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Determinants of predator-prey interaction on salmonid fish species and their survival rate

（サケ科魚類の捕食・被食関係と
生残率決定要因の解明）

Division of Environmental Resources, Graduate
School of Agriculture, Hokkaido University

北海道大学　大学院農学院

環境資源学専攻　博士後期課程

Kouta MIYAMOTO

宮本　幸太

Contents

Abstract

Predator-prey interaction is one of the key factors in ecology. In this study, I aimed to understand the mechanisms of predator-prey interaction on salmonids, and how they are affected by target species and physical habitat. This paper has four Chapters: (1) evaluating the size-selective predation on fish by piscivorous fish and (2) that by riparian wildlife, (3) fish trait variations on their predation risk, and (4) evaluating the effects of physical habitat on predator-prey interaction.

In Chapter-1 my results show that not only predator and prey sizes, but also the interaction between said sizes strongly affected the predation risk of stocked salmon juvenile. Specifically, the survival rates of stocked salmon were extremely low ($\leq 10\%$) when their body size was less than 30% that of predator fish. In Chapter-2 the results suggest that the primary predator in the experimental area was the grey heron, and that their predation resulted in a significantly lower survival rate of large salmon juvenile compared to the small ones. Chapter-3 elaborates on the fact that the most critical determinant for the survival of juvenile fish was their behavior under normal conditions, rather than their hiding behavior after the stimulus. One of the possible reasons for this could be the fact that the primary predator in the experimental area was an ambush predator (grey heron). In Chapter-4 my results show that declining water levels and loss of structural complexity can increase the predation risk of stocked fish by making the process of hunting easier for the grey heron. These results suggest that the predator-prey interaction in my experimental settings is mainly determined by a combination of predator and prey traits, whereas the intensity of predator-prey interaction is mainly determined by physical habitat.

General introduction

Predator-prey interaction is one of the key factors in animal ecology. Understanding predator-prey interaction is essential for the study of ecosystems and evolution in general. As the reasons, predation has a critical effect on ecosystem structure and function (Berger et al., 2001; Hawlena and Schmitz 2010). Predators often control the dynamics of prey populations through their influence on survival (Skelly 1994), growth (Peckarsky et al. 1993), behavior (Werner et al. 1983), size structure (Hall et al. 1976) and distribution (Beauchamp et al. 2007). Meanwhile, prey animals adjust their traits and behaviors as a response to predation risk (Kishida and Nishimura 2004; Bongi et al. 2008; Sönnichsen et al. 2013). Therefore, understanding the mechanisms of predator-prey interaction is necessary for the broader study of animal ecology.

Juvenile fish are vulnerable to predation everywhere and in different manners (e.g. wading birds at the beach, piscivorous fish at reefs; Gibson and Robb 1996; Connell 1998). Predation by riparian wildlife is also widely recognized as a key factor affecting the survival of fish in stream ecosystems (Draulans 1987; Kruuk 1995; Roby et al. 2003; Steinmetz et al. 2003; Sahashi and Yoshiyama 2015). Predation pressure on juvenile salmon, in particular, is of broad interest because of their economic and social values. Declines in salmonid populations as a result of predation by riparian wildlife have been reported not only for stocked salmon populations but also for wild salmon populations (e.g., Osterback et al. 2013; Frechette et al. 2015). It is also noteworthy that stocked salmonids are often intensively preyed upon by fish predators (McCrimmon 1954; Symons 1974; Pepper et al. 1985), bird species (Wood 1987; Martel and Dill 1995; Harvey and Nakamoto 2013), and mammals (Roberts and Garcia de Leaniz 2011). Hatchery-reared salmonid fish are routinely stocked in natural waterways as part of

conservation, propagation, and stock enhancement programs (Brown and Laland 2003; Salvanes and Braithwaite 2006; Naish et al. 2007; Fraser 2008). Therefore, if I can implement a management strategy that decreases local predation pressures on salmon juveniles by understanding the predator-prey interaction between predator animals and stocked salmonid, it will contribute to an improvement of these programs.

Studies examining the predator–prey interaction on fish species have been generally based on either investigation into the predation pressure of wild animals without observing fish responses (Draulans 1987; Post et al. 1998; Harvey and Nakamoto 2013) or these into the mortality rate of fish without observing the behavior of predators (Penaluna et al. 2016). On the other hand, predation risk is influenced by many factors, including conditions of fish (Hostetter et al. 2012), fish migrations (Roberts et al. 2009), the type of local predators and their abundance, the duration of the predators' sojourn (Gawlik 2002; Steinmetz et al. 2003), their habitat [e.g., water depth (Kushlan 1976) and structure complexity (Penaluna et al. 2016)]. However, it is still not clear how these factors fit together to form the predator-prey interaction between predator animals and stocked salmonids. In order to understand the mechanisms of predator-prey interactions, it is necessary to first develop a profound understanding of the interactions between the traits of predators and prey relative to their environment. For example, size of prey fish needs to be taken into consideration in the context of the type of predator and their relative sizes because food size preference depends on predator species (Carss and Marquiss 1991; Sogard 1997) and because the ability of prey to avoid predation depends on size (Dill and Fraser 1984). However, the number of studies on these relationships is limited partly owing to the difficulties in identifying local species of riparian predators and directly observing their predation activities. These surveys are further complicated

because of varieties of cause of migration exhibited by predator species, such as migration for feeding and nesting needs (Collis et al. 2002), migration due to changes in prey densities (Kushlan 1976; Gawlik 2002), and migration resulting from human disturbance (Klein 1993).

Recently, many ecological tools have been developed for better understanding predator-prey interactions. Camera trapping is one of them. Camera trapping that uses fixed cameras, triggered by infrared sensors, to 'trap' images of passing animals has recently provided the opportunity for researchers to collect more detailed information about predators, including species identity, daily presence, appearance frequency and duration of sojourns (Silveira et al. 2003; Wegge et al. 2004). Because camera trapping is a non-invasive technique, it causes minimal environmental disturbance (Henschel and Ray 2003; Silveira et al. 2003). In this dissertation, I conducted a predation test using camera traps on outdoor experimental tanks and a semi-natural stream to investigate the relationships between the predator species, their behavior and traits of stocked salmon.

This dissertation has four Chapters. Chapter-1 covers the evaluation of size-selective predation on masu salmon by piscivorous fish, based on my investigation into the size effects of both predator and prey on the predation risk. Chapter-2 deals with the size-selective predation on masu salmon by riparian wildlife, based on my investigation into the predation risk of stocked masu salmon in outdoor experimental tanks and a semi-natural stream using the camera trapping. In Chapter-3, I inclusively evaluated the effects of fish traits of masu salmon juveniles, including their origin [F1 (fish was raised from eggs obtained from adults that had migrated into river) and F11 (fish was raised for about 11 generations under artificial environment)], behavior (normal and alarmed) and body sizes on their predation risk. Chapter-4 covers the effects of habitat on the predation risk

of fish, based on my investigation into the relationship between water depth, habitat structure complexity and predation risk of white-spotted charr. Finally, in the general discussion and conclusion of this dissertation, I discussed the mechanisms of predator-prey interaction and the effect of physical habitat on the predator-prey interaction.

Chapter-1: *Differentiated predation risk on hatchery-reared juvenile masu salmon by white-spotted charr with different body sizes*

Introduction

The survival rate of stocked salmonids is positively related to their body size at release. This applies to salmonids like coho salmon *Oncorhynchus kisutch* (Hager and Noble 1976; Bilton et al. 1982), chinook salmon *O. tshawytscha* (Martin and Wertheimer 1989; Unwin 1997) and cutthroat trout *O. clarki* (Tipping 1986; Tipping and Blankenship 1993) [for contrary results – steelhead *O. mykiss* (Osterback et al. 2013)]. Similar results were also obtained in masu salmon *O. masou*, leading Miyakoshi (2006) to conclude that predation risk decreases as the body size of stocked masu salmon increases. However, the body size of predator species, which might strongly influence the survival rate of stocked salmon, has not yet been fully taken into account in previous studies of this kind. In fact, some studies indicated that predation losses of stocked salmon by resident salmonids in a river would be high (McCrimmon 1954; Symons 1974; Pepper et al. 1985; Henderson and Letcher 2003). Masu salmon are typically anadromous, although a stream resident types also exists. Anadromous masu salmon spend a variable period in freshwater before migrating to the ocean as smolts. In Japan, masu salmon typically stay in freshwater for 1–2 years before the outmigration (Hayano et al. 2003). Thus, their predation risk at the freshwater phase is expected to be high. In Japan, the white-spotted charr *Salvelinus leucomaenis* is one of the major resident predatory fishes, which often inhabit the same rivers and streams as masu salmon (Nakano 1995a; Kubota et al. 2001; Miyasaka et al. 2003) and indeed prey on them (Ueno et al. 2009). In Chapter-1, in order to understand the predator and prey body size effects on predator-prey interaction, I investigated size-selective predation on masu salmon in experimental settings.

Materials and methods

Test fish

The test fish used in the experiments were hatchery-reared masu salmon from the Shobu-shimizu River that flows into the Lake chuzenji in Tochigi Prefecture (approximately 36°45′N, 139°27′E) and hatchery-reared white-spotted charr from the Sabi River in Tochigi prefecture. It is commonly believed the masu salmon in Lake Chuzenji and its tributaries is an admixture of the two subspecies (*O. masou masou* and *O. masou* subsp.), often called "Honmasu" (Yamamoto et al. 2010). Eggs were obtained from mature adults which migrate into the Shobu-shimizu River and were handled according to standard hatchery procedures dictated by the National Research Institute of Fisheries Science (NRIFS) facility at Nikko. Young-of-the-year (YOY) (n = 520) and one-year-old (OYO) (n = 520) salmon were prey, while two- (n = 6) and three-year-old (n = 11) charr were predators used in this Chapter. The YOY salmon were selected from eight rearing tanks (50 cm width × 120 cm length × 20 cm depth), while the OYO salmon were selected from two large tanks (1.5 m width × 3.0 m length × 0.9 m depth). Two- and three-years-old charr were selected from two separate ponds (2.2 m width × 5.7 m length × 1.0 m depth).

Tank experiment

To evaluate the size effects of both species on the survival rate of masu salmon, the experiments were conducted at the NRIFS facility in Nikko in three outdoor FRP circular tanks (1170 mm in diameter, 500 mm in depth, 400 mm water depth). Each tank was covered with a 5 mm mesh net to avoid fish jumping out, with water (9 °C) pouring in at a rate of 6 L/min. Four different size ranges (fork length or FL hereafter) of masu salmon were used as prey fish: FL 55–75 mm (mean ± SD 62.4 ± 2.3 mm), FL 80–100 mm (89.2

± 2.1 mm), FL 105–125 mm (117.9 ± 5.1 mm) and FL 130–150 mm (139.8 ± 3.7 mm). The YOY fish were FL 55–75 mm and FL 80–100 mm, while the OYO fish were FL 105–125 mm and FL 130–150 mm. On the other hand, white-spotted charr of three different size ranges were used as the predator fish: small (FL, mean ± SD, 162.0 ± 1.4 mm), medium (250.5 ± 8.7 mm) and large (327.8 ± 6.6 mm). The two-years-old charr were used as small predators, while the three-years-old charr were used as medium and large predators. Two white-spotted charr of the same size range were placed in each tank along with ten masu salmon.

The white-spotted charr were acclimatized to the experimental tank 7 to 10 days before the experiment. Food was withheld from predators 24 h before the experiments to standardize their initial hunger levels. When the predator size would increase more than 5 mm during the experiment, the predator fish would be replaced. Each predator-prey size combination (4 FLs of masu salmon × 3 FLs of white-spotted charr) was replicated three times, bringing the total number of experiments to 36. Each experiment was conducted for 24 h, starting at 09:00 h on the first day, between July 18 to August 21, 2014. The survival rate of masu salmon was calculated at the end of each experiment to quantify the predation by white-spotted charr. Relative body size of each combination between masu salmon and white-spotted charr was calculated as follows:

Relative body size (%) = mean FL of masu salmon / mean FL of white-spotted charr × 100

Statistical analyses

Two-way ANOVA and the Bonferroni post hoc tests were used to evaluate the effects of predator and prey fork length class on the survival rate of salmon in the tank test. Data

was arcsine $\sqrt{x}$ transformed prior to analysis (Lundvall et al. 1999). When a significant interaction effects was found, a simple main effect analysis was used, as described by Ishimura (2006). Data analyses were generated using IBM SPSS software (version 21.0 of the IBM SPSS Statistics for Windows). A scatter diagram with the survival rate of masu salmon and the relative body sizes was fitted to a logistic curve using the R (3. 3. 2) software. The relative body size at 50% survival rate was then calculated.

Results

The results of the two-way ANOVA suggested that FLs of masu salmon and white-spotted charr, including their interactions, had significant effects on the salmon survival of salmon ($P < 0.010$, Table 1). As for the size of masu salmon, the survival rate of FL 55–75 mm masu salmon was significantly lower than that of the FL 105–125 and 130–150 mm ones, regardless of predator size ($P < 0.001$ with Bonferroni test, Fig. 1; Table 2). On the other hand, there were no significant differences between the survival rates of masu salmon with FL 105–125 mm and FL 130–150 mm masu salmon (Bonferroni test; small predator, $P = 1.000$; medium predator, $P = 0.910$; large predator, $P = 0.196$). When it comes to the FL 80–100 mm masu salmon, there was no significant difference in survival rates compared to the other sizes, when placed together with the small predator fish (Bonferroni test; FL 80–100 mm v.s. FL 105–125 mm, $P = 0.400$; FL 80–100 mm v.s. FL 130–150 mm, $P = 0.074$; FL 105–125 mm v.s. FL 130–150 mm, $P = 1.000$). However, placed with medium or large predator fish, the FL 80–100 mm masu salmon had significantly lower survival rates compared to the larger masu salmon (Bonferroni test, all $P < 0.010$).

Regarding the size of white-spotted charr, the survival rates of masu salmon with FL ≤100 mm were significantly lower when going up against the large or medium size predators than the small ones ($P < 0.01$ with Bonferroni test, Fig. 1; Table 3). The survival rate of FL 80–100 mm salmon was also significantly lower with the large predators than with the medium predator fish (Bonferroni test, $P < 0.010$). There were no significant differences in survival rates of masu salmon with FL ≥105 mm when going up against medium or large sized predators (Bonferroni test; 105–125 mm, $P = 0.200$; 130–150 mm, $P = 0.804$). The survival rates of masu salmon were ≤10%, when they had a relative body

size lower than 30% of their predator (Fig. 2). On the other hand, the mean survival rates of masu salmon became ≥80%, when they had a relative body size greater than 40% of their predator. The logistic regression resulted in 50% survival of masu salmon when the relative body size of masu salmon was 37.0% of their predators.

Discussion

The upper limit of prey fish size is known to be limited by the relative size to the predator fish. The maximum size of a fish taken by a predator fish was reported to be c.a. 50% of the predator's size (Popova 1978). Similarly, steelhead trout *O. mykiss* (Martin et al. 1993), coho salmon (Pearsons and Fritts 1999) and brown trout *Salmo trutta* (Mayama 1999) can consume salmonids up to 42, 46 and 45.3% of their body size, respectively. However, these studies focused on the upper limit, and they did not take into account the relationship between the relative body size and the number of preys that have been eaten. In order to define the appropriate size of hatchery-reared salmonid fish at release from viewpoints of production cost and predation risk, evaluating the relative body size that decreases the number of salmon preyed upon could be more important than finding the upper limit. In this Chapter, I found that masu salmon exceeding 40% of the predator's size were rarely eaten, and the threshold relative body size was 37%. These results are consistent with the results of a field study that showed the relative size of most individuals of salmonids eaten by c.a. 10,000 coho salmon was under 40% (McConnaughey 1998). Thus, it is realistic to assume that hatchery-reared masu salmon whose relative body size is close to 40% at release will significantly decrease their predation risk, at least from white-spotted charr in the wild. Generally speaking, the lower prey size limit can be set, both by difficulties in retaining the prey (Persson 1987) and by the predator's ability to detect the prey (Breck and Gitter 1983). Capture success has been shown to strongly depend on the relative sizes of predator and prey (Miller et al. 1988; Fuiman 1994) and has generally been suggested to decrease monotonically with prey size (Fuiman and Magurran 1994). In this Chapter, however, the mean survival rate of masu salmon whose relative body sizes were lower than 30% was significantly lower than that of other size

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groups of masu salmon. Therefore, in a case where hatchery-reared masu salmon with FL ≥55 mm were released into a river, the predation risk on the released salmon by white-spotted charr could be high.

In some cases, the body depth of prey fish and predator's gape size were important parameters to estimate the prey size that predator fish can eat (e.g., largemouth bass *Micropterus salmoides* preying on pumpkinseeds Lepomis gibbosus, and pike Esox lucius L. preying on perch *Perca fluviatilis* L. (Hambright 1991, Magnhagen and Heibo 2001). In a case that the predator fish preys upon a slender fish, however, prey body length, rather than prey body depth, might have a bigger impact on the predation risk (Dörner and Wagner 2003). It is suggested that longer fish were hardly preyed on because of their increased swimming performance as they grow (Taylor and McPhail 1985; Brodeur 1991; Lundvall et al. 1999). Similarly, when salmonids prey upon slender fish, the prey body depth did not provide as a good measure of predation risk as prey length (Beamish 1978). It is also suggested that salmon recognize prey body length, based on the fact that coho salmon changed the number of attacks to lures that imitated salmon fry according to the length of the lures and made fewer attempts to attack longer lures (Pearsons and Fritts 1999). These results indicate that the body length of predator and prey are better parameters to use when estimating the predation risk of stocked salmon than the prey's body depth and predator's gape size when it comes to the predator-prey relationship between salmonids.

In Japan, the range of body weight of YOY masu salmon stocked in rivers are about 1–5 g (Nakamura and Iida 2009; Tomida et al. 2016), which is slightly smaller than FL 55–75 mm salmon. FL of white-spotted charr is often about 200–250 mm in rivers around central Japan (Yamamoto et al. 1999; Yagyu et al. 2007). This body size of white-

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spotted charr corresponds to the medium-size predator fish in this Chapter. My results suggest that the survival rate of masu salmon with FL 80–100 mm (relative size is over 30%) is much higher than that with FL 55–75 mm (relative size is under 30%) when placed with medium-size predator fish. Therefore, I expect that the number of masu salmon caught by white-potted charr could be significantly reduced if the body size of YOY masu salmon is further increased before release. There are anadromous white-spotted charr with a body length of over 300 mm in the rivers of north Japan (Yamamoto et al. 1999; Morita et al. 2000). In this case, releasing OYO masu salmon could be effective for reducing their predation risk in the rivers in this region. These results imply that the relative body size is an important component of predator-prey interactions.

The predation risk on masu salmon by white-spotted charr was evaluated in this Chapter, but my result does not necessarily mean that the predation on masu salmon by white-spotted charr dramatically decreases masu salmon numbers in the wild. The period of predation on stocked salmon fry appears to be short [e.g., 2 days long in the case of Atlantic salmon fry (Henderson and Letcher 2003)], so my 24 h experiments might have measured the strongest predation impacts. In the wild, considerations must be made for the impact of predation by other salmonid species, such as OYO masu salmon, brown trout (Mayama 1999) and rainbow trout (Taniguchi et al. 2002). On the other hand, the effect of stocking masu salmon might be a serious problem for YOY white-spotted charr, because the body size of YOY masu salmon is bigger than that of YOY white-spotted charr, and because their habitat is overlapping (Hasegawa and Maekawa 2009). Therefore, in order to fully understand the predator-prey relationship between masu salmon and white-spotted charr, further studies will be needed on the size-structured interaction between both species [see Taniguchi et al. (2002)].

In this Chapter, I reported the results of a predation experiment in an artificial tank environment. In natural environments, the salmon's ability to use shelter (Gregory 1993; Reinhardt 1999; Blanchet et al. 2007), mimic (Miyamoto 2016a) and swim (Plaut 2001) are expected to have a strong effect on the predation risk. Therefore, it is necessary to clarify how much of each factor will affect predation risk in a natural environment. In addition, stocked salmonids can be caught by many other predators; mammals (Aarestrup et al. 2005), birds (Alexander and Shetter 1969; Ayles et al. 1976; Myers and Peterka 1976; Wood 1985, 1987) and other fish species like sculpins (Hunter 1959). Thus, further studies are required to evaluate carefully the most appropriate size of masu salmon at release for reducing the predation risk as a whole.

Chapter-2: *Experimental evaluation of predation of stocked salmon by riparian wildlife: effects of prey size and predator behaviors*

Introduction

In Chapter-1, I discussed the size-selective predation by piscivorous fish. Stocked salmonids can not only be caught by piscivorous fish, but also by mammals (Aarestrup et al. 2005) and birds (Alexander and Shetter 1969; Ayles et al. 1976; Myers and Peterka 1976; Wood 1985, 1987). To evaluate the effects of predation by riparian animals inclusively, it is necessary to evaluate the impact of predation by riparian animals, including mammals and/or birds, not just piscivorous fish; their behaviors and their preferred size of prey in a place that allows for free movement of all parties. Therefore, in Chapter-2, I conducted a predation test using camera traps, outdoor experimental tanks and a semi-natural stream to investigate the relationships among the predator species, their behavior and the size of stocked salmon.

Materials and methods

Test fish

The test fish used in the experiments were hatchery-reared masu salmon (*Oncorhynchus masou*) juveniles from the Shobushimizu River and the Jigoku River in Tochigi Prefecture, Japan (~36°45 'N, 139°27 'E). Eggs were obtained from mature adults that had migrated into the Shobu-shimizu River and the Jigoku River, which were handled according to standard hatchery procedures as directed by the National Research Institute of Fisheries Science (NRIFS) facility at Nikko. In the NRIFS, experimental stock enhancement of masu salmon has been conducted using both hatchery- and wild-born fish since 1886. Artificial crosses were conducted to produce approximately 100 000 fertilized eggs from 70 females and 27 males in 2011, and approximately 45 000 eggs from 53 females and 20 males in 2012 for an experimental stock enhancement program. I used a portion of these eggs (~2000 eggs) for the experiments detailed herein. During the rearing period, to prevent fish diseases, the eggs and fry were treated with povidone-iodine and salt baths respectively. No serious diseases or unusual mortality rates were observed in the experimental population. Young-of-year (YOY; n = 305) and 1-year-old (OYO; n = 305) salmon were used in this Chapter. The YOY and OYO salmon were graded from five rearing tanks (50 cm wide × 120 cm long × 20 cm deep; water supply set at 18 L min^{-1}) and a large tank (3 m wide × 1.5 m long × 0.9 m deep; water supply set at 150 L min^{-1}) respectively. The YOY fish used were 75–100 mm in length [75–100-mm fork length (FL) group hereafter] and the OYO fish used were 135–160 mm long (135–160 mm FL group hereafter). The body size ranges were within the average for masu salmon stocked in the Shobu-shimizu River and the Jigoku River. In addition, the size range of YOY masu salmon is recommended as an effective body size for preventing predation by piscivorous

fish in rivers around central Japan (Miyamoto and Araki 2017). Before the start of the study, fish were fed daily food rations (commercial trout pellets) equal to 1.5–2.0% of their estimated bodyweight.

Tank experiment

Predation tests were conducted in four outdoor tanks for 3 days from August to September 2013. Four fiber-reinforced plastic (FRP) circular tanks (diameter 120 cm, height 15 cm) at NRIFS in Nikko were used in a forested and grassy area and each tank had a thin layer of natural gravel (2–7 cm of gravel and ,13 cm of cobble substrate) on the bottom (Fig. 3a). A circular roof fence (10 cm high in an upright position and an additional 8 cm in length at an angle over the tank) was installed in each tank, both to provide the fish with cover and to prevent them from jumping out. A camera (Trophy Cam HD; Bushnell, Overland Park, KS, USA) was set in the south side of each tank to monitor the entire tank. The cover and gravel substrate were provided so that the masu salmon could show near-natural behavior, allowing them to hide and 'escape' (Miyamoto 2016a), thus minimizing experimental stress. During the study days, the stocked fish were fed daily food rations (commercial trout pellets, ~3 × 4 mm, were scattered all over the tank) equal to ~2.0% of the total bodyweight of all the fish in a particular tank. Spring water, mean (± s.d.) temperature $10.2 \pm 0.38°C$, was introduced into each tank at a rate of 6 L min^{-1}. Two different size groups of masu salmon were used as prey fish, a 75–100-mm-FL group (mean ± s.d., 91.9 ± 7.0 mm FL) and a 135–160-mm-FL group (mean 145.8 ± 6.8 mm FL).

To evaluate the size-selective predation risk, 30 fish (15 from each size group) were placed in each tank. The number of fish of each from each size group that survived

was counted each day for 3 days. The survival rate of salmon was then compared between the two size groups. To identify the predator animals, the number of photographs taken by the camera trap (see below) was counted. Next, to investigate the relationship between the number of stocked salmon and the frequency at which predator animals appeared, three FRP circular tanks were used. These experiments were conducted sequentially using the YOY and OYO fish. In the experiments, the three tanks were stocked with different densities of salmon (10, 5 and 0 individuals). The order of tanks (the density of fish) was randomly selected for each experiment. Three days later, the number of photographs of potential predators taken by the camera trap (see section below) was counted for each tank. This trial was replicated three times for each size group.

Stream experiment

The stream predation test was conducted for 20 days from August to September 2013 using a semi-natural stream (mean ± s.d., 120 m long, 95.8 ± 4.3 cm wide; 2% gradient in a forested and grassy area at NRIFS; Fig. 3b). The stream was constructed of stone, wood and soil. Spring water (mean ± s.d., temperature 10.2 ± 0.38°C) was supplied at a rate of 18 L s^{-1}. The stream was forked equally to allow free passage into four 30-m sections (Sections 1–4 from upper to lower). Each section contained three pools and three riffles. The pools were ~80 cm long and ~60 cm wide, with a maximum depth of ~40 cm. Woody debris (~70 cm long, ~20 cm wide) were placed along the sides of each pool. The riffles were 9–12 cm deep and contained a mixture of 2–7 cm of gravel and ,13 cm of cobble substrate. A 50-cm waterfall was built in the top of the stream with two metal gates (mesh 12 cm) at the top to prevent fish from escaping. The water drain was separated by a metal mesh gate (mesh 1 × 2 cm), with the bottom quarter of the gate covered with

plastic mesh (8 × 8 mm). A total of 400 fish (200 from each size group) was stocked in Section 2 after their FL and bodyweight had been measured. The point-stocking method is the most common stocking method in Japan; therefore, this method was used as part of the experimental design. During the study period, for the purposes of maintaining the semi-natural stream and confirming which fish died from a disease or jumping out, I checked the said stream daily from an overlooking hill.

To investigate the distribution of salmon in the stream, the fish in each section were closed in by fish block nets (mesh 8 × 8 mm) at the end of the experiment to prevent them from moving between the sections. Then, a backpack electrofishing unit (Model 12-A POW Electrofisher, 300 V; Smith-Root, Vancouver, WA. USA) was used to remove and count the fish that had survived. The electric shock was repeated until the fish count was zero twice in a row. When fish were caught, each fish was lightly anaesthetized with 100-ppm 2-phenoxyethanol (Wako Pure Chemical Industries, Tokyo, Japan), its FL and bodyweight measured and the section the fish had been caught in recorded. To identify the predator animals, three cameras (Trophy Cam HD; Bushnell) were arranged in each section and placed at 10-m intervals along the riverside to monitor both sides of the stream. The number of photographs taken by the camera traps (see below) was counted for each section. To estimate the predation behavior of wild animals, the photographs containing potential predators were recorded for the position (at pool or riffle) and time [morning (0300–0900 hours), day (0900–1500 hours), evening (1500–2100 hours) and night (2100–0300 hours)] the predator was observed. Then, to investigate the position that predators used, the proportion of predators located around pools or riffles was calculated in each section, and the average proportions were then compared.

Camera trap

To assess predator encounters during the day and night, potential predators were recoded using motion and infrared sensor camera traps in the tank experiments and the semi-natural stream experiment. Each camera was mounted on a wooden stake so that the camera was 50 cm above the water's surface. Cameras were triggered with a passive infrared motion sensor; the camera was set to wait 15 s after an initial trigger entered its sensor range before attempting to detect additional triggers. To identify predators and estimate the frequency of their visits to the study site, all photographs containing potential predators were checked by the same researcher (KM). For some ambiguous species identifications, additional checking was performed by a local wildlife expert (T. Takeda, Nikko National Park). When more than one potential predator was captured in a photograph, the species and the number of individuals were recorded. In addition, the number of photographs showing predators capturing or eating fish was counted, and the predator species recorded.

Statistical analyses

In the tank tests, two-way analysis of variance (ANOVA) was used to determine the effects of study days and fish FL on the survival of salmon. In addition, a second set of ANOVA tests was used to determine how the number of salmon, and salmon size, affected the number of photographs containing riparian predators in the stream test. One-way ANOVA was used to compare the average proportions of the predators located around pools and riffles in the stream. Count data containing zeros was log (x + 0.5) transformed before analysis (Yamamura 1999) or, when proportions were tested, data were arcsine $\sqrt{x}$ transformed. Tukey's honestly significant difference (HSD) test was used as a post

hoc test. I also used generalized linear models (GLMs) with Poisson distribution (without data transformation) in addition to the ANOVAs described above. Herein I report results from ANOVAs alone because the results were consistent and there was no difference in statistical significance at the 5% level between ANOVAs and GLMs.

For the semi-natural stream experiment, Pearson's Chi-squared test was used to compare the proportion of the number of fish captured at each section. However, the omnibus Chi-squared value does not specify which combination of categories contributes to statistical significance; thus, adjusted standardized residuals (ASR) was used for each value to determine discrepancies between the observed and expected values (Haberman 1973): $|ASR| > 1.96$ and > 2.56 indicate $P < 0.05$ and $P < 0.01$ respectively. Two-sided $P < 0.05$ was considered significant in all statistical analyses. Data were analyzed using IBM SPSS version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

Tank experiment

In the tank experiment, cameras captured grey heron (*Ardea cinerea*), Japanese marten (*Martes melampus*), raccoon dog (*Nyctereutes procyonoides*) and the large-billed crow (*Corvus macrorhynchos*). The total number of photographs containing potential predators was 186 (including three photographs with ambiguous species, which were identified by the local wildlife expert). In total, 173 photographs of grey heron were taken and there were six photographs or fewer of each of the other animals (Japanese marten, three; raccoon dog, four; large-billed crow, six). Twenty-one photographs showed grey herons capturing prey fish. Therefore, in the tank experiments, grey herons were regarded as the main predator.

As for the size-selective predation risk, both the study days (the days elapsed from stocking salmon) and the FL of the stocked salmon had significant effects on fish survival (both $P < 0.001$; Table 4). The survival rate was significantly higher for fish with a 75–100 mm FL than a 135–160 mm FL during the next day or later of the stocking day (Tukey HSD test, $P < 0.050$ for all; Fig. 4a).

In the two-way ANOVA test for frequency of predator appearance, only the number of fish in the tank had a significant effect on the number of photographs containing grey herons (number of fish, $P < 0.001$; others, $P > 0.141$; Table 5) and the number of photographs containing grey herons increased significantly with a larger number of fish in the tanks (Tukey HSD test, $P < 0.001$ for all; Fig. 4b). At the end of the 3-day experiment, there were no surviving salmon.

Stream experiment

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The overall survival of the fish in this experiment was 18.0% (n = 72). In my daily checkups and in the process of catching the fish with an electrofishing unit, I did not find any fish that died from a disease or jumping out of the stream. The survival of fish was significantly higher for the 75- to 100-mm FL group (33.0%; n = 66) than for the 135- to 160-mm FL group (3.0%; n = 6; Pearson's $\chi 2$ = 60.98, d.f. = 1, P < 0.001). There was no significant difference in the proportion of fish recovered in each section between the two groups (Pearson's $\chi 2$ = 0.77, d.f. = 3, P = 0.86). Comparing the actual proportion of fish (75–100 and 135–160 mm FL) recovered in each section with the expected surviving proportion (I hypothesized a uniform 1: 1: 1: 1 ratio) revealed a significant difference (Pearson's $\chi 2$ = 15.43, d.f. = 3, P < 0.001). Accordingly, the number of fish recovered in Section 2 was significantly higher than the average number of fish captured in all sections (ASR = 3.4, P < 0.010; Fig. 5a).

Cameras captured grey heron, brown dipper (*Cinclus pallasii*), Japanese red fox (*Vulpes vulpes japonica*) and large-billed crow during the stream experiment. The total number of photographs containing potential predators was 470. Of them, 455 photographs contained grey heron. There were fewer than 10 photographs of other animals (brown dipper, one; Japanese red fox, seven; large-billed crow, seven). The most grey heron photographs (n = 104) were taken at Section 2 on Day 5 (Fig. 5b). Of all the pictures that contained grey herons, 76.5% were taken at Section 2. Five photographs had two grey herons in each picture, and two photographs had three grey herons in each picture; all photographs with multiple herons were taken in Section 2 between Day 4 and Day 7 after stocking. Seventeen photographs showed grey herons capturing prey fish.

In order to investigate the position that grey herons used, 412 photographs were analyzed (455 photographs minus 43 photographs in which the position could not be

distinguished). The mean (± s.d.) proportion of photographs showing grey heron around pools (6.88 ± 10.68%; Fig. 3c) was significantly lower than that should grey heron around the riffles (93.13 ± 10.68% (Fig. 3d); $F1,6 = 53.13$, $P < 0.010$; Fig. 6). The overall proportion of photographs taken of grey herons in the morning (0300–0900 hours) and evening (1500–2100 hours) was 49.3% (n = 224) and 47.9% (n = 218) respectively.

Discussion

In the two experiments, most fish were lost and the grey heron was the most frequently visiting predator. It has previously been reported that salmonid populations can be seriously damaged by avian predation (Feltham 1995; Stewart et al. 2005), but quantitative assessments of the effect of avian predation on fisheries resources with predator identification are rare (Hodgens et al. 2004). The results of the Chapter suggest that in my experimental setting, grey herons can significantly reduce salmonid populations by consuming juvenile fish. It is important to note that water depth can strongly affect wading birds' selection of foraging habitat (Master et al. 2005; Gawlik and Crozier 2007). The length of a grey heron's legs restricts the maximum depth at which they can forage, up to a depth of 17 cm (Ntiamoa-Baidu et al. 1998), thereby limiting the habitat suitable for hunting. My observation that grey herons showed a significant preference for riffles, rather than pools, in the semi-natural stream is consistent with previous studies. Thus, where local fish reside in shallow water, it is reasonable to expect that grey herons exert a high predation pressure.

On the first day of the tank experiments, grey herons preyed upon large salmon more often than upon small salmon. In both the tank and stream experiments, the survival rate of large salmon was less than that of small salmon. Birds of the heron family (Ardeidae), including the grey heron, have been reported to show a preference for larger mosquito fish *Gambusia affinis* (Britton and Moser 1982) and sailfin molly *Poecilia latipinna* (Trexler et al. 1994) as prey. It is suggested that larger fish are easier for avian predators to detect than small fish (Magnhagen 1988). In addition, it has been reported that the larger fish can monopolize foraging spaces near inflow sections (Nakano 1995ab). In the tanks, the current derived from the water filling port flowed through the center of

the tanks; therefore, if the large salmon monopolized the center of the tank, this may have made them more visible to the grey herons. However, after the second day of the tank experiment, both sizes of salmon showed similar levels of decline in survival rate. One possible explanation for this is the decreased opportunities to prey upon larger fish. The results suggest that size-selective predation by grey herons depends on the density of the preferred prey size, and it appears that size-selective predation occurred in the stream experiment as well as in the tank experiments. Therefore, if fish with different body sizes are stocked in rivers, it may be important to consider how size composition will potentially affect the behavior of predators.

It is established that larger fish are safer from fish-on-fish predation than smaller fish (Peterson and Wroblewski 1984; Houde 1987; Miller et al. 1988; Miyamoto and Araki 2017). One potential cause for the higher survival rate of larger fish is improved swimming ability, which allows them to better avoid predators as they grow (Beamish 1978; Lundvall et al. 1999). However, the grey heron is an ambush predator that usually stands upright and waits for a fish to approach (Tojo 1996), so the swimming ability that a fish possesses to avoid aquatic predators has significantly limited benefits to them in this case (Miyamoto 2016b), and large size may make fish more detectable. Therefore, these results imply that what traits are considered to be beneficial for survival changes depending on the local predator species and their habitat.

Generally, larger fish tend to avoid exposure to a predatory threat and reduce their growth rate, whereas smaller fish are less cautious and maintain their growth rate even in the presence of a threat (Reinhardt 1999). Thus, it is expected that the larger fish are more difficult for grey herons to catch than the smaller fish. In contrast, it has also been reported that the hatchery environment selects for bolder individuals that spend more

time in open areas and are more active than their wild counterparts (Sundström et al. 2004). So, the bold behavior of stocked fish may also depend on their rearing period in the hatchery environment (Roberts et al. 2014). Therefore, to fully understand the predation risks for stocked fish in general, further studies on individual behaviors of fish (e.g. bold or shy) and their adaptation to artificial environments (Berejikian et al. 1999; Roberts et al. 2011) are needed.

In the tank experiment where different numbers of salmon were stocked, the number of grey herons present or the duration of their sojourn at the tanks was positively correlated with the number of stocked fish. In other studies, large numbers of fish have been observed to remain close to where they were stocked (Cresswell 1981), and piscivorous water birds have exhibited similar density and temporal trends in response to stocking (Draulans 1987; Gawlik 2002). This suggests that for the duration of my stream experiment many salmon stayed in Section 2, because multiple grey herons simultaneously frequently appeared in that section. I also captured more fish in Section 2 by electrofishing than in any other section on the final day of the stream experiment. These results indicate that most stocked fish gathered without migrating from the initial stocking site and continued to be preyed upon there. This phenomenon may have serious implications for the conservation and propagation of economically and socially important fish species. Although the reason why grey herons were most frequently observed as predators in the stream experiment is unknown, it is possible that territorial behavior of grey herons excluded other predator species from their feeding territories (Marion 1989). It is also plausible that predator species with the highest predation pressure differ among prey fish species and ecosystems, and that the observation of grey herons as a primary predator species may be restricted to the conditions of the present study. Naturally

predators will vary regionally and across ecosystems, so it is important to further

investigate the spatial and temporal relationships among stocked fish, local predators and

their local environments to further understand the generalizability of experimental results.

Chapter-3: *Behavioral variation in masu salmon affected their survival in a semi-natural environment with ambush predators*

Introduction

In Chapters-1 and -2, I explained the effects of prey size on predation risk. Through the experiments, the importance of considering the effects of fish traits (origin and individual behavior) on their predation risk has become evident. Regarding the prey's behaviors in response to predation risks, it has been reported that prey animals vary daily in their behaviors, through habitat selections (Svanbäck and Eklöv 2003; Bongi et al. 2008) and changing the levels of their activities (Lima and Dill 1990; Liley and Creel 2007; Sönnichsen et al. 2013). When evaluating the predator avoidance behaviors of prey animals and their relation to fitness in the wild, researchers have mainly focused on behaviors of prey after their sensing of predators (Leduc et al. 2007; Kuehne and Olden 2012; Christensen et al. 2014; Salvanes 2017). The same is true for aquatic organisms such as fish (Berejikian et al. 1999; Mirza and Chivers 2000). The difference being that less is known about natural behaviors of aquatic species in the wild.

For salmonid species, previous studies have demonstrated that many factors, including body color, size, shape and age, can influence their predation risk (Donnelly and Whoriskey 1991; Pearsons and Fritts 1999; Adriaenssens and Johnsson 2013; Roberts et al. 2014; Miyamoto 2016a; Miyamoto and Araki 2017; Miyamoto et al. 2018). It is also known that captive rearing has some influence on the behavior of salmon. In particular, relaxed natural selection in captivity tends to increase the behavioral variance in individual captive fish, which, in natural conditions, can translate into maladaptive behaviors such as extreme boldness (Cusato and Morrow 2003; McPhee 2003),

eventually affecting the survival and reproduction rates of fish after their release (Lee and Berejikian 2008). However, empirical studies on the relationship between multiple fish traits (e.g. behavior, body size and origin) and their predation risk are scarce at best.

The fitness advantage of prey's traits would depend on the local predators (Carss and Marquiss 1991; Sogard 1997; Miyamoto et al. 2018). In addition, the behaviors of wild animals are influenced by their habitat and the time of day (Thurow and Schill 1996; Bradford and Higgins 2001). When predators change their feeding habits (feeding area, feeding time and feeding style), the vulnerability of prey also changes (Harvey and Stewart 1991; Blake and Gabor 2014; Belgrad and Griffen 2016). Therefore, in order to understand the relationship between prey's behavior and their predation risk in the wild, it is necessary to also have knowledge about local predator species and their feeding habits.

Chapter-3 elaborates on my use of hatchery-reared, juvenile masu salmon (*Oncorhynchus masou*) and a camera trapping system to investigate predator-prey interactions, after releasing the hatchery fish in a semi-natural stream. First, I measured fish traits in an aquarium (origin, body size, structure use time with/without an artificial predation stimulus) before releasing the fish in the semi-natural stream. The camera trapping captured photographs of natural predators at the stream, providing information about them such as species, time, frequency and area of appearance. After 56 days of observation, I captured the surviving fish from the stream and evaluated their markings.

Materials and methods

Test fish

Two types of young of the year (YOY) hatchery-reared masu salmon were used in this Chapter. They were both from the Shiribetsu River, in southwestern Hokkaido, Japan, but one type of fish (F11 fish, hereafter) was raised for about 11 generations in the National Research Institute of Fisheries Science (NRIFS) facility at Nikko since 1979 and 1980 (Masaoka et al. 1997). The other type of fish (F1 fish, hereafter) was raised from eggs obtained from mature adults that had migrated into the Shiribetsu River in 2013. Both F1 and F11 fish were reared in the same manner, following the standard hatchery procedures directed by the NRIFS facility at Nikko. Note that in Shiribetsu River, the stock enhancement of masu salmon has been conducted using hatchery-born and wild-born fish that had migrated in to the Shiribetsu River. Thus, F1 fish might not necessarily be wild-born.

The F11 fish (n = 200) and F1 fish (n = 200) were independently sampled from each rearing tank (50cm width × 120cm length × 20cm depth). There was no significant difference in body size at the beginning of this study between the F11 and F1 fish groups (Table 6). The two types of fish were reared separately in tanks at 9.0°C and on a 14 h light: 10 h dark cycle, and fed daily food rations (commercial trout pellets) equal to 1.0–2.0% of their body weight until the beginning of the following experiments.

Aquarium experiment

In order to evaluate the individual fish behavior, a single fish was placed in the aquarium, the time that the fish used the structure (shelter gravel or both, hereafter TFUS) was measured for three minutes. The aquarium experiment was conducted using 50

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randomly selected fish from each origin (F11 or F1) in May to June, 2014. Two identical aquaria were used in the study. They had a capacity of 360 liters of water per aquarium (120 cm wide × 50 cm long × 60 cm high), with a layer of natural gravel at the bottom (30–40 mm deep). Water (9.0°C) was introduced into each aquarium at a rate of 4 L/min. The observation area for each aquarium was 120cm wide × 35cm long × 40cm deep. A ceramic object (20 × 20cm) with four glass legs (10cm long) was placed in the center of each aquarium as a shelter (Fig. 7). The aquaria and recording room were separated by a vinyl sheet, and the behavior of fish was observed through a makeshift window (length 10cm × width 20cm) on the said sheet.

The fish were considered hiding when using the shelter (Brown and Smith 1998) or the gravel on the bottom (DeGraaf and Bain 1986; Hayes and Baird 1994; Martel and Dill 1995). To be recorded as selecting the gravel sites, the fish had to be partially concealed in the gravel (Miyamoto 2016b). The fish behaviors were observed before (as a normal TFUS) and after the test of a mimicked predation stimulus (as a post-stimulus TFUS). For acclimation, the normal TFUS of fish was measured after 90 minutes since the test fish was placed in the aquarium following Olla and Davis (1989) and Miyamoto (2016b). To investigate the behavior of fish that being subjected to the stimulus, a remote-controlled vinyl bird model (32cm long and 58cm wide) was dropped into the aquarium and allowed to splash onto the water surface. The model was removed from the aquarium at ten seconds after dropping, and then the behavior of fish was recorded. After the aquarium experiment, all test fish were lightly anesthetized with 100ppm 2-phenoxyethanol (Wako Pure Chemical Industries, Tokyo, Japan), marked on the abdominal side with visible implant elastomer (VIE; Northwest Marine Technology, Inc.) for individual marking, and used for the stream experiment below.

Stream experiment

After the aquarium experiment, stream observation was conducted for 56 days from June to July, 2014, using a semi-natural stream (mean ± standard deviation [SD]: 120m-long, 112.5 ± 8.7cm-wide; 2% gradient drain) in a forested and grassy area at NRIFS. The stream was constructed of stone, wood, and soil. Spring water, at 10.3 ± 0.5°C (mean ± SD) was drained at 18 L/s. The stream was split into four sections, each of which having 30m in length (Sec. 1–4 hereafter, starting from the uppermost section). Each of the four sections contained three pools and three riffles, which were created by flow over an obstruction system using buried logs (Chapter-2 for more details). The pools were about 80cm-long and about 60cm-wide with maximum depth of approximately 40cm. Woody debris (c.a. 70cm long and c.a. 20cm wide) were placed at the sides of each pool. The riffles were 9–12cm deep and contained a mixture of 2–7cm gravel and about 13cm cobble substrate. A 50cm waterfall was built in the top of the stream with two metal gates (1cm × 2cm mesh) to prevent fish from escaping. The water drain was separated by a metal mesh gate (1cm × 2cm mesh) with the bottom quarter of the gate covered with plastic mesh (8mm × 8mm mesh).

At the beginning of the stream experiment, I released 400 fish into Sec. 2. They consisted of 100 fish from the aquarium test above (50 F11 fish and 50 F1 fish), and 150 additionally selected fish from each origin (150 F1 fish and 150 F11 fish). The adipose fin of the 150 F11 fish were clipped prior to the release for group marking. Previous studies have shown that adipose fin removal has only minor effects on survival (Vander Haegen et al. 2005) and susceptibility to predation (Shetter 1951). During the study period, for the purposes of maintaining the semi-natural stream and confirming which fish died

from a disease or jumping out, I checked the said stream daily from an overlooking hill. At the end of the study period, I investigated the distribution of fish in the stream in the following manner. First, each section was split using fish block nets (8mm × 8mm mesh) to prevent fish from moving between the sections. Then, a backpack electrofishing unit (model 12-A POW Electrofisher, 300V, Smith-Root, Vancouver, WA, USA) and 100ppm 2-phenoxyethanol (Wako Pure Chemical Industries, Tokyo, Japan) were used to catch and anesthetize fish. To investigate the migration of stocked fish in the stream, I recorded the section where fish were caught. The electrofishing was repeated until the fish count was zero twice in a row. Finally, I checked the individual and group markings of stocked fish, counted the number of fish in each group and measured their fork length and body weight.

Camera trapping

To assess predator encounters during day and night, I recorded potential predators using motion and infrared sensor camera traps in the stream experiment. Each camera (Trophy Cam HD, Bushnell, Overland Park, KS, USA) was mounted on a wooden stake so that the camera was about 50cm above the water's surface. Cameras were triggered with a passive infrared motion sensor; the camera was set to wait 15 seconds after an initial trigger entered its sensor range before attempting to detect additional triggers. The three cameras were arranged in each section and placed at 10m-intervals along the stream, so that they could cover the surface of whole stream section (Miyamoto et al. 2018).

The photographs by camera traps were counted for each section. To identify predators and evaluate the frequency of their visits to the study site, all the photographs containing potential predators were checked by the same researcher (KM). In this Chapter,

to focus on the photographs of potential predators, I analyzed the photographs of carnivorous or omnivorous animals. For some ambiguous species identifications, additional checking was performed by a local wildlife expert, Dr. T. Takeda from Nikko National Park. In addition, the number of photographs that showed predators capturing or eating fish was counted, and the predator species was recorded. To evaluate the predation behavior of wild animals, the photographs that contained potential predators were recorded for the position (at pool or riffle) and the time (following the categories of morning: 3:00-9:00, day: 9:00-15:00, evening: 15:00-21:00 and night: 21:00-3:00) the predator was observed. To investigate the position predators utilized in each section, the percentage of predators located around the pool or riffle was calculated.

Statistical analyses

Two-way analysis of variance (ANOVA) was used to evaluate the effect of time (before and after the stream observation), difference of origin (F11 or F1), and their effect on the body size of survivor fish in the stream. However, this test contained a risk that size-selective predation affects the body size composition of fish. Therefore, analysis of covariance (ANCOVA), using body size of pre-stocked fish as the covariate, was conducted to evaluate the effect of fish origin on the growth of fish in the stream based on the data of surviving individually marked fish. Additionally, two-way repeated measures factorial ANOVA was used to evaluate the effect of the existence of predatory stimulus, difference of origin, and their interaction on TFUS in the aquarium test. In order to compare different pairs of means, Bonferroni test was used as a post-hoc test. When significant interaction effects were found, the simple main effect analysis was used as described by Ishimura (2006).

In the stream experiment, the Pearson's χ^2 test was used to compare the proportion of the number of fish captured at each section. However, the omnibus χ^2 value does not specify which combination of categories contributes to statistical significance. Thus, the adjusted standardized residuals (ASR) were used for each value to determine discrepancies between the observed and expected value (Haberman 1973). $|ASR| > 1.96$ and > 2.56 correspond to $P < 0.050$ and $P < 0.010$, respectively. Data analyses were generated using IBM SPSS software (version 21.0 of the IBM SPSS Statistics for Windows).

In order to evaluate the effect of body size, origin, behaviors and their interactions on the survival of test fish in the stream (Table 7), the GLM (Generalized Linear Model; glm function; family: binominal) was used. GLM is widely used to model binary outcome variables. The 'dredge' function from the 'MuMIn' package was used to test all possible combination of the variables included in the full model using the software R (3. 3. 2). The lower value of the Akaike Information Criterion (AIC) was used, which indicates the most parsimonious model (Burnham et al. 2011). To screen covariates for multicollinearity with variables I used Variable Inflation Factor (VIF), where I removed variables with VIF > 10 (Eyduran et al. 2012) using the 'vif' function from 'car' package. The significance of the explanatory variables in the GLM was evaluated using the Wald test. Finally, to assess the association between the survival of masu salmon and TFUS, TFUS (%) was calculated as follows:

$$TFUS (\%) = TFUS/180 \times 100$$

where 180 was the full observation time in seconds in the aquarium test. The correlation between survival of masu salmon and TFUS (%) was evaluated using Spearman non-parametric correlation coefficients. The TFUS was compared between the groups of

individually marked fish (fish that died or survived) using Student's *t* test.

Results

Aquarium experiment

Observed variation in TFUS suggested two extreme strategies, with the majority of fish using structure within 30s or hiding all the time (Fig 8). There was a weak, but significantly positive correlation between TFUSs with and without the predatory stimulus (Fig. 9a, Spearman's r = 0.22, p < 0.050). The results of the two-way repeated measures factorial ANOVA showed that the interaction between the existence of the stimulus and the origin (F11 or F1) had significant effects on the TFUS ($F_{1,\,98}$ = 4.121, P < 0.050) (Fig. 10). As for the stimulus, the TFUS was significantly longer than that of normal status in both origins as expected (Bonferroni test, F11: 26.26 + 43.6 [mean + SD] seconds without stimulus, 80.12 + 78.9 seconds with stimulus, P < 0.050; F1: 40.78 + 66.1 seconds without stimulus, 126.46 + 74.5 seconds with stimulus, P < 0.001). With regard to the origin, the post-stimulus TFUS of the F1 fish was significantly longer than that of the F11 fish (Bonferroni test, P < 0.050). On the other hand, there was no significant difference between the time periods at which two origins of fish used structure at the normal TFUS observation (Bonferroni test, P = 0.905)

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Stream experiment

Capturing fish after stream observation suggested that 207 out of 400 fish survived (i.e., 48.5% of released fish were lost in the 56 days). In my daily checkups and in the process of catching the fish with an electrofishing unit, I did not find any fish that died from a disease or jumping out of the stream. The survival rates were not significantly different between the fish origins (F11 fish, n = 106; F1 fish, n = 101; Fisher's exact test, P = 0.689). There was no significant difference in the proportion of recovered fish in each

section between both fish origins (Peason's $\chi 2$ test, $\chi 2 = 4.375$, df = 3, $P = 0.224$).

Comparing the proportion of all recovered fish (F11 fish and F1 fish) in each section and a hypothetical proportion that all captured fish distribute with uniformity (1:1:1:1 ratio) in each section, there was significant heterogeneity among sections (Peason's $\chi 2$ test, $\chi 2 = 29.834$, df = 3, $P < 0.001$). The proportion of recovered fish in Sec. 2, where all the fish were released, was significantly higher than the expected number of fish without any bias among sections (ASR = 4.8, $P < 0.010$) as observed in the previous study (Miyamoto et al. 2018) (Fig. 11).

The two-way ANOVA revealed that the time had a significant effect on the fork length of stocked fish (time, $F_{1, 603} = 365.242$, $P < 0.001$; origin, $F_{1, 603} = 0.067$, $P = 0.796$; time × origin, $F_{1, 603} = 0.314$, $P = 0.575$) and on the body weight of stocked fish (time, $F_{1, 603} = 325.171$, $P < 0.001$; origin, $F_{1, 603} = 0.064$, $P = 0.800$; time × origin, $F_{1, 603} = 0.178$, $P = 0.673$), indicating growth of the fish during the observation. Fish increased roughly 20% of their fork lengths in the 56 days (F11 fish: from 74.73 + 7.20 mm [mean + SD] to 88.54 + 10.37 mm; F1 fish: from 74.12 + 6.8 mm to 88.76 + 12.19 mm). The average body weights of stocked fish changed from 4.33 + 1.23 g (mean + SD) to 7.63 + 3.24 g for F11, and from 4.20 + 1.23 g to 7.65 + 3.41 g for F1. These changes were all statistically significant ($P < 0.001$ by the Bonferroni test).

The ANCOVA, using the data of surviving individually marked fish, indicated that the origin of fish had a non-significant effect on the growth of fork length (origin, $F_{1, 53} = 3.077$, $P = 0.085$) and body weight (origin, $F_{1, 53} = 2.534$, $P = 0.117$). The fork length (F11 fish: from 79.10 + 5.22 mm [mean + SD] to 95.79 + 10.46 mm; F1 fish: from 78.96 + 7.83 mm to 92.44 + 13.30 mm) and body weight (F11 fish: from 5.28 + 1.09 g [mean + SD] to 10.20 + 3.75 g; F1 fish: from 5.09 + 1.64 g to 8.79 + 3.89 g) of fish at the post-

release measurement were significantly larger than those of fish before release in both origins (Bonferroni test; both $P < 0.001$).

Cameras captured grey heron (*Ardea cinerea*), brown dipper (*Cinclus pallasii*), Japanese red fox (*Vulpes vulpes japonica*), raccoon dog (*Nyctereutes procyonoides*), Japanese marten (*Martes melampus*), large-billed crow (*Corvus macrorhynchos*), grey wagtail (*Motacilla cinerea*) and sika deer (*Cervus nippon*) during the stream observation. To investigate the behavior of potential predators, the photographs containing animals excluding sika deer (herbivore) were analyzed. The total number of photographs containing potential predators was 466 (including eight photographs with ambiguous species, which were identified by the local wildlife expert). Among them, 267 photographs (57.3%) contained grey heron. There were 3-95 photographs for each of the other species (brown dipper: 4, Japanese red fox: 95, raccoon dog: 58, Japanese marten: 3, large-billed crow: 34, grey wagtail: 5). There was no photograph of two or more animals in a photograph frame. There were 10 photographs showing grey herons holding fish prey in their beaks. The 267 photographs with grey heron showed a significant heterogeneity among the sections (Peason's $\chi 2$ test, $\chi 2 = 63.885$, df = 3, $P < 0.001$), for Sec. 2 being the section of the most frequently photograph-taken (Fig. 11b). The proportion of the taken photographs in Sec. 2 was significantly higher than the expected number of photographs without any bias among sections (ASR = 4.8, $P < 0.010$), so that it may synchronize with the number of fish in Sec. 2 (Fig. 11).

The overall proportions of photographs of grey herons in the morning (3:00– 9:00) and evening (15:00–21:00) were 32.6 % (n = 87) and 67.4 % (n = 180), respectively. There were no photographs taken during day and night (Fig. 12). To investigate the position grey herons utilized, 267 photographs were analyzed (34 photographs were

excluded because their positions were indistinguishable). The average percentage of the photographs showing grey herons located around the riffles was significantly higher (98.8 + 1.37 [mean + SD] %) compared to the pools (1.19 + 1.37 [mean + SD] %) (Mann-Whitney's U-test, $U = 16$, $P < 0.050$). There was no photograph taken in the water for species other than grey heron during the study period.

In the GLM analysis, the interactions (normal TFUS × post-stimulus TFUS , vif =11.2; normal TFUS × fork length, vif =161.9; post-stimulus TFUS × fork length, vif =211.4; origin × fork length, vif =209.8) were removed to avoid multicollinearity. The result of GLM suggested that the best model for explaining the survival of fish after release includes the TFUS, not in post-stimulus, but in normal and the fork length of fish just before stocking as the significant variables. Although the normal TFUS had a significant effect on the survival of the masu salmon (Wald test, coefficient = 0.011, $z = 2.282$, $P < 0.050$), there was no significant contribution of fork length in the best model (Wald test, coefficient = 0.540, $z = 1.685$, $P = 0.092$) (Table 8). The positive coefficient for the normal TFUS indicates that individuals exhibiting risk-adverse behavior even without a predation stimulus had a high survival rate after release. There was significant correlation between the survival of the masu salmon and the normal TFUS (%) (Spearman's r = 0.261, $p < 0.010$) but not between the survival and the post-stimulus TFUS (%) (Spearman's r = 0.040, $p = 0.693$) (Fig. 13). In fact, the normal TFUS of the fish that survived in the stream test was more than two times longer than that of the fish that died (fish that died: 19.8 + 40.9[mean + SD] seconds, fish that survived: 44.3 + 64.1 seconds, Student's t test, $t = 2.2$, $P < 0.050$) (Fig. 14).

Discussion

After the 56-day observation in the semi-natural stream, I found that 48.3% of the fish was lost. According to the camera trapping, the grey heron was the most frequently visiting predator. In addition, the photographs showing animals capturing fish and standing in the water were only those of the grey heron. These results were consistent with my previous study, suggesting that the grey heron can exert high predation pressure when salmon juveniles are in shallow water (Miyamoto 2016a; Miyamoto et al. 2018). Furthermore, the few reports that exist on predation pressure from the grey heron are in line with the notion that the grey heron has a major negative effect on freshwater fish populations in natural conditions (Feunteun and Marion 1994). My results indicate that, at least in my experimental setting, the grey heron can significantly reduce salmonid populations by consuming a large number of salmon juveniles.

One of my previous studies, comparing multiple salmonid species, indicated that normal fish behavior without any predation stimulus, not their avoidance behavior, was the most important contributor to their survival (Miyamoto 2016a). However, the Chapter suggests that such a phenomenon might exist even at the individual level within the same species. More specifically, the results suggest that the behavior of salmon juveniles under normal circumstances was the most important determinant of their predation risk, at least in a shallow and small stream often visited by the grey heron for predation. These results indicate the possibility that suitable behavioral traits of fish depend on predator species and their environment.

The predation style of local predators might have a strong impact on the relationship between fish behaviors and the survival of fish after release. The grey heron is an ambush predator that usually stands upright and waits for fish to approach (Tojo

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1996). Therefore, it is reasonable to assume that the grey heron can easily prey on the stocked fish before being noticed. Additionally, the grey heron was frequently observed during the late afternoons of this study, and because the study area was inside a forest, it would start getting dark earlier than usual. In fact, the grey heron photographs were often taken in night vision mode. One of the previous studies investigated the predator avoidance behavior of the bluegill *Lepomis macrochirus* and found that the use of shade by fish was less frequent in low illuminance conditions (McCartt et al. 1997). These studies are consistent with the scenario that stocked fish have difficulties fully exerting their predator avoidance behavior against an ambush predator. Furthermore, this underpins the importance of identifying the predator species and their feeding habits when studying the predator-prey interaction (Blake and Gabor 2014; Belgrad and Griffen 2016).

The aquarium test revealed two extreme strategies exhibited by the hatchery salmon juveniles (bold and shy). There was a significant correlation between TFUSs with and those without the mimicked bird attack stimulus, suggesting clearly identifiable behavioral characteristics among fish individuals as reported earlier (Roberts et al. 2011). Furthermore, the time in which the fish used structures after the mimicked bird attack stimulus was longer for the F1 than for the F11 fish. This possibly indicates that the multi-generational domestication process had weakened the predator avoidance capabilities of the F11 fish. In addition, this is consistent with previous studies showing a cumulative fitness effect of captive breeding through domestication (Araki et al. 2007). The captive breeding programs might create fish with phenotypes maladaptive in the wild, such as lower sensitivity to a predation stimulus (Roberts et al. 2011). This is further supported by a case of masu salmon reared for several generations exhibiting abnormal behaviors (Reinhardt 2001; Yamamoto and Reinhardt 2003). These results imply that post-stimulus

predator avoidance behavior might work effectively only when fish are attacked by non-ambushing predators (e.g. pursuit predators such as the great cormorant and the river otter). To fully understand the predator-prey interactions, further investigation into the environment-specific relationship between the behaviors of prey and those of local predator species would be required.

For salmonid species in general, competition for food is known to be a major determinant of growth in the wild (Reinhardt 2001). The larger (Elliott 1990; Huntingford et al. 1990) or more aggressive (Fleming and Einum 1997) fish have a greater advantage in competition for feeding sites where they have priority access to a wider range of food items (Reinhardt 2001). In these cases, the increase in mortality and emigration of smaller fish of the same species might have been due to larger fish having an enhanced growth rate (Elliott 1990; Sandercock 1991). It has been reported that the growth rate of farmed fish is higher than that of wild ones in natural conditions (Einum and Fleming 1997; for contrary results - Yamamoto et al. 2008). However, there was no significant difference in the amount of growth between F11 and F1 fish in this Chapter. Both origins showed significant growth during the study period. In terms of body weight, fish of both origins gained c.a. 180% of their body weight during the study period. Therefore, the food conditions in the semi-natural stream seem to have been good for the juvenile fish, especially when compared to a river nearby that was used to study Honmasu salmon (*O. masou masou* × *O. masou* subsp.) juveniles (Munakata et al. 2000). One potential cause for the substantial growth is that the aggressive behavior for territory and food did not occur very often in the study area, because such competitive exclusion and domination of food resources is weakened under frequent predation threat (Reinhardt 1999). In fact, the stocking section of the semi-natural stream had a much higher density of stocked fish

compared to the other sections.

At the end of the study, more fish (F11 and F1) were caught in Sec. 2 by electrofishing than in any other section. Moreover, the grey heron most frequently appeared in Sec. 2 during the study. These results suggest that for the duration of my stream observation many salmon stayed in Sec. 2 despite it having the highest predation pressure from the grey heron. In other study as well, large numbers of fish have been observed to remain close to where they were originally stocked (Cresswell 1981), and piscivorous water birds have displayed similar temporal trends in response to stocking (Draulans 1987; Gawlik 2002). These results indicate that most of the stocked fish chose not to migrate from the initial stocking site despite being continuously preyed upon. This phenomenon presents a serious problem for the stocking of large rivers, as they tend to need a large number of stocked fish, which in turn is going to attract more predation. Therefore, if a large number of stocked fish with barely any tendency towards migration is released into a stream, they will make the initial stocking location into an attractive feeding site for predator animals. This will happen mainly because their large numbers and high density, due to very low migration to other parts of a stream, will make them an easy target for predators. To make matters worse, it has been reported that the number of grey herons, their frequency of appearance and duration of sojourns at a fish stocking site seemingly correlates to the number of stocked salmon (Miyamoto et al. 2018). Therefore, more serious damages might be inflicted on the population of stocked fish by piscivorous water birds at an actual stocking site.

My observation suggested that the grey heron significantly preferred the riffles for predation, as opposed to the pools. It has been reported that water depth can strongly influence wading birds' choice of a foraging habitat (Master et al. 2005; Gawlik and

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Crozier 2007). As previously mentioned, the length of the grey heron's legs restricts the maximum depth at which it can forage down to 17 cm (Ntiamoa-Baidu et al. 1998), thereby limiting its habitat range for hunting. Therefore, it is reasonable to infer that the grey heron exerts higher predation pressure in riffles than it does in pools. These results indicate that, apart from the combination of predator and prey traits, habitat is also an important component of overall predator-prey interaction. Finally, the results of this Chapter suggest that predator-prey interaction can be interpreted as a combination of prey's individual behavioral traits, predator's feeding habits and their habitat.

Chapter-4: *Effects of water depth and structure complexity on survival and settlement of white-spotted charr*

Introduction

In Chapters-1, -2 and -3, I focused on what effects traits of both predators and prey have on the predator-prey interaction. However, it is presumable that their physical habitat conditions also affect predation risk through the predator-prey interaction. Most age-0 salmonids use shallow pools at shorefronts of small tributaries as habitats (Nagoshi et al. 1988; Kubota et al. 2001; Hasegawa and Maekawa 2009). Therefore, declining water levels, caused by reducing river flows, might negatively influence salmonid populations in small tributaries. In addition, declining water levels possibly increase predation risk by bird species (Kushlan 1976). However, there are few studies on the relationship between physical habitat and predator-prey interaction (riparian predatory animals and stocked salmonids). Previous studies indicate that the greater structural complexity leads to an increase in population density (Kalleberg 1958; Dolinsek et al. 2007; Finstad et al. 2007) due to a greater degree of protection from predators (Beukers and Jones 1997; Finke and Denno 2006). Therefore, it is important to investigate the effects of habitat structural complexity in order to evaluate the effect of declining water levels on the predator-prey interaction properly.

In this Chapter, I focus on the effect of water depth on the predator-prey interaction between riparian predatory animals and age-0 white-spotted charr (*Salvelinus leucomaenis*) by using outdoor tanks together with camera trapping. Additionally, I also evaluate the effects of structural complexity on the predator-prey interaction in shallow water.

Materials and methods

Test fish

Test fish used in experiments were age-0 hatchery-reared white-spotted charr from the Yanagisawa River in Tochigi Prefecture, Japan (approximately 36°44'N, 139°24'E). Fish were reared in four rearing tanks (50 cm wide × 120 cm long × 20 cm deep, the supply amount of water was set at 18 L/min) according to standard hatchery procedures as directed by National Research Institute of Fisheries Science (NRIFS) facility at Nikko. Test fish were 50–65 mm in fork length and 1.05–2.9 g in body weight, roughly similar in body size to age-0 stocked white-spotted charr in Japan (Nakamura and Iida 2009). Before the start of the study, fish were fed daily food rations (commercial trout pellets) equal to 1.5–2.0% of their estimated body weight.

Tank experiment

I conducted tank experiments with 960 fish (in total) in four Fiber-Reinforced Plastic (FRP) circular tanks (120-cm in diameter, 50 cm high) at the riverside of the Jigoku-gawa River in the premise of NRIFS in Nikko in a forested and grassy area for 32 days, from June to August 2017. Each tank had a thin layer of natural gravel (fragments 2–7-cm in diameter) substrate and nine 15-cm cobbles, placed at the bottom as shelters. Each tank had a 10-cm high fence and an 8-cm roof with green artificial leaves installed, to provide fish with cover and prevent them from jumping out. Furthermore, each tank had a camera (Trophy Cam HD, Bushnell, Overland Park, KS, USA) to monitor the whole tank from its side. Cover and gravel substrate allowed the charr to show near-natural behavior, by providing options to hide or escape (Miyamoto 2016a), thus minimizing experimental stress. The 30 fish were placed in each tank. The density was relatively high

for salmonid fry, but was within the range observed in nature (Korsu et al. 2010). During the study, fish were fed daily food rations (commercial trout pellets were scattered all over the tank) equal to 1.0–2.0% of total body weight of all fish present at the time of feeding in a particular tank. Spring water was introduced into each tank at a rate of 6 L per minute; water temperature of the tank was 10.4 ± 0.5°C (mean ± SD) during the experiments.

To evaluate the relationship between water depth and predation risk, four different water depths (15 cm, 25 cm, 35 cm and 45 cm) were maintained by bedding bottoms of each tank differently with bags of gravel and then putting a layer of gravel on top of the bags. Each tank's water depth was changed randomly at the beginning of every trial. Fish that survived were removed and counted separately for each tank at the end of each day for four days, the duration of a single trial period, and the trial was replicated four times. To identify predator animals, photographs taken by the four cameras were evaluated (described below) at the end of each experiment.

The second tank experiment investigated the relationship between habitat structure complexity and predation risk. In the experiments, four FRP circular tanks with 15 cm water depth were used. To create artificial structures as cover for the fish, each individual cobble was covered in twelve 30 cm strings of biodegradable Ringlong tape (made from polylactic acid by Tokokosen Inc.) tied in place with flaxen rope. For this experiment, two tanks lacked artificial structures (hereafter referred to as control tank) and two tanks had artificial structures (hereafter referred to as AS tank). Cobbles with Ringlong tape were used only in the AS tank, while regular cobbles (without tape) were used in control tanks. Tanks were positioned in a configuration alternating between AS and control tanks (AS, control, AS, control). This configuration was reversed at the

beginning of each trial (AS, control, AS, control <> control, AS, control, AS). Furthermore, the number of surviving fish in each tank was counted at the end of each trial, along with the number of photographs of potential predators taken by the cameras (described in the section below). This trial was replicated four times.

Camera traps

To assess daytime and nighttime predator encounters, potential predators were recorded using motion and infrared sensor cameras (Trophy Cam HD, Bushnell, Overland Park, KS, USA) for tank experiments. Each camera was mounted on a wooden stake about 50 cm above the water's surface. Cameras were triggered with a passive infrared motion sensor. Cameras were set to wait 15 seconds after an initial trigger entered its sensor range before attempting to detect additional triggers. To identify predators and evaluate the frequency of their appearances at the study site, all photographs containing potential predators were checked, sorted, and counted. For photographs of two or more animals, the species, number, and number of photographs were recorded. In addition, the number of predators capturing or eating fish was counted and identified. For the first tank experiment, to evaluate the relationship between water depth and predator behavior, the number of predators inside tanks was counted separately for each tank.

Statistical analyses

Effects of water depth and habitat structure complexity on populations of charr were evaluated using a generalized linear mixed model (GLMM), with individual trials and tanks or ponds as random intercepts. Significance of explanatory variables was evaluated using a likelihood ratio test.

To evaluate effects of water depth on predation risk of charr in the first tank experiment, a GLMM was fitted with a binomial distribution. The model was described as logit(p) = a + b1Water depth category + Si, with p = probability of survival, a = regression constant, b1 = regression coefficient, and Si = study period (i.e. first, second, third and fourth trial). Water depth represented the water depth of each outdoor tank (i.e. 15 cm, 25 cm, 35 cm, or 45 cm). Post-hoc comparisons of all possible combinations of paired comparisons between numbers of surviving fish from each tank were made using the Games-Howell test.

For evaluating effects of water depth on the number of photographs showing the primary predator species in the first tank experiment, a GLMM was fitted with a Poisson distribution. The model describes the following: log(n) = a + b1Water depth category + Si, with n = the number of photographs of the primary predator animal appearing around or in tanks, a = regression constant, b1 = regression coefficient, and Si = the study periods. Post-hoc comparisons of all possible combinations of paired comparisons between the numbers of photographs showing the primary predator for each tank were made using the Games-Howell test.

To evaluate effects of habitat structure complexity on predation risk in the second tank experiment, a GLMM was fitted with a binomial distribution. The model describes the following: logit(p) = a + b1Structure category + Si + Tj, with p = the probability of survival, a = regression constant, b1 = regression coefficient, Si = study period, and Tj = tank. Structure category represents the AS and control tank. Post-hoc comparisons of all possible combinations of paired comparisons between the numbers of surviving fish from each tank were made using the Games-Howell test.

To evaluate effects of habitat structure complexity on the number of photographs

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showing the primary predator species in the second tank experiment, a GLMM was fitted with a Poisson distribution. The model describes the following: $\log(n) = a + b1\text{Structure category} + S_i + T_j$, with n = the number of photographs showing the primary predator species appearing around or in the tanks, a = regression constant, b1 = regression coefficient, S_i = study period, and T_j = tank. Structure category represents the AS and control tank. Post-hoc comparisons of all possible combinations of paired comparisons between the numbers of photographs showing the primary predator for each tank were made using the Games-Howell test. All statistical analyses were performed using the lme4 package for R ver. 3.4.3 for Windows (www.r-project.org).

Results

In the first tank experiment, mean fork length of the charr was 57.9 ± 3.9 (mean $\pm$ SD) mm, and mean body weight was 1.86 ± 0.4 g. The water depth had a significant effect on the probability of survival (Table 9a). The number of surviving fish for the 15 cm water depth tank was significantly lower than for the other three water depth tanks (Games-Howell test; 15 cm vs. 25cm, $t = 17.58$, $p < 0.001$; 15 cm vs. 35 cm, $t = 18.36$, $p < 0.001$; 15 cm vs. 45 cm, $t = 18.36$, $p < 0.001$) (Fig. 15a).

Cameras captured photographs of grey heron (*Ardea cinerea*), raccoon dog (*Nyctereutes procyonoides*) and sika deer (*Cervus nippon*) during the first tank experiment. Of 255 photographs containing potential predators, 222 photographs (87.1%) contained grey heron, 31 photographs contained sika deer, and 2 photographs contained raccoon dog. No photographs included two or more individual animals. There were: 49 photographs containing a predator inside a tank (all of them featuring the grey heron inside the 15 cm water depth tank); 42 photographs of the grey heron holding fish in its beak. The water depth had a significant effect on the number of photographs containing the grey heron (Table 9b). The number of photographs containing grey heron in and/or around a tank was significantly greater for the 15 cm water depth tank than for the other 3 water depth tanks (the Games-Howell test; 15 cm vs. 25 cm, $t = 4.51$, $p < 0.05$; 15 cm vs. 35 cm, $t = 4.30$, $p < 0.05$; 15 cm vs. 45 cm, $t = 4.57$, $p < 0.05$) (Fig. 15b).

In the second tank experiment, mean fork length of the charr was 58.9 ± 4.0 (mean $\pm$ SD) mm, and mean body weight was 1.92 ± 0.4 g. The structure had a significant effect on the probability of survival (Table 10a). The number of prey that survived was higher for the AS tanks (Fig. 15c). Cameras captured grey heron, Asiatic black bear (*Ursus thibetanus*), grey wagtail (*Motacilla cinerea*), and sika deer during the second tank

experiment. Of 1260 photographs containing potential predators, 1247 photographs (99.0%) contained grey heron, three photographs contained Asiatic black bear, five photographs contained grey wagtail, and five photographs contained sika deer. No photographs contained two or more individual animals at the same time. There were 68 photographs of the grey heron holding fish in its beak. The structure had a significant effect on the number of photographs containing the grey heron (Table 10b), and the number of photographs containing grey heron was significantly higher for the AS tanks than for the control tanks (Fig. 15d).

Discussion

In the experiments in this Chapter, I found that the grey heron was the most frequently observed predator. Additionally, photographs of predators in an instance of predation or inside the tanks were only those of the grey heron. This result was consistent with Chapter-2 in which the grey heron exerted high predation pressure on fish in shallow water. Other studies have also found that predation by the grey heron negatively affects freshwater fish populations in natural conditions (Feunteun and Marion 1994). These results indicate that the grey heron can significantly reduce salmonid populations by consuming a large quantity of juvenile fish.

In the first tank experiment predation risk was the greatest for fish in the 15 cm water depth tank. Water depth can strongly influence wading birds in their selection of foraging habitats (Master et al. 2005; Gawlik and Crozier 2007). Generally, predation risk from water birds and mammals increases in shallow areas (Harvey and Stewart 1991). This is also true for the case of a water bird like the grey heron, mainly because the length of its legs restricts the maximum depth at which it can hunt for food down to 17 cm (Ntiamoa-Baidu et al. 1998), thereby limiting its habitat range for hunting. For that reason, it is likely that the 15 cm water depth tank was the only place where the grey heron had the least difficulty hunting for food. In fact, photographs of the grey heron standing inside a tank were only those of the 15 cm water depth tank, which also had the greatest number of photographs showing the grey heron in general (not only inside, but also around a tank). Another study also suggests that serious damage can be inflicted on populations of freshwater fish in relatively shallow streams (15 to 30 cm water depth) by predation of water birds (Penaluna et al. 2016). Additionally, age-0 salmonids were reported to live in water depths roughly within the range of 5-30 cm (Wakabayashi et al. 2003; Kawai et al.

2007). These studies collectively indicate that declining water levels due to reducing river flows could increase the predation risk of salmonid juveniles in small streams. In contrast, declining water levels might decrease the predation risk for juvenile fish in rivers where the primary predator is piscivorous fish, which seem to pose a lesser predation risk in shallow waters (Harvey and Stewart 1991). Therefore, the effects of declining water levels on predation risk will also depend on the predator species.

Results of the second tank experiment suggest that artificial structures in the AS tank mitigated predation risk. Generally speaking, salmonids including the cultured white-spotted charr tend to avoid predators by using shelter and concealing themselves among gravel (Miyamoto 2016ab). Therefore, fish could have avoided predator attacks if they had noticed their predators in time, even inside the control tanks, by using cobbles, gravel, and shade as shelter. However, only a few fish survived each trial in the control tanks. This result implies that charr had difficulty avoiding the grey heron, probably because the grey heron is an ambush predator that usually stands still in an upright position while waiting for fish (Tojo 1996). Therefore, the charr was most likely caught before noticing the grey heron. On the other hand, water birds, including birds such as the grey heron and the kingfisher, are obstructed in their hunting of fish by ruffling water surfaces (Sawara et al. 1990), turbid waters (White 1936; Cezilly 1992), and submerged aquatic vegetation (Lantz et al. 2011). These results were consistent with the theoretical predictions that structures mitigate predation risk by obstructing the view of potential predators (Stoner 1982). Furthermore, the number of photographs showing the grey heron around or inside a tank was slightly higher for AS tanks than for the control ones. This result indicates that the grey heron had much less success and needed to expend more time and energy hunting inside an AS tank than inside a control one, possibly because

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artificial structures obstructed its view.

In any case, these results suggest that the intensity of predator-prey interaction can be indirectly determined by physical habitat conditions (water levels and structural complexity, in this case). Thus, it is necessary to consider the physical habitat conditions to evaluate predator-prey relationships, in addition to the predator and prey traits.

General discussion and conclusion

The predator-prey interaction, in my experimental settings at least, was mainly determined by a combination of predator and prey traits (behavior, density and body size), while the intensity of predator-prey interaction was mainly determined by physical habitat.

Regarding the relationship between body size and predator-prey interaction, the prey body size preference differed depending on whether the main predator was the piscivorous fish (Chapter-1) or the grey heron (Chapter-2). Furthermore, predation risk has been shown to depend on the relative body sizes between piscivorous fish and stocked fish (Chapter-1), whereas size-selective predation by the grey heron did not only depend on the size of prey fish, but also on their density (Chapter-2). These results imply that predator-prey interaction, in the experimental settings in Chapters-1 and -2, was comprised of prey body size, prey density, predator species and predator body size.

The results in Chapter-2 suggest that grey heron exhibits similar frequency of appearance and temporal trends in response to the number of fish. Therefore, the results of Chapters-2 and -3 indicate that most stocked fish settled around the stocking site, leading to there being a high concentration of grey heron visitations at that site. Regarding the individual fish behavior, the results in Chapter-3 suggest that fish behavior observed in the aquariums contributed to their predation risk. In particular, the normal TFUS of prey fish significantly affected their predation risk, presumably because the primary predator was an ambush predator, the grey heron. On the other hand, regarding predator avoidance behavior, as elaborated in Chapter-3, F11 salmon had a weaker response than F1 salmon to the predator stimulus in the aquarium test. The reason for this could be that the predator avoidance behavior of farmed salmon might have been weakened by domestication. Therefore, in a situation where fish behavior has a major effect on their

predation risk, it might be necessary to consider the effects of domestication on their behavior.

Regarding the relationship between physical habitat and predator-prey interaction, the results in Chapter-3 show that the primary feeding sites of the grey heron were the riffles, not the pools. The main reason for this could be that traits determining the behavior of the grey heron make hunting in deeper waters unpreferable, which is further supported by the results of Chapter-4. Regarding structural complexity, the results in Chapter-4 suggest that structural complexity mitigated predation risk by obstructing the vision of grey heron. In addition, it also seems that the survival of fish which use structures as part of their normal TFUS (Chapter-3) is associated with structural complexity of their habitat. Based on these results, I suggest that physical habitat conditions such as water depth and structural complexity serve as the function of intensity of predator-prey interaction.

The results of chapters 2-5 reaffirm the effectiveness of camera trapping to evaluate predator-prey interaction in a semi-natural stream. Although there were multiple photos of the grey heron holding fish in its beak, the number of those photos was not enough to account for all of the lost fish during the study, presumably because the grey heron's act of predation is instantaneous and difficult to capture on camera. In fact, there were many photos depicting the grey heron with its beak in the water, probably just before the act of predation. Therefore, I was unable to precisely evaluate the predation pressure by the grey heron. In the future, video footage might be able to solve this issue.

Finally, while I was investigating the determinant factors of predator-prey interaction in limited experimental conditions, the main predator was the grey heron in Chapters-2, -3 and -4. The grey heron is native throughout temperate Europe, Asia and

parts of Africa. According to the latest available records (1987-2007), the population size of grey heron has been increasing in Northern/Western Europe and Western/South-western Asia (Heron Conservation 2012 https://www.heronconservation.org/). In Japan, according to the latest available records, grey heron habitats have expanded considerably (Biodiversity center of japan 2004) and might continue to expand in the future. This phenomenon results in difficulties for fishermen and fish farmers because of increased consumption of fish by the grey heron, leading to actions being taken (usually hunting) in trying to manage grey heron populations. Therefore, in order to avoid conflicts between grey heron and humans, the results of this study should prove useful in developing non-invasive methods of mitigating predation on fish by the grey heron.

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Table 1 Results from two-way ANOVA to determine the effect on the survival rate of

masu salmon by charr size (FL), salmon size (FL) and their interaction.

Variable	df	MS	F	p
Charr FL	2	1.24	44.63	< 0.001
Salmon FL	3	2.51	90.20	< 0.001
Charr FL × Salmon FL	6	0.13	4.48	0.004
Error	24	0.02		

Table 2 Statistical test for the difference in survival rate of masu salmon among the different FL-classes.

*Results of Bonferroni post hoc tests, survival rate data were arcsin $\sqrt{x}$ transformed prior to analyses.

Charr FL (mm)	Salmon FL (mm)		p *
Small	55-75	80-100	**0.018**
(160-180)	55-75	105-125	**< 0.001**
	55-75	130-150	**< 0.001**
	80-100	105-125	0.400
	80-101	130-150	0.074
	105-125	130-150	1.000
Medium	55-75	80-100	**< 0.001**
(240-260)	55-75	105-125	**< 0.001**
	55-75	130-150	**< 0.001**
	80-100	105-125	**0.006**
	80-101	130-150	**< 0.001**
	105-125	130-150	0.910
Large	55-75	80-100	1.000
(320-340)	55-75	105-125	**< 0.001**
	55-75	130-150	**< 0.001**
	80-100	105-125	**< 0.001**
	80-101	130-150	**< 0.001**
	105-125	130-150	0.196

Table 3 Statistical test for the difference of the survival rate of masu salmon among the different charr (predator) FL-classes.

*Results of Bonferroni post hoc tests, survival rate data were arcsin $\sqrt{x}$ transformed prior to analyses.

Salmon FL (mm)	Charr FL		$p*$
55-75	Small	Medium	**< 0.001**
	Small	Large	**< 0.001**
	Medium	Large	1.000
80-100	Small	Medium	**0.009**
	Small	Large	**< 0.001**
	Medium	Large	**< 0.001**
105-125	Small	Medium	0.455
	Small	Large	**0.007**
	Medium	Large	0.200
130-150	Small	Medium	1.000
	Small	Large	0.200
	Medium	Large	0.804

Table 4 Results from two-way ANOVA to determine the effect of study days, fork length

(FL) and their interaction on the survival of masu salmon (*Oncorhynchus masou*).

*Mean Square.

Variable	d.f.	MS*	F	P-value
Study days	2	0.38	10.56	**0.001**
FL	1	1.31	36.67	**< 0.001**
Study days × FL	2	0.01	0.18	0.833
Error	18	0.04		

Table 5 Results from two-way ANOVA to determine the effect of number of salmon, fork length (FL) and their interaction on the number of photographs containing grey herons (*Ardea cinerea*).

*Mean Square.

Variable	d.f.	MS*	F	P-value
Number of salmon	2	1.11	149.26	**< 0.001**
FL	1	0.02	2.48	0.141
Number of salmon × FL	2	0.00	0.02	0.981
Error	12	0.01		

Table 6 The mean ± SD fork length and body weight of F11 and F1 fish.

Statistics were performed with a Students t-test.

	F11	F1	t	p
Fork length (mm)	74.73 ± 7.20	74.12 ± 6.75	0.874	0.383
Body weight (g)	4.33 ± 1.23	4.20 ± 1.23	1.023	0.307

Table 7 The valuables of generalized linear model for the effects of behavior factors, origin, fork length and their interactions on the survival of stocked masu salmon.

Variable
Dependant value:
Survival of stocked fish (fish that survived or not)
Independant value:
Normal TFUS (TFUS without stimulus)
Post-stimulus TFUS (TFUS with mimicked predation stimulus)
Origin (F1 of F11)
Fork length (Before the release)
Normal TFUS × post-stimulus TFUS
Normal TFUS × origin
Normal TFUS × fork length
Post-stimulus TFUS × origin
Post-stimulus TFUS × fork length
Origin × fork length

Table 8 The best model of a generalized linear model selected by the AIC.

The table indicates the Akaike's information criterion scores (AIC), the difference

between the AIC for a given model and the best model (Δ AIC).

Model	AIC	Variable	Coefficient	z	p
Best model	135.1	Normal TFUS	0.011	2.306	**0.021**
		Fork length	0.064	1.685	0.057
		Constant	-5.119		
Second-best model	135.6	Normal TFUS	0.011	2.372	**0.018**
		Origin	-0.313	-0.739	0.460
		Fork length	0.065	1.923	0.054
		Constant	-5.035		
Third-best model	136.0	Normal TFUS	0.009	2.056	**0.040**
		Constant	-0.035		
Fourth-best model	136.1	Normal TFUS	0.004	0.606	0.544
		Origin	-0.608	-1.251	0.211
		Fork length	0.060	1.742	0.082
		Normal TFUS × origin	0.012	1.221	0.222
		Constant	-4.478		
Fifth-best model	136.2	Normal TFUS	0.011	2.293	**0.022**
		Post-stimulus TFUS	0.000	-0.171	0.864
		Fork length	0.064	1.858	0.063
		Constant	-5.001		

Table 9 Results of a generalized linear mixed model: effects of the water depth category (i.e. 15cm, 25cm, 35cm and 45cm) on charr probability of survival (a) and on the number of photographs containing grey heron (b) (G2, the likelihood-ratio statistic).

[1]Standard deviations of the random effects: 0.641 for trials.

[2]Coefficient value: 0 for 15cm, 6.203 for 25cm, 6.619 for 35cm and 45cm; standard error (SE): 0.728 for 25cm, 0.836 for 35cm and 45cm.

[3]Standard deviations of the random effects: 0.462 for trials.

[4]Coefficient value: 0 for 15cm, -1.812 for 25cm, -1.735 for 35cm, -2.197 for 45cm; standard error (SE): 0.215 for 25cm, 0.208 for 35cm, 0.255 for 45cm.

	Variable	G2	df	coefficient	SE	p
(a)[1]	Water depth category[2]	124.7	3	0 to 6.619	0.728 to 0.836	< 0.001
	Constant			-2.363	0.471	
(b)[3]	Water depth category[4]	171.1	3	-2.197 to 0	0.208 to 0.255	< 0.001
	Constant			3.548	0.247	

Table 10 Results of a generalized linear mixed model: effects of the structure category (control tank / artificial structure tank) on charr probability of survival (a) and on the number of photographs containing grey heron (b) (G2, the likelihood-ratio statistic).

[1]Standard deviations of the random effects: 0.395 for trials, 0.010 for tanks.

[2]Coefficient value: 0 for control tank, 2.813 for artificial structure tank; standard error (SE): 0.285 for artificial structure tank.

[3]Standard deviations of the random effects: 0.445 for trials, 0 for tanks.

[4]Coefficient value: 0 for control tank, 0.315 for artificial structure tank; standard error (SE): 0.057 for artificial structure tank.

	Variable	G2	df	coefficient	SE	p
(a)[1]	Structure category[2]	97.77	1	0 to 2.813	0.285	< 0.001
	Constant			-2.516	0.403	
(b)[3]	Structure category[4]	30.29	1	0 to 0.315	0.057	< 0.001
	Constant			3.925	0.337	
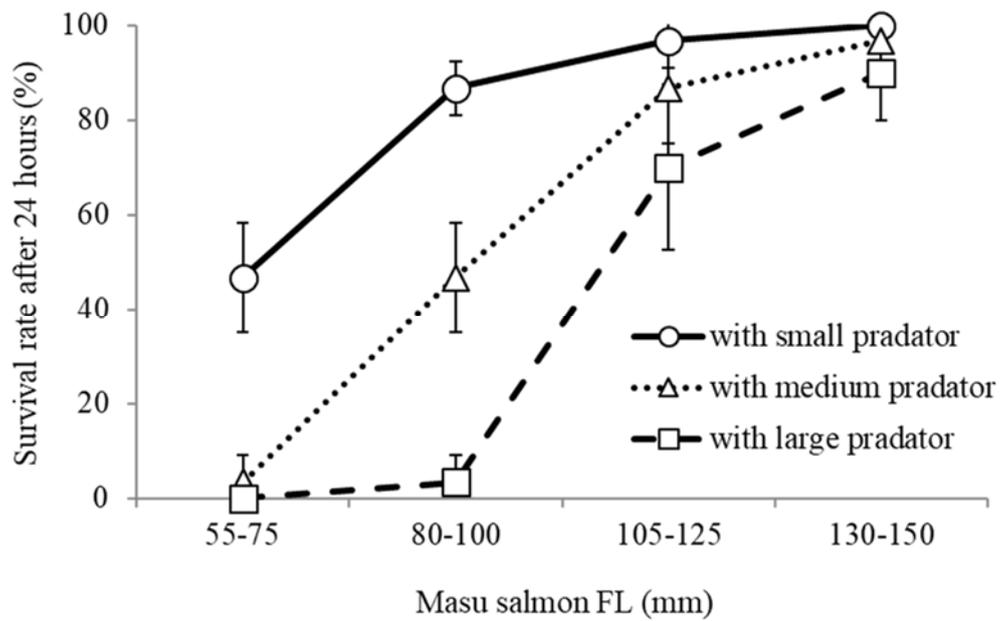
Fig. 1 Survival rate of masu salmon with different body sizes. Circles and solid line: using small predator (white-spotted charr); triangles and plot line: using medium predator; squares and broke line: using large predator.
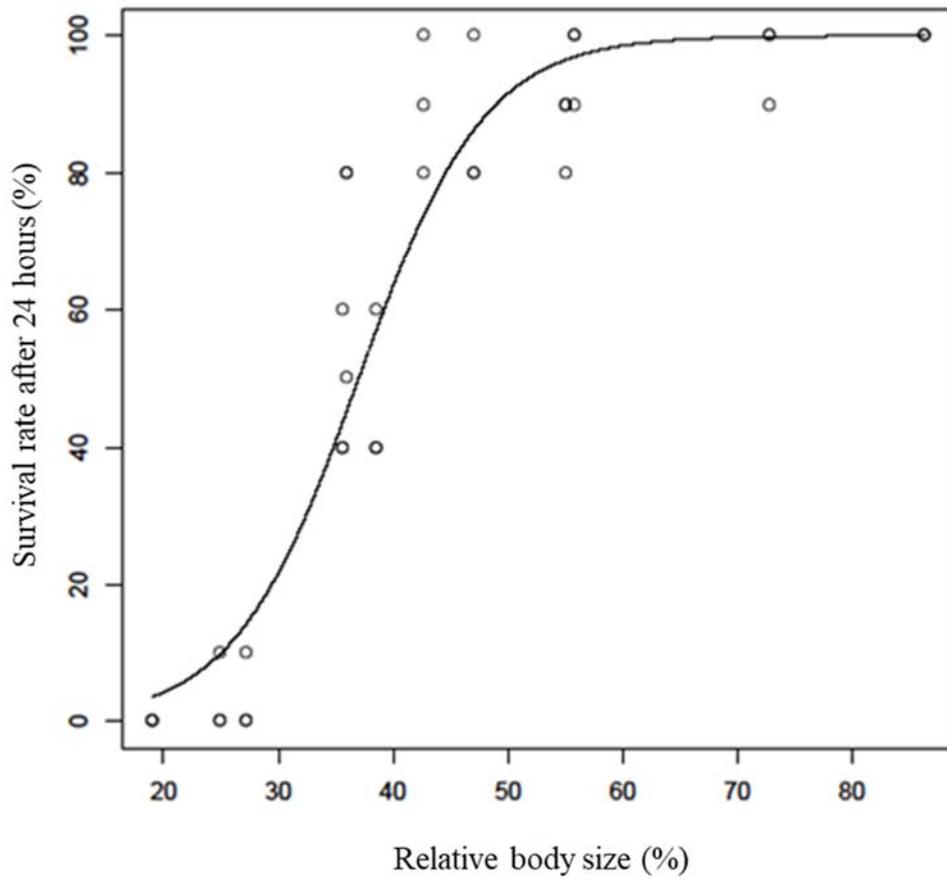
Fig. 2 Relationship between survival rate of masu salmon and relative body size of masu salmon to the predator (white-spotted charr).

Fig. 3 a and b: outdoor fibre-reinforced plastic circular tank (a) and semi-natural stream (b) at National Research Institute of Fisheries Science in Nikko. c and d: examples of photograph of grey heron in the pool (c) and in the riffle (d) in the semi-natural stream.
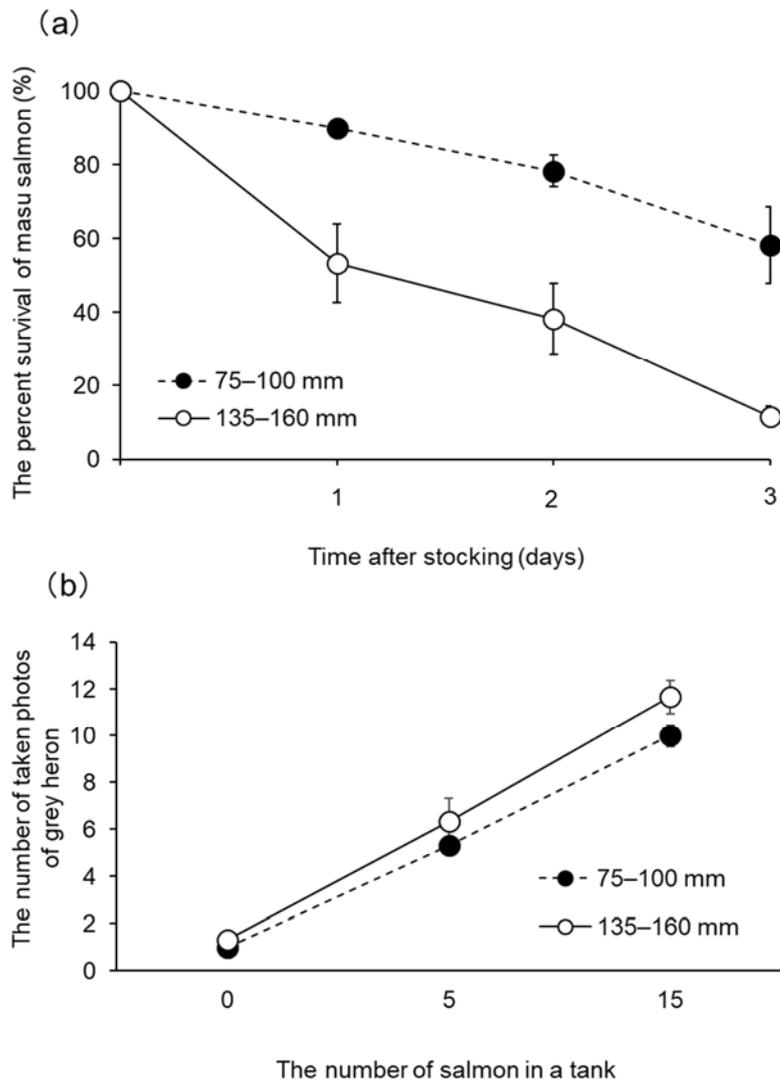
Fig. 4. The results of tank experiments. (a) Survival rate of masu salmon (*Oncorhynchus masou*) with different body sizes groups in outdoor tanks. Circles and solid line: using 75–100 mm salmon; black circles and dashed line: using 135–160 mm salmon. (b) Relationship between the number of taken photographs of grey heron (*Ardea cinerea*) and the number of masu salmon (*Oncorhynchus masou*) in outdoor tanks. Circles and solid line: using 75–100 mm salmon; black circles and dashed line: using 135–160 mm salmon. Data are means ± standard error.
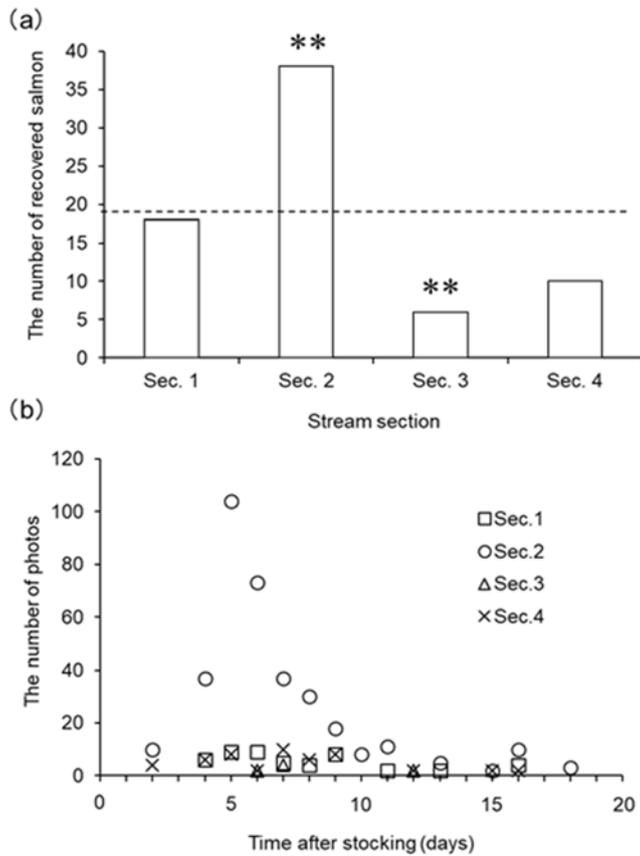
Fig. 5. The results of stream experiments. (a) The number of juvenile masu salmon (*Oncorhynchus masou*) recovered at each section in the semi-natural stream. In each section of the stream, fish number was calculated as the sum of fish remaining from each size category (75-100 and 135-160 mm). Broken line indicates the expected surviving proportion (we hypothesized a uniform 1:1:1:1 ratio). Asterisk denotes a significant deviation from the average value (** $p < 0.010$). Each section (Sec.) was named from the upper most section in the stream, Sec. 1, Sec. 2, Sec. 3 and Sec. 4, Sec. 2 was the stocking location. (b) Number of photographs of grey heron (*Ardea cinerea*) in each study section of semi-natural stream. X-axis show the number of days elapsed from stocking (day 0) to completion at day 20. Data are means ± standard error.
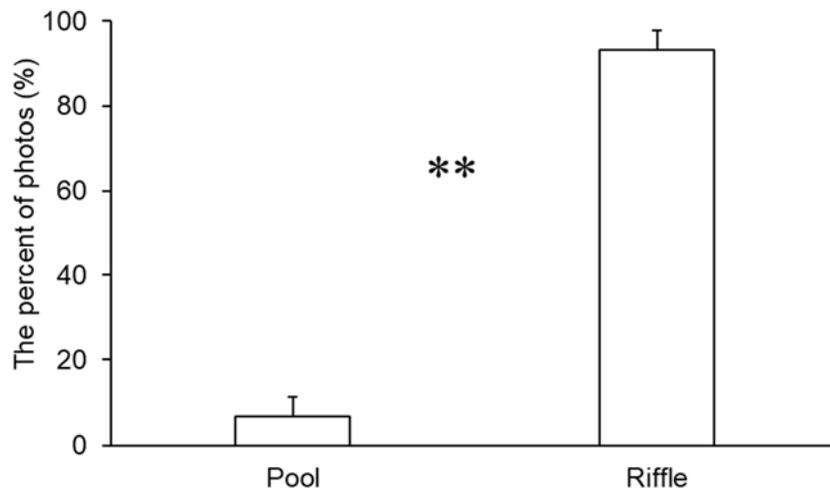
Fig. 6. The percent of the photographs that showed grey heron located around the pool or riffle across each study section of the semi-natural stream. Asterisk denotes a significant difference (** $p < 0.010$). Data are means ± standard error.
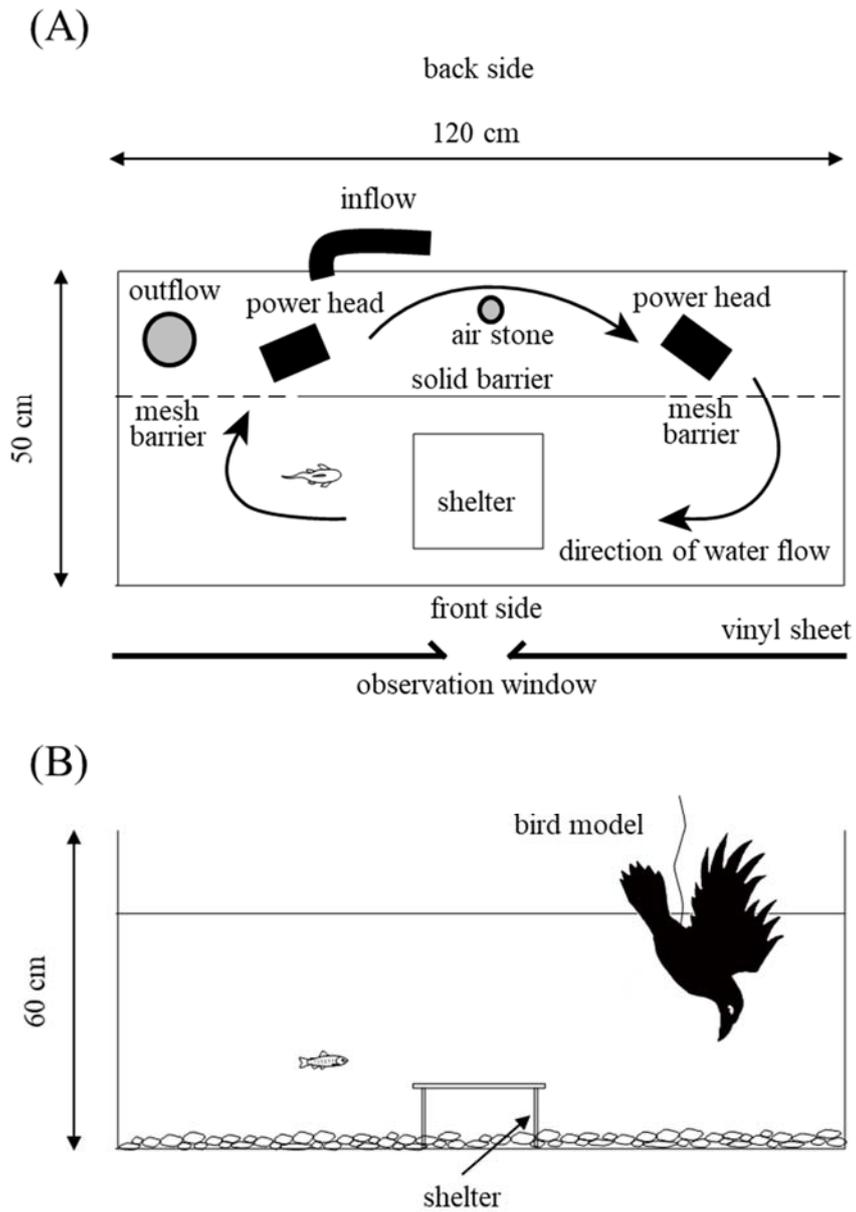
Fig. 7. Illustration of the aquarium used for behavioral observations. (A) Top view. (B) Front view when the bird model was dropped as the mimicked bird attack stimulus. Two aquarium powerheads were located in the aquarium, were used to create flow throughout the aquarium.
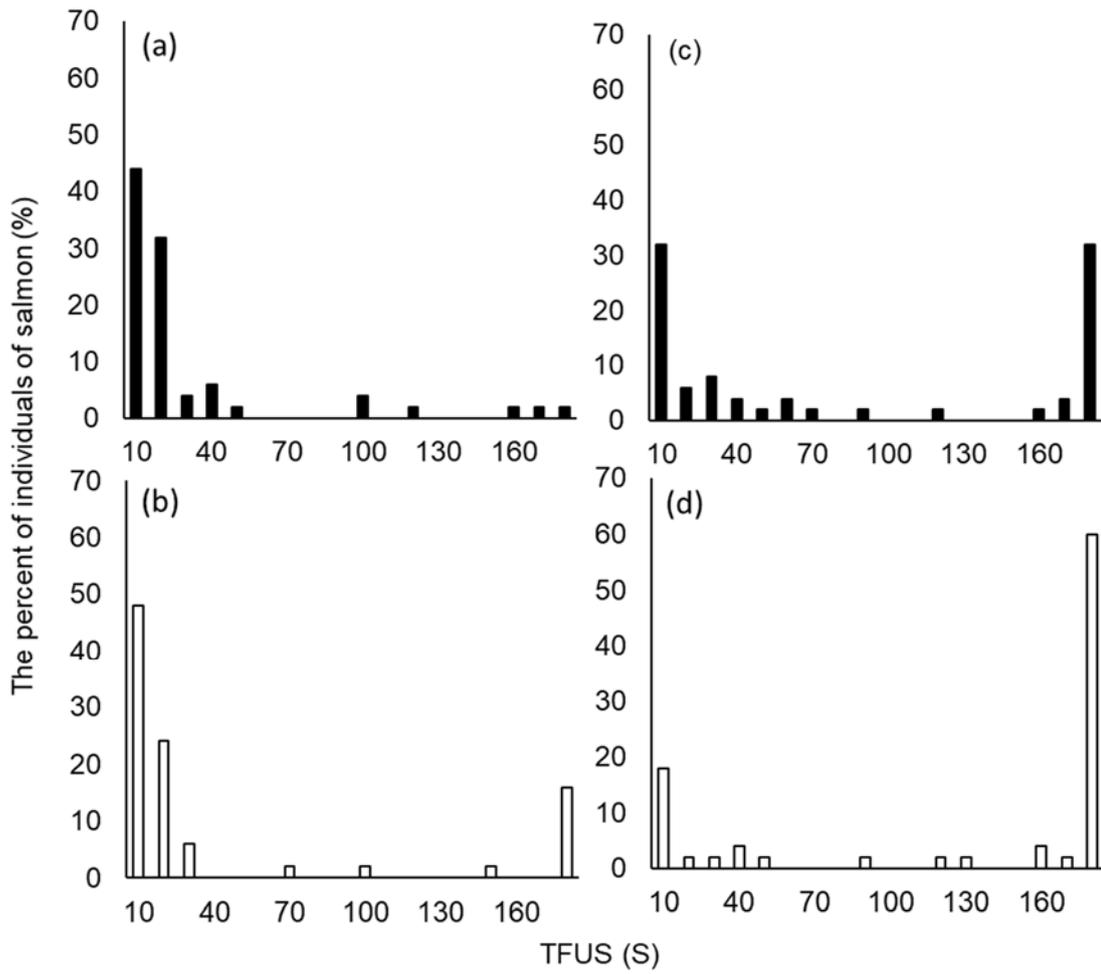
Fig. 8. The percent of the individuals of salmon that showed the time that fish used structure under the normal condition (a for F11 and b for F1), and that after the mimicked bird attack stimulus (c for F11 and d for F1) in the aquarium test. Each observation was made for 180 seconds.
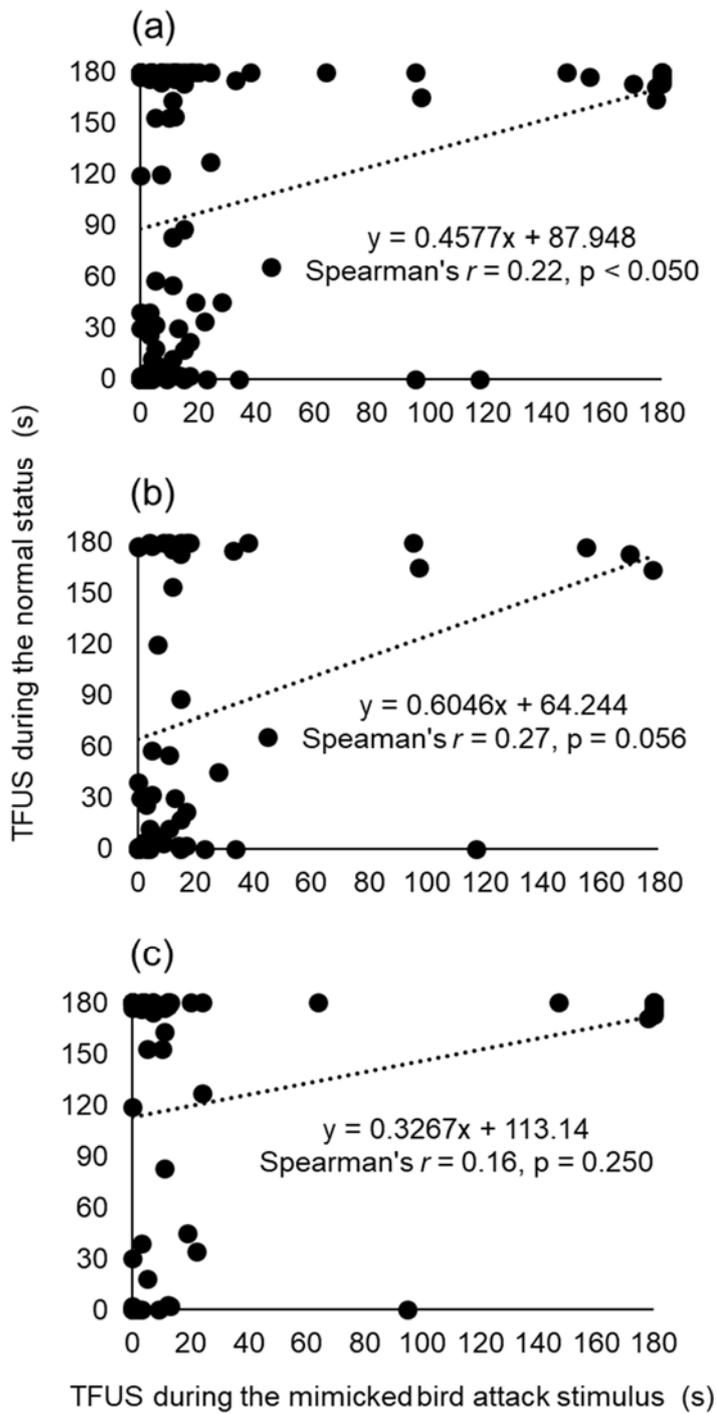
Fig. 9. The relationships between the time that fish used structure (TFUS) under the normal condition and after the mimicked bird attack stimulus of total fish (a), F11 fish (b) and F1 fish (c).
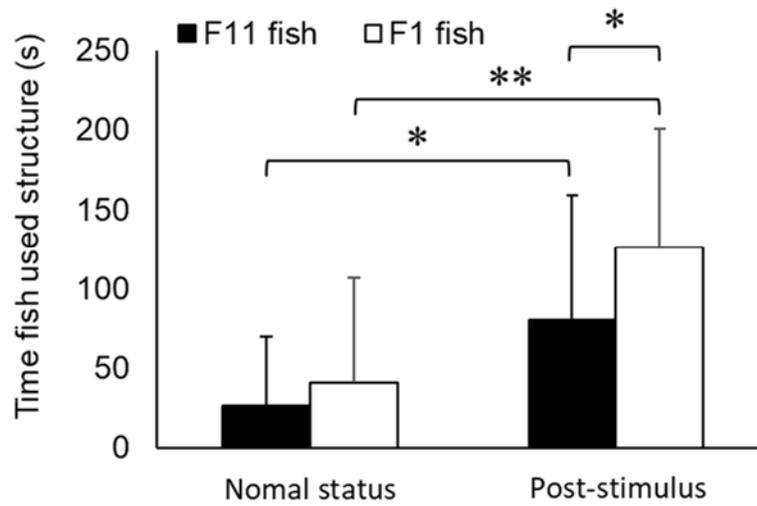
Fig. 10. The time that masu salmon used structure under the normal condition and after the mimicked bird attack stimulus in the aquarium test. Each observation was made for 180 seconds. Black bar: F11 fish; open bar: F1 fish. Error bars represent standard deviations. * $p < 0.05$, ** $p < 0.01$.
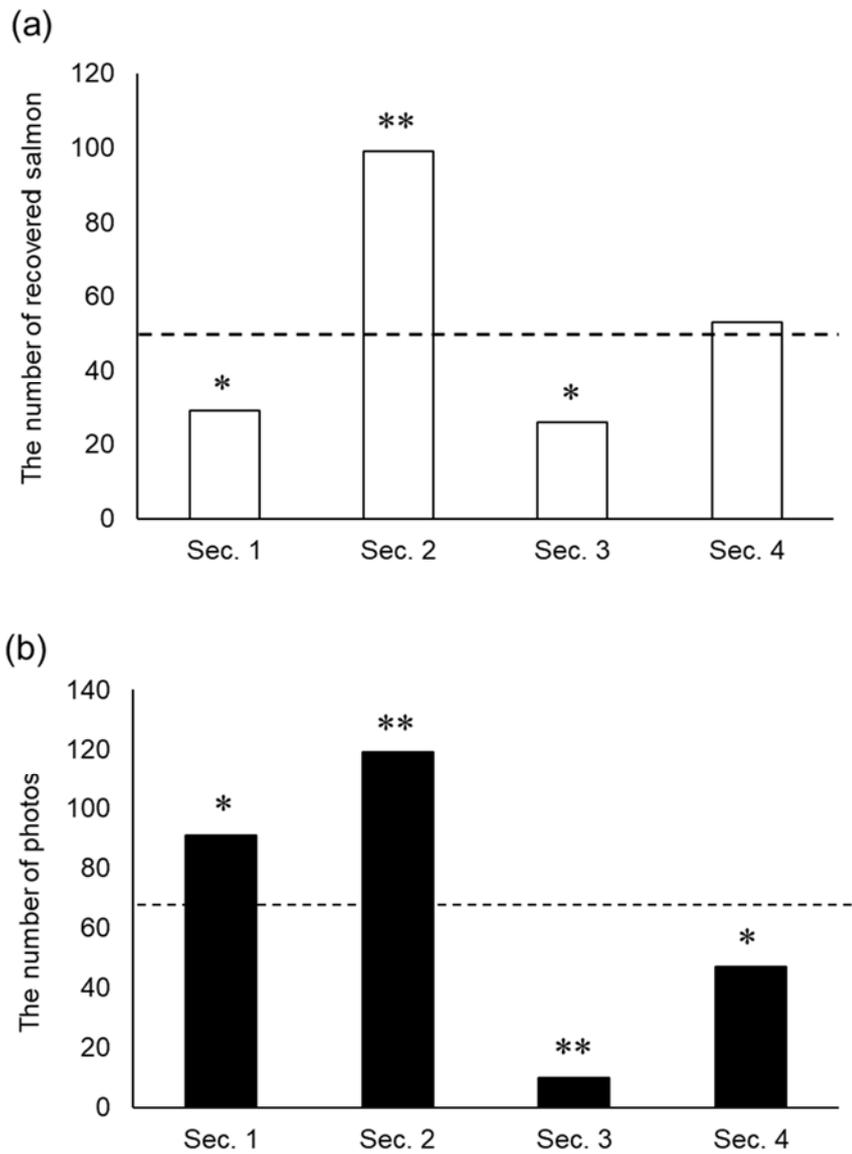
Fig. 11. The total number of fish recovered (a) and of photographs of grey heron (b) at each section of the semi-natural stream. Broken lines indicate the averages over the sections (expected values under uniform distribution). Asterisk denotes a significant deviation from the average value (* $p < 0.05$, ** $p < 0.01$). Each section (Sec.) was named from the upper most section in the stream, Sec. 1, Sec. 2, Sec. 3 and Sec. 4. Sec. 2 was the stocking location.
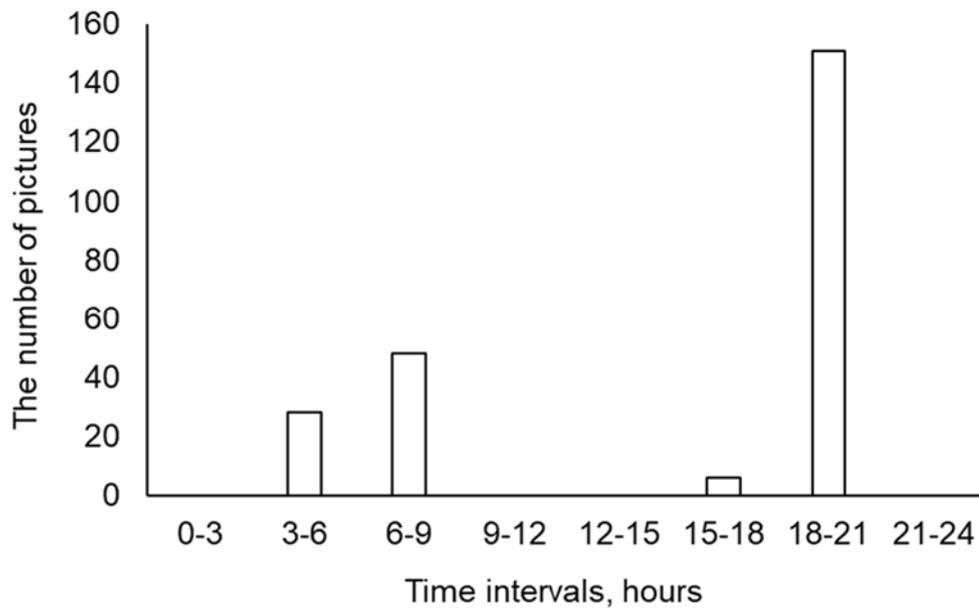
Fig. 12. Number of photographs of grey heron (*Ardea cinerea*) taken in different time range in the semi-natural stream.
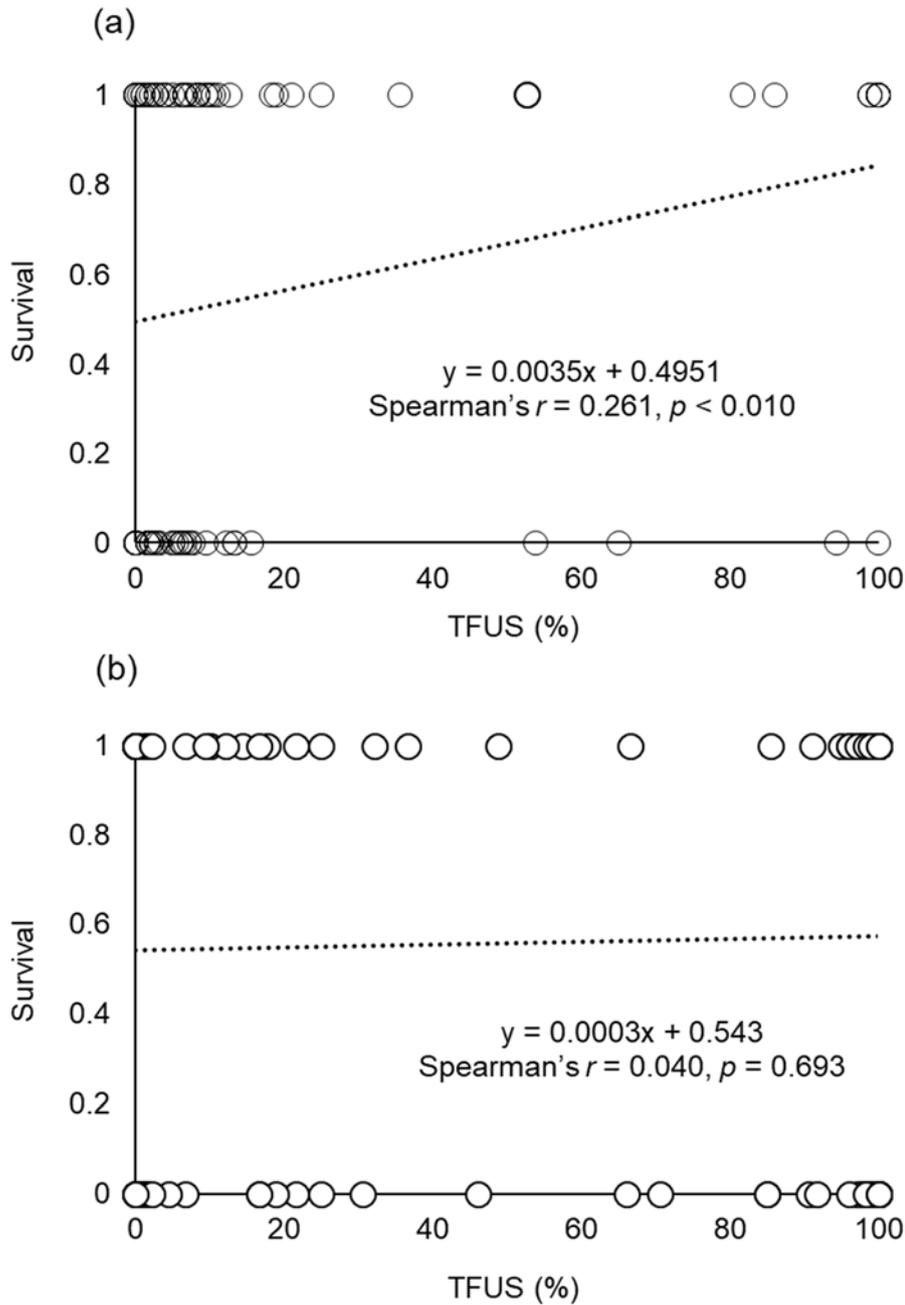
Fig. 13. The relationships between the survival of masu salmon after the stream observation and the time that fish used structure (TFUS %) under the normal condition (a) and after the mimicked bird attack stimulus (b) of surviving individually marked masu salmon. Circle: survival of masu salmon (survival: 1 or not: 0).
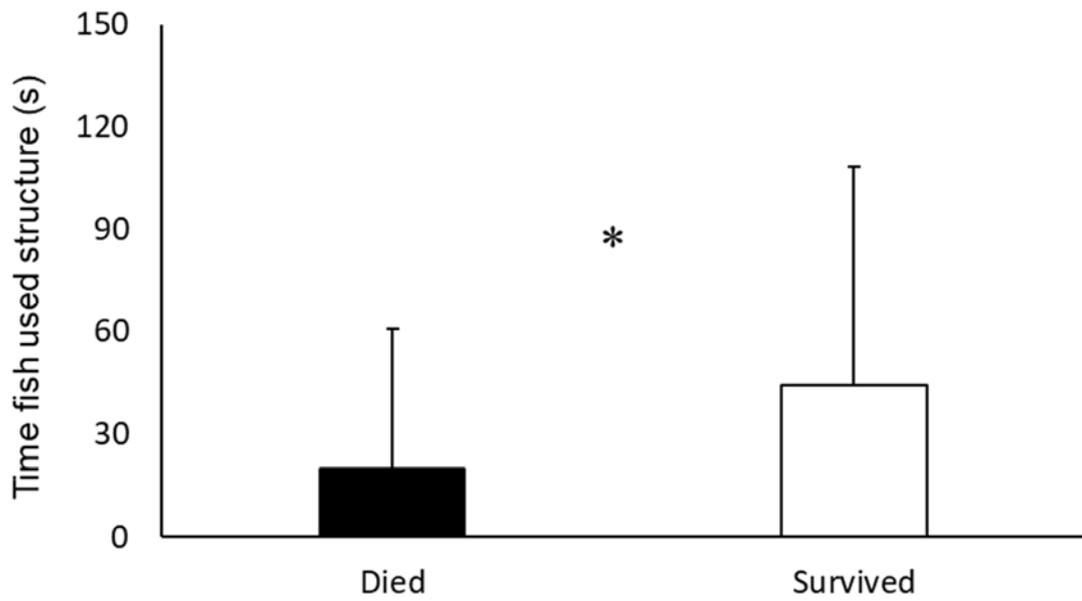
Fig. 14. The relationship between the time that masu salmon used structure under the normal condition and the survival of individually marked fish (survived or not) stocked in the stream. The behavior observation was made for 180 seconds. Black bar: the fish died; open bar: the fish survived. Error bars represent standard deviations. * $p < 0.05$).
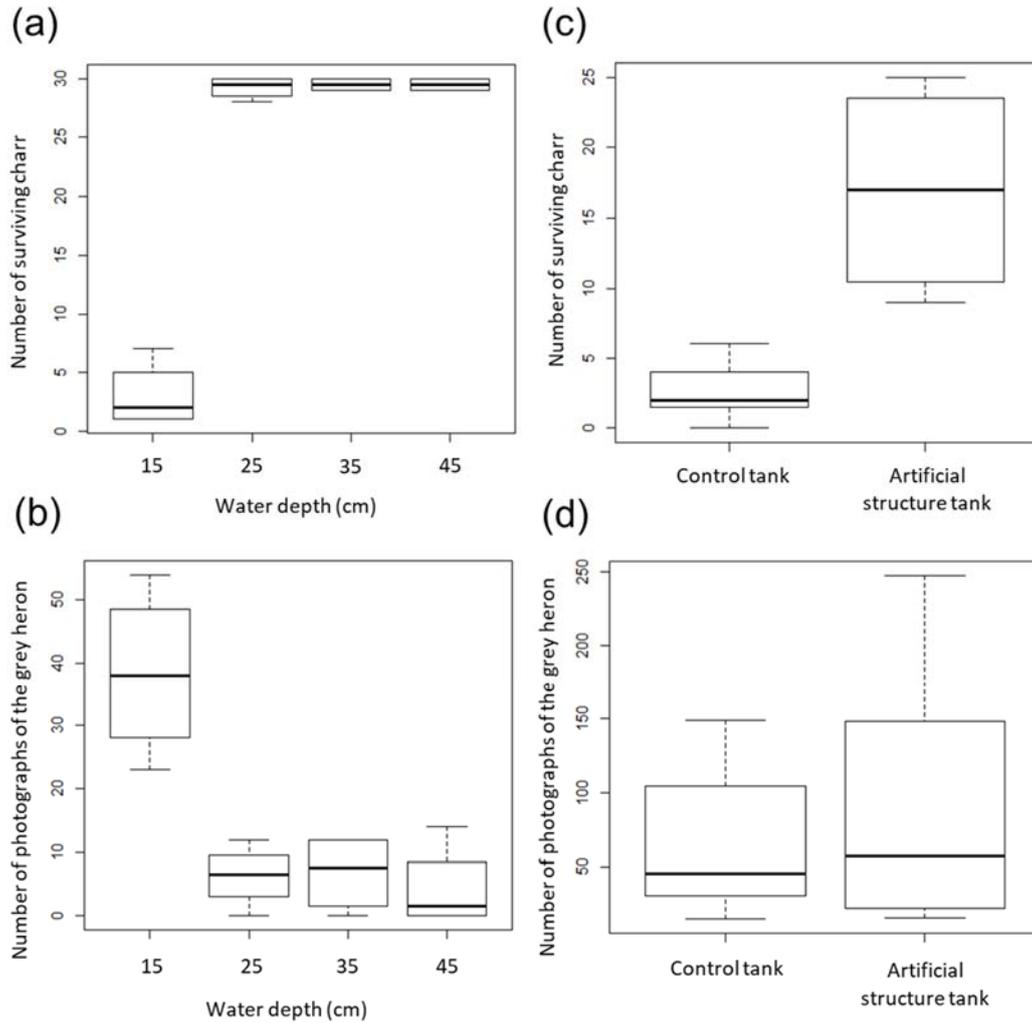
Fig. 15. Box plots illustrating (a) the number of surviving charr for each of the four water depth tanks, (b) the number of photographs containing grey heron for each of the four water depth tanks, (c) the number of surviving charr for the control tanks and the artificial structure tanks and (d) the number of photographs containing grey heron for the control tanks and the artificial structure tanks.