area, probably because of fights between males. The presumed time of death for this individual was early August 2017, when fights between males were likely to increase before the breeding season (Tochimoto 1995).

Our study shows that hybrid giant salamanders in the lower reaches of the Kamo River tended to stay in the upstream area of a section (i.e., within 50 m downstream of a weir) during the pre-breeding (May-August) and post-breeding (September-December) seasons, whereas hybrids stay in the mid-downstream area within a section during the non-breeding season (January-April). Home ranges tend to be larger during the post-breeding season than during the non-breeding season. Moreover, three salamanders moved downstream during the post-breeding season. These movement behaviors indicate a similarity in the movement of hybrid giant salamanders to A. japonicus, which usually moves upstream during the pre-breeding season, downstream during the postbreeding season, and does not move much during the non-breeding season (Taguchi 2009). The similarity in reproductive migration between hybrid giant salamanders and A. japonicus supports the claim of Matsui (2017) that backcross breeding occurs in the Kamo River.

Weirs apparently limit upstream movement of hybrid giant salamanders during the pre-breeding season. Hybrids moved upstream across only two weirs (T7 and T8). One of these weirs was damaged (the height of T7 was reduced because a V-shaped portion was missing), and sediment and thick vegetation accumulated on the left bank of T8. The reduced height of these two weirs, approximately 35 cm, allowed salamanders upstream passage by climbing over the weirs and grasping either broken, ragged sections or thick plants. Taguchi and Natuhara (2009) and Takahashi et al. (2016) reported that artificial river structures hamper the breeding migration of A. japonicus; however, irregular surfaces on weirs provide footholds allowing giant salamanders to traverse barriers. We observed H3 fail to climb a weir 2 July 2017. Moreover, we captured

H1 13 August 2017 below a weir and confirmed it to be sexually mature (the area around cloaca opening was swollen and doughnut-shaped, characteristic of mature males during the pre-breeding season). Therefore, weirs potentially prevent hybrid giant salamanders from moving upstream to suitable breeding sites in the upper reaches of the Kamo River.

Our data do not indicate any effect of water level or season on the distance or direction of movement of hybrid giant salamanders. Hybrids generally moved < 10 m/3 d, an indication that they potentially exhibit sedentary behavior, as observed in A. japonicus (Tochimoto 1992) and reintroduced A. davidianus (Zheng and Wang 2010). Additionally, when typhoons and heavy rains increased water level and flow, some hybrids exhibited upstream movements across weirs, but no large downstream movements. Therefore, when the water level rises, hybrid giant salamanders may take refuge at locations where the current is slow, or where there is land along the shore. We observed a giant salamander, which was not telemetered, on land along the shore immediately after the typhoon 29 October 2017. This observation is in contrast to the aggregation of individuals in the downstream area after a typhoon in the Hyogo Prefecture (Ikuno. 9 Jun 2005. op. cit.). Our observations suggest that giant salamanders are seldom washed downstream by heavy rainfall. In contrast, they may aggregate at weirs to traverse them, or take detours by land, taking advantage of rising water levels. Therefore, individuals that aggregate on the downstream side of the structures in Hyogo Prefecture might not have come from upstream, but from downstream. Because of our small sample size and the possibility of water levels increasing beyond those observed during the study, more extensive and long-term monitoring with more salamanders is necessary to investigate the movement patterns of hybrid giant salamanders and determine the effect of flooding on their movement.

Our study indicated that weirs potentially limit hybrid giant salamanders in the lower reaches of the Kamo River from moving upstream. We did not find any spawning nests or eggs of hybrid salamanders in the study area, although they were found approximately 8 km upstream of the study area (pers. obs.). We suggest that river structures likely prevent hybrids from moving to suitable breeding sites. This is the case for isolation by river structures, which contribute to the prevention of interbreeding, as observed by Fausch et al. (2006). River structures may also limit native *A. japonicus* from moving upstream. Therefore, it is necessary to establish sidewalls or detours around river structures where interbreeding does not occur. In rivers where interbreeding is occurring, it may be necessary to transport only *A. japonicus* to the upper section of the river to prevent hybrid giant salamanders from moving upstream during breeding. To effectively capture hybrid giant salamanders in the Kamo River, we propose leaving weirs in the lower reaches intact and conducting a thorough survey near weirs and breeding sites during the breeding season. Hybrid giant salamanders may gradually move downstream because they are prevented from moving upstream by weirs; thus, it is also necessary to conduct regular surveys around confluences with other rivers to prevent further expansion of interbreeding.

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