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Changes in post- and pre-capture escape ability over development in juvenile Japanese eels

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ABSTRACT: Predation is one of the major forces driving the evolution of defensive tactics in prey. Recently, post-capture active escape behaviors of different prey animals from the predator's digestive tract have been reported. However, no studies have yet examined when these escape behaviors are developed through the ontogeny of the prey. This study examined the ontogenetic change in a unique defensive tactic of juvenile Japanese eels *Anguilla japonica* in which they escape via the predator's gills after being captured. We used *A. japonica* ranging in stage from pre-settlement glass eels to post-settlement yellow eels (based on their habitat change from tidal estuaries to freshwater rivers). The results indicated that individuals in the 2 earliest stages of glass eels (stages VI_{A0} and VI_{A1}) never escaped after being captured, while 28.1% of individuals after the middle glass eel stages (VI_{A2} and later stages) escaped via the predator's gill. The ontogenetic timing of the development of escape ability coincides with when eels settle into benthic riverine and estuarine habitats as suggested by previous studies. Additionally, the pre-capture attack avoidance ability of Japanese eels improved rapidly with growth in the subsequent elver and yellow eel stages. These results suggest that the unique post-capture defensive tactic of eels may be particularly important during the vulnerable period around the settlement phase when they are less capable of avoiding predator attacks. Our study offers valuable insights into the behavioral ecology and conservation of anguillid eels, which have faced considerable population declines.

KEY WORDS: Escape behavior · Predator—prey interaction · Recruitment · Behavioral ontogeny · *Anguilla japonica* · Post-capture defensive tactics · Metamorphosis · Settlement

1. INTRODUCTION

Predation is one of the greatest selective forces that affect the survival of prey species. Predation pressure has resulted in the evolution of a variety of effective defensive tactics in prey (Abrams 2000). These tactics can be divided into 2 categories: those focusing on the pre-capture phase, such as counter-adaptations against searching, recognition, and attack by predators (Davies et al. 2012, Cooper et al. 2015), and those that are effective after prey have been captured. Compared to the pre-capture defen-

sive tactics of prey (e.g. mimicry, schooling, and escape responses) (Joron & Mallet 1998, Mallet & Joron 1999, Davies et al. 2012, Cooper et al. 2015), there have been fewer reports on post-capture defensive tactics. Moreover, most of those tactics are limited to passive mechanisms, such as the use of spines (Hoogland et al. 1956, Forbes 1989, Morgan 1989, Bosher et al. 2006) and poison (Losey 1972, Sugiura & Sato 2018) to prevent being swallowed or using protective armors (Norton 1988, Silva et al. 2019, Lovas-Kiss et al. 2020) to prevent digestion and allow passage through the gut.

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Recently, some active behaviors to allow escape from the digestive tract of predators after being captured have also been reported. For example, an aquatic beetle captured by frogs can move through their digestive tract toward the end of the intestine and be expelled alive in a short period of time (Sugiura 2020). The parasitic gordian worm can escape through the mouth, nose, or gills when the host insect is captured by fish or frogs (Ponton et al. 2006). The Japanese eel *Anguilla japonica* can escape through a predator's gill clefts by navigating backward from the stomach of a predatory fish after being swallowed (Hasegawa et al. 2022, 2024) (see Fig. 1). However, there are only limited reports of these active escapes, and detailed information regarding their ontogeny, mechanisms, and evolution is still largely lacking.

Generally, the ability of prey to avoid predation improves with ontogeny in the prey species due to the development of their physical and sensory organs and the acquisition of experience and learning (Werner & Gilliam 1984, Warkentin 1999, Pongrácz & Altbäcker 2000, Lind & Cresswell 2005). Given that prey with underdeveloped escape abilities are vulnerable to a wide range of predators, the early life history period is a major bottleneck that exerts particularly strong selection pressure on most prey species (Folkvord & Hunter 1986, Fuiman & Magurran 1994, Cowan et al. 1996, Hinke et al. 2020). This intense selection pressure during early life history has shaped the evolution of a variety of morphological (e.g. armors, mimicry, and coloration) and behavioral (schooling, freezing, use of the refuges, and parental care) defensive tactics in prey species, enabling them to survive this vulnerable period (Fuiman & Magurran 1994). Therefore, exploring the anti-predator tactics accompanying the development of prey is essential for understanding their survival strategies in predator–prey interactions.

Anguillid and other eels have a pelagic larval phase, and these larvae are referred to as leptocephali (Bertin 1956, Tesch 2003). After birth in offshore pelagic waters, anguillid leptocephali are transported toward their continental recruitment areas by ocean currents before they metamorphose into transparent glass eels (Miller & Tsukamoto 2017). After glass eels reach riverine and estuarine habitats, they gradually increase body pigmentation and become pigmented elvers (Fukuda et al. 2013). Once the eels complete the pigmentation process, they are referred to as yellow eels. Throughout these developmental stages, their morphology and behaviors change (Imbert et al. 2008, Hatakeyama

et al. 2022). It is possible that these changes influence their defensive tactics against predators. Moreover, fishes with a pelagic larval phase develop considerably in their swimming and sensory performance leading up to settlement in new habitats (Fuiman & Magurran 1994, Leis 2006, 2010). This suggests that during the settlement phase, individual traits become more crucial in response to various natural selection pressures, including predation, compared to during the pelagic phase, which is more influenced by random events (e.g. passive transportation). Therefore, juveniles around the settlement phase are a particularly important stage with which to investigate defensive behavior.

Temperate anguillids (e.g. Japanese eels *A. japonica*, European eels *A. anguilla*, and American eels *A. rostrata*) are well-known for their high commercial value, and their populations have drastically declined in recent decades (Haro et al. 2000, Kaifu & Yokouchi 2019, Righton et al. 2021). In response to these population declines, various factors affecting their population levels have been studied, including migration patterns, habitat selection, and the amount of fishery catches (Righton et al. 2021). However, surprisingly little is known about their defensive tactics against predators, even though predation is a crucial factor for the survival of prey species in general.

Previously, we discovered a unique defensive behavior of juvenile Japanese eels in which more than half of the captured individuals escaped via the predator's gill by moving between the gill arches (Hasegawa et al. 2022) (Fig. 1). In all successful escapes, the tails of the eels emerged first through the gill, and then their whole bodies slipped out in a backward direction. Further experiments using an X-ray video system revealed that the eels escape by moving back up through the digestive tract from the stomach towards the gills (Hasegawa et al. 2024). Although we have clarified fundamental information (e.g. escape rate, time, route, and behavioral characteristics) about this unique escaping behavior, it is still not clear how this defensive tactic develops through ontogeny.

In this study, we investigated changes in the defensive ability of juvenile Japanese eels through their development. We conducted a predator–prey interaction experiment to examine how escape behavior after capture varies across different developmental stages of *A. japonica* juveniles, from pre-settlement glass eels to post-settlement yellow eels. Furthermore, we evaluated changes in their ability to evade predator attacks before capture across various developmental stages.
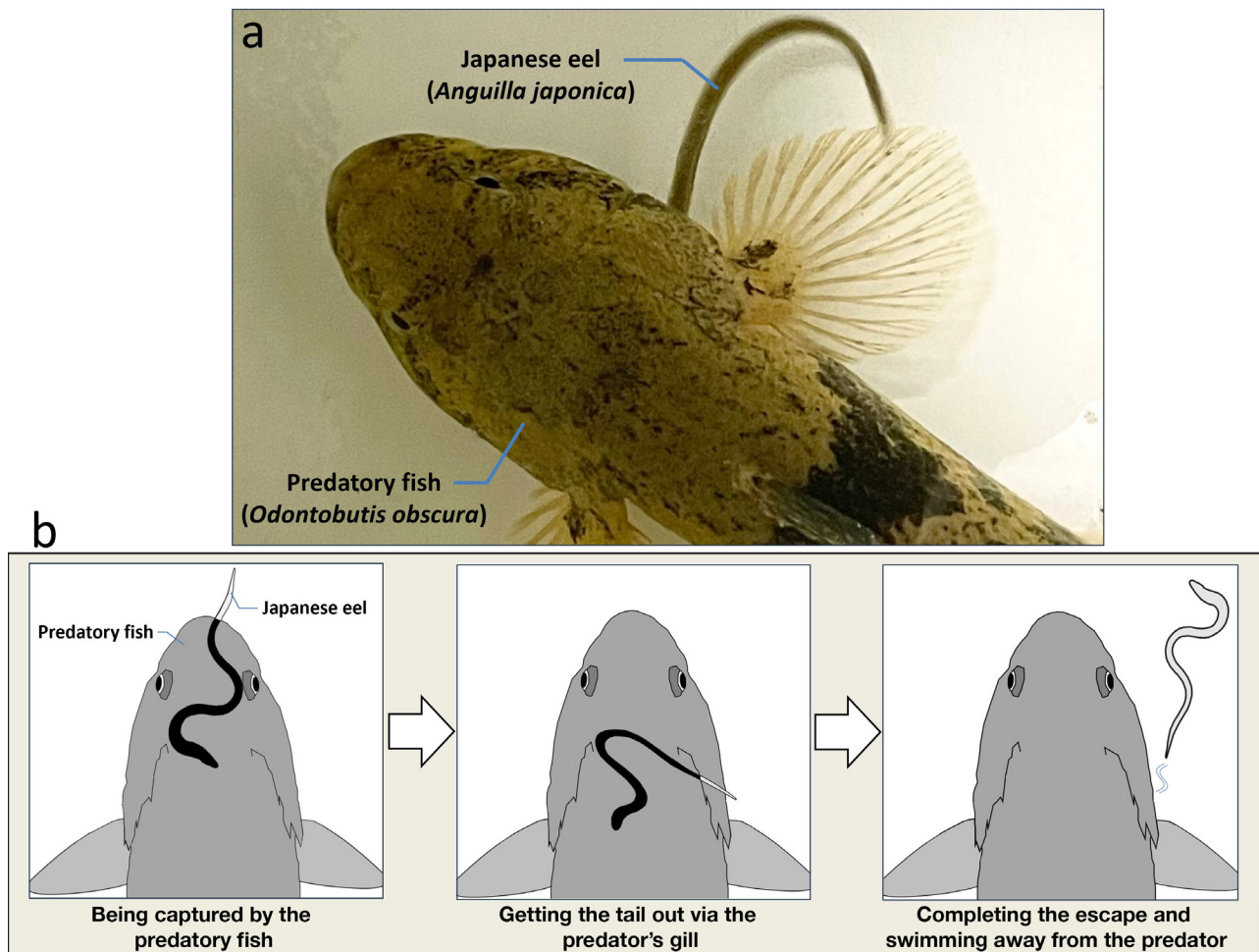
Fig. 1. Escape of Japanese eel *Anguilla japonica* juvenile via the predator's gill after being captured. (a) Snapshot of the post-capture escape of *A. japonica*. (b) Mode and sequence of escape, with parts of the eel inside the predatory fish shaded black

2. MATERIALS AND METHODS

2.1. Fish samples

We used *Anguilla japonica* juveniles (mean ± SD: 60.3 ± 6.2 mm, n = 302) that were reared by recruiting early stage glass eels each year over 3 seasons in 2021−2023. They were purchased from licensed local fishermen at the Tone River estuary in Chiba, Japan. The eels were maintained in glass aquariums (600 × 300 × 360 mm) and were fed frozen *Chironomus* sp. larvae once every 1−2 d. For the predator species, we used adult dark sleeper, *Odontobutis obscura* (167.4 ± 22.6 mm, n = 14; see Table S1 in the Supplement at www.int-res.com/articles/suppl/m752p137_supp.pdf), a nocturnal carnivorous fish that is a common predator in southwestern Japanese rivers. In the laboratory, *O. obscura* actively prey on *A. japonica*, and the eels have been confirmed to escape via the predator's gill following predation (Hasegawa et al. 2022). All *O. obscura* were kept in 10 l holding tanks (270 × 200 × 200 mm) after being collected using a hand net in the Urakami River in Nagasaki, Japan. Only those individuals that ate food under observation were transferred to the experimental tanks. *O. obscura* were initially fed frozen Manila clams, which have high palatability in the early rearing period, and were fed live Japanese killifishes *Oryzias latipes* for several days before starting the experiment.

2.2. Experimental procedure

Each experimental trial began with the retrieval of an *A. japonica* individual from the rearing aquarium

using a hand net. After anesthetization with 2-phenoxyethanol, its total length (TL) and body weight (BW) were quickly measured, and photographs were taken from both side and top views with a stereomicroscope (Zeiss Stemi 305; Carl Zeiss). The developmental stage was then determined as one of 7 stages (VI_{A0}, VI_{A1}, VI_{A2}, VI_{A3}, VI_{A4}, VI_B, and yellow eels; see Table 1) based on the progression of their pigmentation (Fukuda et al. 2013). Each measured individual was used in the experiment after recovering in a small tank (200 × 150 × 120 mm) for 1 d. Predator—prey experiments were conducted in a large glass aquarium (900 × 600 × 300 mm, 20.5 ± 1.4°C) divided into 8 sections by nylon mesh fabric (250 × 170 × 300 mm, mesh opening: 0.55 × 0.75 mm; Nagasaki Tenmaku). The measured individual was introduced into a PVC pipe (30 mm diameter), set in the center of one section of the experimental tank containing a predator, and left to acclimate for at least 10 min. Then, the trial was started by slowly removing the PVC pipe to release the eel, and the predator—prey interaction was recorded using a video camera (DSC-RX0; SONY) from the dorsal view. If *O. obscure* showed no attack behavior towards *A. japonica* or failed to capture the eel after 20 min, the experiment was finished. In cases where the predator attacked the eel, we counted the number of attacks by the predator (i.e. the number of attack-evasion instances by *A. japonica*) until the end of the trial. If the predator captured *A. japonica*, we continued recording their interaction for at least 3 min. This recording time was adopted based on the maximum escape time of *A. japonica* (130 s) in our previous studies (Hasegawa et al. 2022, 2024). All individuals that escaped via the predators' gill were kept in plastic tanks (200 × 150 × 120 mm) for at least 48 h to investigate whether they could survive after the trial. While *O. obscura* individuals were repeatedly used (167.4 ± 22.6 mm, n = 14; Table S1), each *A. japonica* individual (60.3 ± 6.2 mm, n = 302) was used only once.

The experiment was conducted over 3 seasons from 2021 to 2023 to collect behavioral data on *A. japonica* at various developmental stages, ranging from early glass eels to yellow eels. In 2021, monthly experiments were conducted from May to September, using 20 or 30 eels each time (n = 120). The following year, in 2022, the experiment was carried out from March to May with 15 eels used each week (n = 180). Additional experiments were conducted in July and September 2022 (n = 15) and in February 2023 (n = 15) due to a lack of data on early glass eels and yellow eels.

2.3. Ethical statement

Animal care and experimental procedures were approved by the Animal Care and Use Committee of the Faculty of Fisheries, Nagasaki University (Permit No. NF-0054) in accordance with the Guidelines for Animal Experimentation of the Faculty of Fisheries and the Regulations of the Animal Care and Use Committee of Nagasaki University.

2.4. Statistical analysis

Given that there was a small number of individuals in the earliest stage (VI_{A0}, n = 5), this stage was combined with VI_{A1} as stage VI_{A01} in all statistical analyses. A generalized linear mixed model (GLMM) analysis with a binomial distribution and logit link function was used to test whether the developmental stage of *A. japonica* influenced escape success via the predator's gill. Escape success and failure were designated as one and zero, respectively, and were considered objective variables. In this model, the developmental stage of *A. japonica* was regarded as an explanatory variable (i.e. fixed effect), and the predator identification was included as a random effect. The significance of the developmental stage was assessed by removing it from the model and comparing the change in deviance using a likelihood ratio test (LRT) with a χ^2 distribution.

Additionally, we analyzed the effect of the developmental stage of *A. japonica* on their defensive behaviors by categorizing interactions with predators into 3 phases (Fig. 2): (1) pre-captured attack evasion, (2) tail emergence via the predator's gill after being captured, and (3) completing the escape. In each model, the predator's identification was included as a random effect. In the first phase analysis, we tested whether the developmental stage of *A. japonica* influenced the evasion behavior before being captured during an attack by *O. obscura* by using the mixed-effect Cox regression model (Fig. 2a). This model is based on a widely used semi-parametric survival model, the Cox proportional hazard model (Cox 1972). It can include individuals that never undergo the event of interest as sources of censored data. Censoring allows for the calculation of probability functions without attributing event times. Although time is usually used as the axis of the abscissa in this model, in this study we utilized the number of times each individual successfully evaded a predator's attack by the end of the experiment. This approach was adopted to quantify the ability of *A. japonica* to escape before they were captured
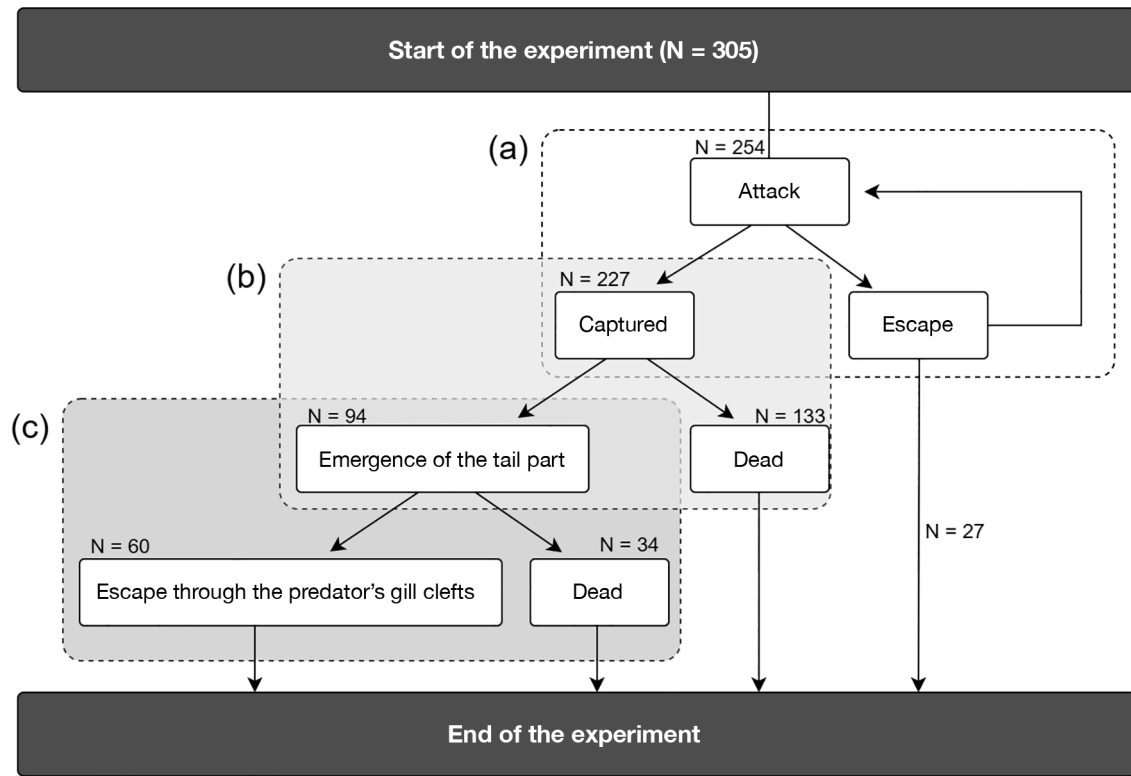
Fig. 2. Interactions between *Anguilla japonica* and the predatory fish *Odontobutis obscura* throughout the experiment, showing (a) pre-captured attack evasion phase, (b) tail emergence phase via the predator's gills after being captured, and (c) escape completion phase

using anti-predator behaviour. The definition of attack avoidance was that the eels could try to evade capture by showing some response behavior against the approaching predator with its mouth open. Survival without being eaten by *O. obscura* until the end of the experiment was regarded as censoring. The LRT with a χ^2 distribution was used to test the significance of the developmental stage. A hazard ratio was calculated based on the exponentiated parameter coefficients. The hazard ratio indicates the relative risk of the event occurring (i.e. the risk of being captured by the predator) for different stages of eel development compared to the reference group. A hazard ratio greater than 1 indicates an increased risk, while a hazard ratio less than 1 indicates a decreased risk compared to the reference group.

In order to identify factors that influenced the escaping success of *A. japonica* after capture, we analyzed each of the 2 phases, tail emergence (Fig. 2b) and escape completion (Fig. 2c), by using a binomial GLMM analysis with a logit link function. Whether the tail part emerged or not and final escape success or failure were the objective variables, designated as 1 and 0, respectively. As in the pre-captured analy-

sis, we assessed the significance of the developmental stage of *A. japonica* using the LRT with a χ^2 distribution.

All analyses were conducted using R v.4.2.1 (R Core Team 2022) with the package 'survival' for the mixed effect Cox regression model, the package 'lme4' for the GLMM.

3. RESULTS

3.1. Developmental changes in escape success after capture

During the 3 yr experiment, across 305 trials, we observed 254 interactions between *Anguilla japonica* and the predator *Odontobutis obscura*, and 227 instances of escape behaviors after capture were recorded from 7 pigmentation stages (Table 1). These trials and interactions included 5 stages of glass eels (VI$_{A0}$, VI$_{A1}$, VI$_{A2}$, VI$_{A3}$, VI$_{A4}$), elvers (VI$_{B}$), and yellow eels. Of the 227 eels that were captured by predators, 26.4% (60 out of 227) of the individuals escaped via the predator's gill. Individuals in the 2 earliest devel-

Table 1. Summary of parameters at each developmental stage. Only data from trials where the predator exhibited attack behavior at least once were used. Refer to Table S2 for the summary of each year. TL: total length; BW: body weight

Stage	Trials	Captured	Post-capture escape (Number [%])	Escape time (s) (mean ± SD)	TL (mm) (mean ± SD)	BW (g) (mean ± SD)
VI_{A0}	4	3	0 [0]	–	60.29 ± 0.98	0.17 ± 0.02
VI_{A1}	14	14	0 [0]	–	58.18 ± 2.67	0.14 ± 0.02
VI_{A2}	10	10	3 [30]	56.7 ± 11.6	58.45 ± 2.79	0.14 ± 0.03
VI_{A3}	21	18	3 [16]	21.0 ± 3.7	58.10 ± 1.81	0.12 ± 0.03
VI_{A4}	38	35	6 [17]	28.0 ± 14.7	57.23 ± 2.06	0.10 ± 0.03
Elver (VI_B)	162	144	47 [33]	32.1 ± 18.3	61.07 ± 6.62	0.15 ± 0.10
Yellow eel	5	3	1 [33]	26.0	73.18 ± 9.66	0.38 ± 0.14

opmental stages (VI_{A0} and VI_{A1}) never escaped after they were captured (Fig. 3). Individuals at the VI_{A3} and VI_{A4} stages showed lower escape rates (16 and 17%, respectively) than those in the other developmental stages of VI_{A2}, VI_B, and yellow eel (30, 33, and 33%, respectively) (Fig. 3). Escape probability was significantly affected by the developmental stage (GLMM: $\chi^2 = 15.47$, df = 5, p < 0.01), but there was no effect of stage on the escape time through the predator's gill (GLMM: $\chi^2 = 6.82$, df = 4, p = 0.15). In morphometric measurements, the TL and BW of *A. japonica* continued to decrease during the glass eel stage, and then these values increased sharply during the elver and yellow eel stages (Fig. S1).

3.2. Developmental changes in pre- and post-capture escape abilities

In the attack avoidance phase against predators prior to capture (Fig. 2a), there was a significant effect of the developmental stage of *A. japonica* on attack avoidance probability (LRT: $\chi^2 = 16.4$, df = 5, p < 0.01; Fig. 4). Based on calculations using hazard ratios, the capture risk for *A. japonica* glass eels (VI_{A01}, VI_{A2}, VI_{A3}, and VI_{A4}) and elvers (VI_B) was approximately 3.7 times and 2.4 times higher, respectively, than that of yellow eels during the trials (Fig. 4, Table S3).

After being captured by the predatory fish (Fig. 2b), 41.4% of individuals (94 out of 227) were able to extend their tail through the predators' gill clefts; 63.8% of those that achieved tail-emergence succeeded in escaping completely (60 out of 94; Fig. 2c), while 36.2% of individuals were swallowed again (i.e. died). Although the developmental stage of *A. japonica* significantly influenced the tail emergence phase through the predator's gill (GLMM: $\chi^2 = 17.2$, df = 5, p < 0.01), no significant effect was observed in the subsequent escape completion phase (GLMM: $\chi^2 = 6.7$, df = 5, p = 0.24) (Fig. 5).

4. DISCUSSION

This study tested the ability of eels at 7 different developmental stages — from early-stage glass eels to young yellow eels — to avoid the attacks of a predator or subsequently escape via the gill of the predator. The results indicated that the post-capture escape ability of *Anguilla japonica* changes with development, and underdeveloped glass eels (i.e. VI_{A0} and VI_{A1}) are incapable of escaping via the predator's gills (Figs. 3 & 6). Although the precise timing of their settlement in freshwater habitats is still lacking, most individuals that are caught at the surface of estuaries during the recruitment period across a wide range of regions are glass eels up to the VI_{A1} or VI_{A2} stage (Fukuda 2010, Aoyama et al. 2012, Leander et al. 2013, Fukuda et al. 2016, Guo et al. 2024), whereas in the lower reaches of the river, most individuals were found to be at or beyond the VI_{A3} stage (Fukuda 2010). These reports, together with our results, suggest that *A. japonica* move up into freshwater areas during the middle stage of the glass eel period (i.e. around VI_{A1}–VI_{A3}) and develop the ability to escape through the gills of predators during this transitional period (Fig. 6).

Predator–prey interactions can be divided into 4 stages: searching, recognition, catching, and handling (Davies et al. 2012). Prey species have counteradaptations at each of these stages. Generally, because prey in their early life history are vulnerable to predator attacks, they have developed defensive tactics to avoid being searched for (e.g. crypsis and habitat selection) or recognized (e.g. warning signals and mimicry) by their predators (Gotceitas & Brown 1993, Fuiman & Magurran 1994, Palma & Steneck 2001, Randall 2005, Lindstedt et al. 2008, Miller et al. 2013, Ruxton et al. 2019). Similar to those prey species, to survive the vulnerable period when eels are less capable of avoiding attacks from predators, the evolution of the unique anti-predator tactic focusing on
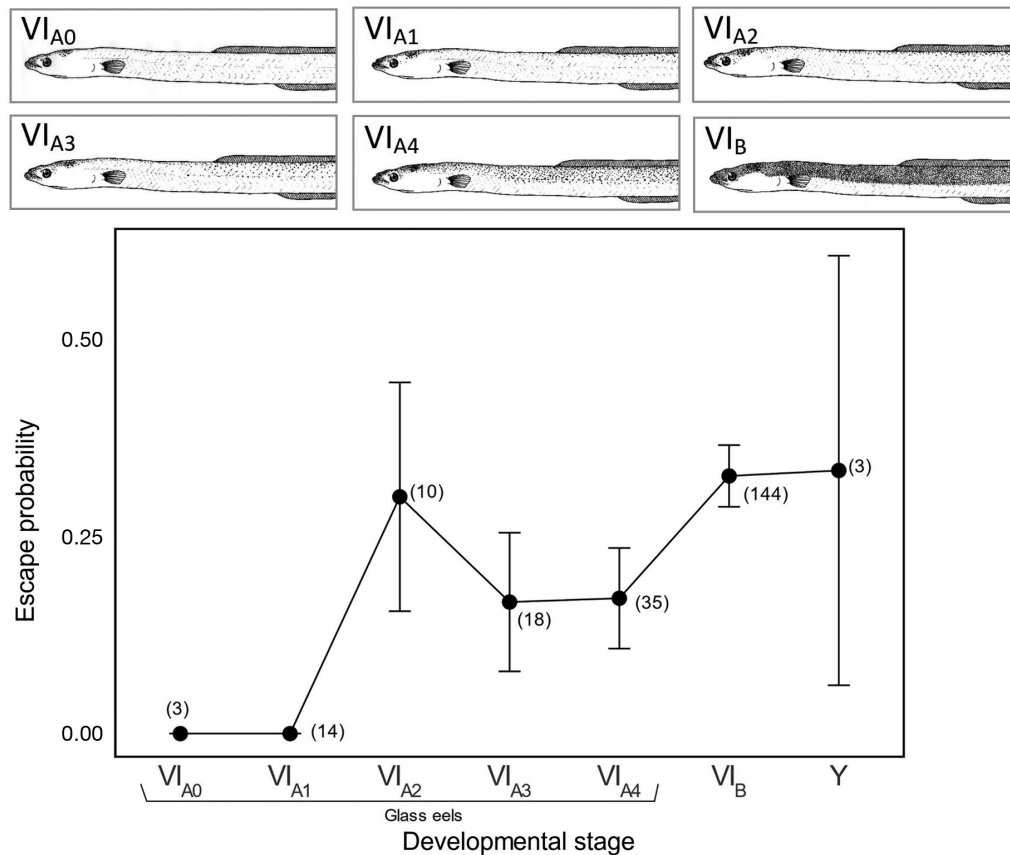
Fig. 3. Mean (±SE) probability of *Anguilla japonica* escaping via the gill of the predatory fish in each developmental stage (shown as illustrations based on progression of pigmentation; Fukuda et al. 2013). As the transition from elver (VI_B) to yellow eel (Y) results from guanine deposition in the peritoneal membrane rather than on the body surface, an illustration for Y is not included. Numbers in parentheses: number of captures by predatory fish for each stage
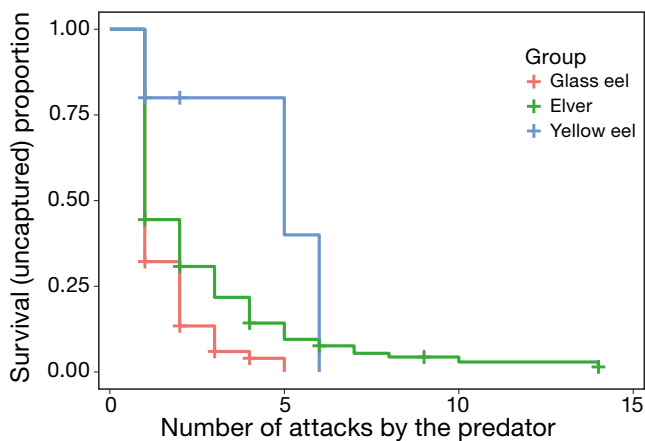

Fig. 4. Changes in the survival (uncaptured) rates of *Anguilla japonica* at each developmental stage following predator attacks. There was a significant developmental effect on the probability of attack evasion (likelihood ratio test [LRT]: $\chi^2 = 13.0$, df = 2, p < 0.01). As the developmental stage did not significantly affect attack avoidance probability during the glass eel period (i.e. VI_{A0}, VI_{A1}, VI_{A2}, VI_{A3}, and VI_{A4} stages; see Fig. S2, LRT: $\chi^2 = 4.49$, df = 4, p = 0.3), we grouped these stages together as 'glass eel' for viewability

handling, whereby they escape via the gill of predatory fish, might have been selected for. This hypothesis is supported by the fact that eels develop the post-capture escape ability (VI_{A2} stage) earlier than they improve their pre-capture attack avoidance ability (elver and yellow eel stages) (Fig. 6). Although knowledge about the predators of eels around the settlement phase is quite limited, recruited eels would likely encounter a variety of predators in coastal, estuarine, and riverine environments (Needler 1929, Miyake et al. 2018). Conducting similar predator—prey experiments with various types of predators would further strengthen our hypothesis that this behavior contributes to juvenile eel survival in the wild.

In the detailed analysis that divided the escape behavior into 2 phases (i.e. tail emergence and escape completion) (Fig. 5), only the tail emergence phase was significantly affected by the developmental stage of eels. Observations of morphological changes with development in *A. japonica* suggest that changes involved in their swimming and locomotor performance, such as increased mucus cells and
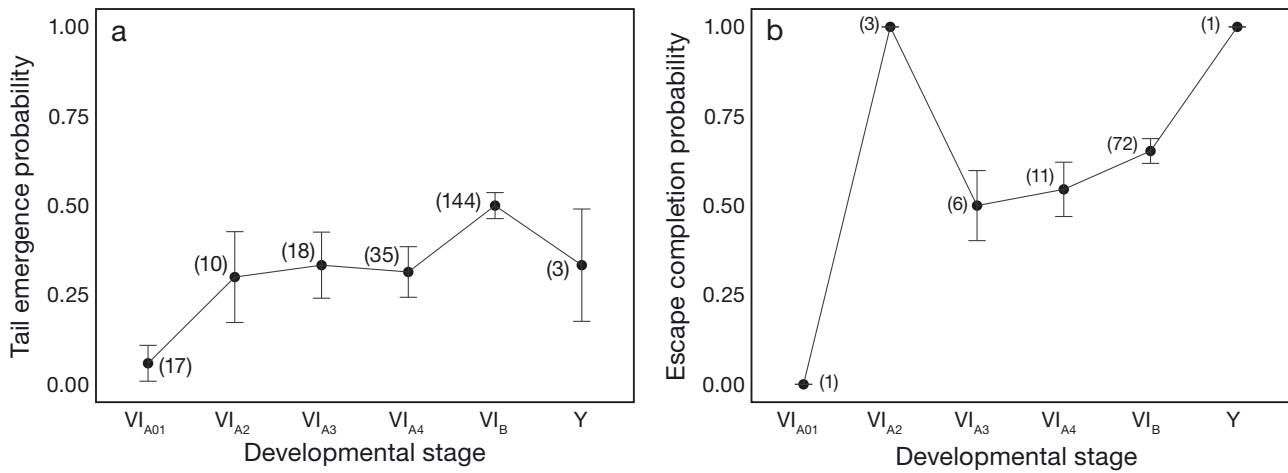
Fig. 5. Mean (±SE) probability of (a) tail emergence and (b) escape completion in each developmental stage of *Anguilla japonica*. Numbers in parentheses represent the data points (denominators for probability) for each stage: (a) number of captures and (b) number of tail emergences
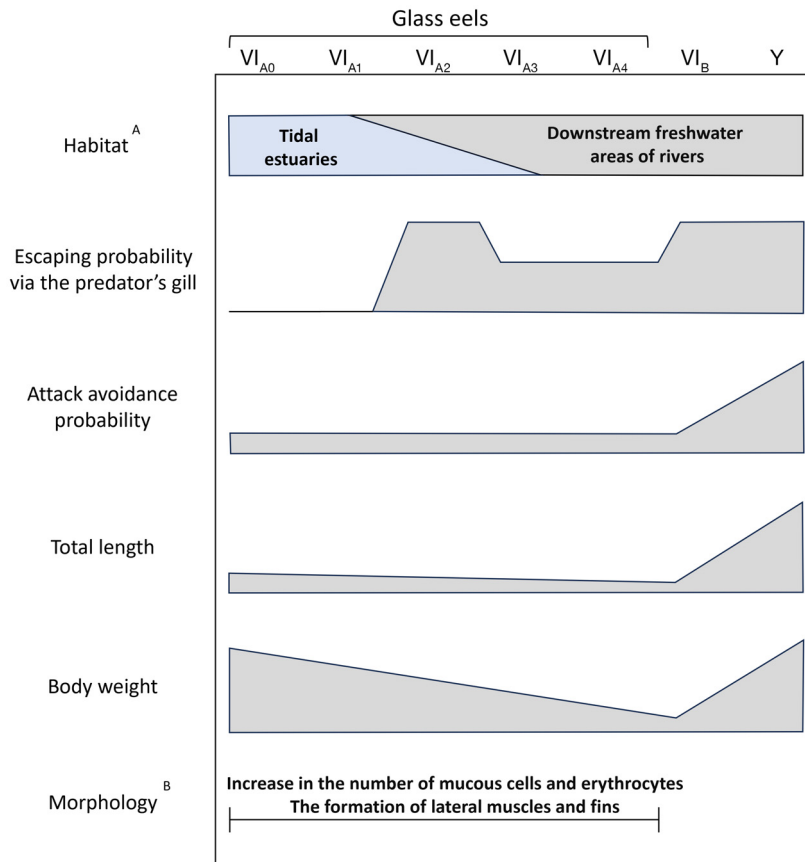

Fig. 6. Developmental changes in the ecology, behavior, and morphology of juvenile eels. [A]Habitat schematic was created based on previous studies (Fukuda 2010, Aoyama et al. 2012, Leander et al. 2013, Fukuda et al. 2016, Guo et al. 2024); [B]morphology was designed based on Hatakeyama et al. (2022)

erythrocytes as well as the formation of lateral muscles, occur during the glass eel period (Hatakeyama et al. 2022). These findings suggest that morphologi-

cal changes and improved locomotor performance with development might be necessary for *A. japonica* to navigate back through the predator's digestive tract toward the gill after they are captured (Hasegawa et al. 2024). On the other hand, the success of completing the escape after the tail emerges might be influenced by factors other than the developmental stage of the eels, such as the energy expended until tail emergence. Further experiments are required to identify the detailed traits of *A. japonica* that enable post-capture escape by comparing escape success after measuring their behavioral performance (e.g. endurance and burst swimming) and physiological development (e.g. mucus and muscle mass) across various developmental stages.

The change in the developmental stage of eels also significantly improved their ability to avoid attacks from predators before capture. This is likely due to the improvement in escape performance (e.g. response, velocity, and acceleration) or the emergence of a specific response, similar to other larval and juvenile fish (Webb 1976, Fuiman 1993, Hale 1999, Kopf et al. 2014). Although the escape behavior of eels differs from that of general fish (e.g. C-start behavior) in that eels instantaneously retract their head and swiftly swim backward against stimulation

(D'Août & Aerts 1999, Ward & Azizi 2004), there is no knowledge of developmental changes in these behaviors. The formation and increase in lateral muscles with development in eels (Fig. 6) may have resulted in the emergence or improvement of their escape behaviors, including backward swimming and head retraction, thereby increasing the escape probability against attacks by predators.

In line with previous studies on *A. japonica* (Fukuda et al. 2013, Hatakeyama et al. 2022, Guo et al. 2024), although the TL and BW of *A. japonica* decreased during the glass eel stages, a marked increase in these values was observed during the elver (VI$_B$) and yellow eel stages (Fig. S1 and Fig. 6). Such decreases in size during and after metamorphosis have been observed in other anguillid species (Tesch 2003) and other fish having a leptocephalus phase (Pfeiler 1984, Miller 2009). These changes can be attributed to variations in body water content as well as modifications in muscles and other organs (Pfeiler 1984, Hatakeyama et al. 2022). Interestingly, the late glass eel stages, just before these values start to increase, showed the lowest escape success rates (VI$_{A3}$, 16%; VI$_{A4}$, 17%, respectively) compared to other stages that can successfully escape (32% on average; Fig. 3, Table 1). This suggests that *A. japonica* may have a vulnerability, at least for escaping via the gills of *O. obscura*, at the end of their reduction in TL and BW (i.e. the late glass eel stages). The fact that defensive abilities, once developed, decline in certain stages is intriguing and open to further research, including the investigation of vulnerabilities to other factors affecting their survival (e.g. feeding and intraspecific competition) during this period.

Knowledge about ontogenetic changes affecting survival is important from a conservation perspective. In temperate eels, especially European eels, restocking (trap-and-transport) programs are actively conducted (Pedersen 2000, Desprez et al. 2013, Ovidio et al. 2015, Nzau Matondo et al. 2019, 2021, Delrez et al. 2021). This strategy for resource restoration is to capture pre-settlement glass eels and release them back into inland rivers. Although protecting the period during which they are most vulnerable to various natural selection forces in the wild could improve their survival rates after release, the specific developmental stages at which they are most vulnerable and truly need protection are still unclear. Further research on the behavioral, physiological, and physical changes in eels through ontogeny can provide insights into selecting the appropriate developmental stages and individuals for release. Therefore, our research has the potential to help establish effective and efficient approaches for restoring populations of temperate eels.

Overall, this study reveals that Japanese eels develop the ability to escape after being captured at a certain developmental stage, and this stage might coincide with their settlement timing in riverine and estuarine habitats. Some invertebrate prey also possess the ability to actively escape from the digestive tract of their predators (Ponton et al. 2006, Sugiura 2020). Our study offers some fundamental insight that serves as an important guideline for investigating developmental changes in these active escape behaviors of prey species. Moreover, we found that the developmental stage of *A. japonica* significantly influenced both pre-capture attack avoidance and post-capture escape behaviors. Our findings indicate the vulnerability of the early stage eels to predatory fish immediately after reaching freshwater habitats, thereby providing valuable insights into the ecology and conservation of anguillid eels, which have faced remarkable population declines.

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