

# Effects of stocking density and rearing factors on aggressive behaviour and cannibalism in the Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel) larvae

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## Research Article

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# Abstract

We studied the effects of stocking density and multiple factors on the aggression and cannibalism of Pacific bluefin tuna, *Thunnus orientalis* (Temminck & Schlegel) larvae. In feeding experiment, if the appropriate food was abundant and the fish a uniform size, the frequency of aggressive behaviour was unaffected by the increase in stocking density from low-(0.1 fish/L) to high-density (0.5 fish/L). However, high-density conditions significantly increased aggressive behaviour when food was scarce. Aggressive behaviour increased only slightly due to fish size differences, and there was no increase due to high-density conditions. However, when fish of different size were present under a restricted feeding regime, aggressive behaviour significantly increased compared to the case of restricted feeding alone and was further amplified at high-density. Cannibalistic mortality was more likely for small-size fish, which was significantly increased by high-densities. In particular, when fish size differences and feeding shortages overlapped, cannibalism increased sharply as does aggressive behaviour. Even if the fish are reared at high-density, if the shortage of appropriate food and the spread of fish size difference are prevented, the aggressive behaviour is suppressed, as the result, it is possible to rear at least 0.5 fish/L with thorough food management.

## Introduction

Pacific bluefin tuna (PBT) *Thunnus orientalis* (Temminck & Schlegel) is regarded as a superior foodstuff and is used for sushi and sashimi, mainly in Japan. Consequently, this is a fish species with extremely high commercial value. Therefore, PBT have been caught internationally by a variety of fishers, and consequently, their population levels have remained low for decades (Nakatsuka et al. 2017). Successful PBT aquaculture could reduce fishing pressure and provide a stable supply of fish. However, in recent years, even in Japan, more than half of the young fish used for aquaculture depended on natural resources (Sawada et al. 2005).

Several institutions continue to research seedling production in *T. orientalis*, including Kinki University where PBT was successfully completely farm-raised for the first time in the world, Unfortunately, translating this into mass production is difficult. The high mortality in the larval and juvenile stages (Harada et al. 1971; Miyashita 2002; Sawada et al 2005; Ishibashi 2012) and consequent required numbers of fish prevent the process from being profitable. The number of fish larvae was depleted as a result of floating and sinking deaths in pre-flexion larvae, and cannibalism in post-flexion larvae to juveniles (Sawada et al. 2005; Ishibashi et al. 2008; Sabate et al. 2010; Tanaka et al. 2018). In the juvenile stage, cannibalism, and mass death occurs due to fractures and skin lacerations caused by contact with rearing tanks and offshore net cages (Ishibashi 2012; Okada et al. 2020a, b). However, there are effective approaches to instances of mass death in PBT such as the addition of feed oil to prevent floating deaths (Kurata et al. 2012), and 24-hour lighting in the rearing environment to prevent sinking deaths (Kurata et al. 2017). Furthermore, to prevent deaths from cannibalism, we ensured that the fish were of uniform size and adequately fed (Ishibashi et al. 2013a). Deaths caused by fractures and skin lacerations can be prevented by using particular colours and patterns in the rearing tanks (Ishibashi et al. 2013b; Okada et al. 2015), and

providing light at night in the sea net cages (Ishibashi et al. 2009; Honryo et al. 2020). Although several research and private institutions have introduced these technologies into PBT seedling production, their production efficiency is still low compared with that of other marine fish.

Seedling production is carried out on land and the size of the facility cannot easily be increased, owing to restrictions such as the size of the land and running costs. Furthermore, PBT is a multiple-spawning fish, with a spawning season that is limited to approximately 60 days along the coast of Wakayama Prefecture (Miyashita 2002). However, it takes approximately 30 to 40 days after the eggs are fertilized in the production facility tanks for them to be raised to juveniles ready for transfer to the marine cages (Miyashita 2002). Consequently, it is not possible to have many production rounds. Therefore, seedlings have to be produced at a higher stocking density to increase the production efficiency. However, PBT larvae have been empirically bred at a much lower density than that of other marine fish. Chasing behaviour is observed in PBT larvae after the post-flexion stage (Sabate et al. 2010), and, hatched larvae of other fish species are fed as live baits (Sawada et al. 2005). Therefore, mass death due to cannibalism is a concern (Ishibashi 2012; Tanaka et al. 2018).

Cannibalism is a serious problem in seedling production (Baras and Jobling 2002) and is an important factor for survival. This phenomenon is accompanied by aggressive behaviour as a precursor and is affected by the rearing environment. For example: in Koi carp (*Cyprinus carpio*) and Asian seabass (*Lates calcarifer*) cannibalism is promoted by high stocking density (Damme et al. 1989; Khan et al. 2021) while differences in fish size promotes it in the orange-spotted grouper (*Epinephelus coioides*), (Hseu 2002). In yellowtails (*Seriola quinquevadiata*) and greater amberjack (*Seriola dumerili*), differences in fish size and starvation increased the frequency of aggressive behaviour, but a higher stocking density decreased it (Sakakura and Tsukamoto 1998; Miki et al. 2011). Stocking densities that are too high can stress fish and adversely affect their survival and growth (Barton and Iwama 1991).

Many fish species, such as the gilthead sea-bream (*Sparus aurata*), Atlantic cod (*Gadus morhua*), and Nile tilapia (*Oreochromis niloticus*), slow down growth as stocking density increases (Canario et al. 1998; Lambert and Dutil 2001; El-Sayed 2002). Conversely, increasing stocking density promotes growth in Arctic char (*Salvelinus alpinus*) (Wallace et al. 1988), Atlantic Salmon (*Salmo salar*) and European perch (*Perca fluviatilis*) had no effect on growth (Kjartansson et al. 1988; Baras 2003). It is necessary to take note of the developmental stage and the degree of stocking density of the test fish used in each experiment to make comparisons, but whether the increased stocking density affects survival and growth depends on the fish species.

In PBT larvae, aggressive behaviour have been shown to increase by feeding is restricted, and survival rates are reported to deteriorate further when size differences occur (Ishibashi et al. 2013a). Therefore, it is possible that PBT larvae can be produced at a higher density by feeding them sufficiently and selecting and rearing them such that the size difference is small. However, the effects of stocking density on aggressive PBT behaviour and cannibalism is not well understood. In this study, to ensure sufficient production and reduce the unit price per seedling, we paid particular attention to the stocking density of larvae in the post-flexion stage, where mass deaths due to cannibalism is a concern. PBT larvae were

reared at various densities, and the effects of stocking density on aggressive behaviour, cannibalism, survival and growth were investigated. Moreover, factors such as feeding restrictions and fish size differences in addition to stocking density were considered to have a significant effect on the aggressive behaviour, cannibalism, survival and growth of PBT larvae (Ishibashi et al. 2013a).

## Materials And Methods

### Fish

*Thunnus orientalis* larvae at 16 days post hatch were supplied by the Kindai University Aquaculture Technology and Production Center, Ohshima Station. The larvae were produced in 30-kL volume concrete tanks, and fed *Artemia* spp. as well as the Japanese striped knifejaw (*Oplegnathus fasciatus*) larvae. The PBT larvae were softly transported in the rearing water using bowls and buckets so as not to come into direct contact. Simultaneously, the larvae were visually sorted into three groups according to size based on the total length (TL). From each group, 9–13 larvae were randomly selected and used to measure TL and body weight (BW) at the start of the experiment. The required number of larvae divided into the three groups was transferred to the experimental tanks.

### Experimental design

The experiment was performed in ten groups with a single replicate. Nine of the groups were a combination of density (low, medium, high), feeding (satiation, restriction), and fish size (same, diverse) conditions (Table 1). The tenth group was established to ensure that very delicate, small-sized PBT larvae could be reared in the experimental tank without problems (Table 1). Specifically, there were 10 groups with control = low-density (C), low-density + restricted feeding (F), low-density + diverse size (S), low-density + restricted feeding + diverse size (FS), medium-density (M), high-density control (H), high-density + restricted feeding (HF), high-density + diverse size (HS), high-density + restricted feeding + diverse size (HFS), and small size only (SSO) (Table 1). Fish per liter (L) was set to 0.10 fish in the low-density groups, 0.25 fish in the medium-density groups, and 0.50 fish in the high-density groups, respectively (Table 1).

### Housing

The transparent cylindrical 200 L or 100 L tanks used in the experiment were made of polycarbonate. A flowing water system (approximately 400 ml/min) was adopted, and tanks were aerated. The seawater injected into each tank was filtered by sand and sterilised by ultraviolet rays, and then distributed using natural seawater of the same nature. Experimental tanks were set indoor where no light enters, and the water temperature was kept at  $27.1 \pm 0.1$  °C and the dissolved oxygen (DO) was maintained at  $7.9 \pm 0.8$  mg/L during the experimental period. The light was set to approximately 1000 lx on the water surface using a fluorescent lamp, which was turned on from 0800 h to 2000 h.

### Fish size

Larvae were sorted into large (TL:  $17.4 \pm 1.1$  mm, BW:  $47.3 \pm 7.8$  mg), medium (TL:  $13.7 \pm 1.1$  mm, BW:  $23.0 \pm 8.1$  mg) and small (TL:  $10.8 \pm 1.0$  mm, BW:  $11.7 \pm 3.1$  mg) based on the TL, these were significantly

different in the Game-Howell test. Medium-sized PBT larvae were used in the same size groups, and the same number of large-and small-sized larvae were contained in the diverse size groups, except for the SSO group, which contained only small-sized larvae. The fish were placed in their respective containers at 0600h on the first day of the experiment.

## Feeding

Regarding feeding conditions, 400 the Japanese striped knifejaw larvae were fed per PBT larva in one feeding session in the satiation groups and 40 in the restricted groups. Feeding was performed 3 times a day at 0600, 1300 and 1600 h.

## Aggressive behaviour

All experimental tanks were visually divided into three parts with black plastic tape on the exterior surface. Aggressive behaviour was categorized as aim, attack, chase, and bite, with reference to observations in PBT (Ishibashi et al. 2013a). "Aim" is defined as an action that mainly aims at the opponent from behind or from the side while the swimming speed is not changed or stopped. "Attack" is defined as an action that pecks or hits the opponent during high-speed swimming. "Chase" is defined as chasing the opponent at a faster swimming speed than usual, while "bite" is defined as biting the opponent from either direction. In each group, aggressive behaviour was visually measured by three people twice a day at 0800 and 1500 h (on the first day of the experiment, only once at 1500 h for acclimatisation) for 5 min. In other words, the aggressive behaviour was observed from 1500 h on the 1st day to 1500 h on the 5th day at 9, 26, 33, 50, 57, 74, 81, 98 and 105 h after the start of the experiment. The nine observation data of aggressive behaviour were converted into the frequency per larva per hour, and then the frequency of the four patterns of behaviour were evaluated separately or in total.

## Survival and growth rate

Dead fish were removed from the tanks as soon as they were found to avoid damage and deterioration of rearing conditions, and the number was recorded. For the analysis related to survival, we used data that aggregated the numbers twice a day at 0800 h and 1500 h, based on the observation of aggressive behaviour. Mortality (%) in each group was calculated as the total number of dead fish/(number of initially accommodated fish) × 100. At the end of the experiment (1600 h on the 5th day of the experiment), the number of surviving PBT larvae was counted, and some or all of the larvae in each group were measured to determine their TL and BW.

## Statistical analysis

The data are presented as mean ± standard deviation, with a significant difference ( $p < 0.05$ ), and was calculated using statistical software. Welch's test was used to determine significant differences in fish size and aggressive behaviour in each section. In addition, the test for each experimental group showed a significant difference by multiple comparison using the Game-Howell test. Survival status was analyzed using the Kaplan-Meier method, and the significant difference between the groups was determined by log-rank test.

## Results

### Aggressive behaviour and cannibalism affected by stocking density

The frequency of aggressive behaviour between C (0.1 fish/L), M (0.25 fish/L) and H (0.50 fish/L) groups was not significantly different (Games-Howell test,  $p > 0.05$ ; Fig. 1). There was no difference in the frequency of aggressive behaviour between the C group containing only medium-sized fish and the SSO group containing only small-sized fish (Games-Howell test,  $p > 0.05$ ; Fig. 1). However, there were effects resulting from feeding conditions and size differences for each stocking density. In the low-density groups (C, F, S, FS and SSO), frequencies of aggressive behaviour were significantly increased in the FS group with feeding restrictions and overlapping size differences. The second most frequent aggressive behaviour was observed in the F group with restricted feeding (Fig. 2).

In the high-density groups (H, HF, HS, and HFS), the frequencies of aggressive behaviour were significantly higher in the HF group with restricted feeding and the HFS group with overlapping feeding restrictions and size differences (Fig. 2). When the effects of food shortage and size differences were compared by stocking density, it was shown that there was a significant difference in the frequencies of aggressive behaviour between the F and HF groups, and that stocking density had an effect (Welch's test,  $p < 0.05$ ; Fig. 2), but there was no significant difference in the frequencies of aggressive behaviour between the FS group and HFS groups (Welch's test,  $p > 0.05$ ; Fig. 2).

### Survival rate affected by stocking density

The survival rates of the C, M and H groups were 80%, 72% and 86%, respectively, with no significant differences between them (log-rank test,  $p > 0.05$ ; Fig. 3A). The survival rate of the SSO group containing only small-sized larvae was 80%, and it was confirmed that the small-sized larvae could be reared even in the small tanks used in the experiment (Table 2). The survival rate of the FS group, which was reared at a low density and had overlapping feeding restrictions and fish size differences, was lower than that of the C, F and S groups, and there was a significant difference between the S and FS groups (log-rank test,  $p < 0.05$ ; Fig. 3B). However, the survival rate of the HFS group (reared at high density with overlapping feeding restrictions and fish size differences, was significantly lower than that of the H group, and that of the HF group with restricted feeding and the HS group with fish size differences were also significantly lower than that of the H group (log-rank test,  $p < 0.05$ ; Fig. 3C). The survival rates in the F and HF groups, which compared the effects of food shortage by stocking density were 75% and 46%, respectively, and the survival rate in the high-density HF group was significantly lower (log-rank test,  $p < 0.05$ ; Fig. 3D). The survival rates of the S and HS groups, which compared the effects of fish body differences by stocking density, were 85% and 62%, respectively, and the the high-density HS showed a lower survival rate (Fig. 3E). However, the survival rates of small-sized fish in the S and HS groups were 90% and 36%, respectively, clearly significantly lower in the denser HS group and the effect of small-size became more pronounced (log-rank test,  $p < 0.05$ ; Fig. 3E). Comparing the effects of duplication of food shortage and size differences by stocking density, the survival rates of the FS and HFS groups were both 50%, showing no difference

(log-rank test,  $p > 0.05$ ; Fig. 3F), but the survival rates of small-sized fish were both 0%, showing a significant difference (log-rank test,  $p < 0.05$ ; Fig. 3F).

A high positive correlation was found between the frequencies of aggressive behaviour (including all attack types) and mortality ( $r = 0.87$ ,  $p < 0.01$ ; Fig. 4), and increased frequencies of aggressive behaviour accrued mortality.

## **Growth affected by stocking density**

There was no difference in the total length and weight of the larvae reared in the low-density C, medium-density M and high-density H groups (Games-Howell test,  $p > 0.05$ ; Table 2). There was no difference in growth at the density under the experimental conditions where the food was abundant and there was little difference in fish size. The weight of larvae reared in the F group with restricted feeding tended to be lower than that of the C group, and the weight of the HF group with high-density and restricted feeding was significantly lower than that of the H group. This indicated a substantial effect resulting from restricted feeding (Games-Howell test,  $p < 0.05$ ; Table 2). The total length and weight of large and small-sized larvae reared in the high-density HS group tended to be smaller than those of the low-density S group. In addition, the total length and weight of large-sized larvae in the high-density HFS group were significantly smaller than those in the low-density FS, S and high-density HS groups (Games-Howell test,  $p < 0.05$ , Table 2), indicating that the density factor may have affected them.

## **Discussion**

### **Relationship between inducing factors of aggressive behaviour and stocking density**

As the result of this experiment, it was suggested that if the appropriate food is abundant and the fish size is uniform, even if the stocking density increases from 0.1 to 0.5 fish/L, it does not affect the aggressive behaviour (Fig. 1). In contrast, in both low and high-density groups, the frequencies of aggressive behaviour increased when food was insufficient due to restricted feeding (Fig. 2), which was consistent with the results of our previous report (Ishibashi et al. 2013a). Furthermore, in the state of food shortage, the frequencies of aggressive behaviour varied greatly depending on the stocking density, suggesting that high-density significantly increased aggressive behaviour (Welch's test,  $p < 0.05$ ; Fig. 2). In the previous report (Ishibashi et al. 2013a), the frequency of aggressive behaviour in restricted feeding group was 0.25 fish/L that was significantly higher than that in the control group, the degree showed an intermediate value between low-density (0.1 fish/L) and high-density (0.5 fish/L) under restricted feeding in this experiment, and it was confirmed that it changed depending on the density.

Even if the difference in fish size increased, the increase in aggressive behaviour was slight, and there was no increase due to high-density (Fig. 2). In other words, it seems that a single factor, size difference, does not significantly increase aggressive behaviour. However, when the fish body difference widened under restricted feeding, the aggression significantly increased even at low-densities compared to restricted

feeding alone, and the same effect was observed at high-densities (Fig. 2). From the above, it is suggested that the aggressive behaviour remarkably increases due to lack of appropriate food, but it is difficult to increase only by the spread of fish body difference, it increases remarkably when the spread of fish size difference is added to the lack of appropriate food. Furthermore, these effects are amplified at high-density. Like Japanese flounder (*Paralichthys olivaceus*) (Dou et al. 2000), PBT is considered to be a type in which aggressive behaviour is intensified when the stocking density is high. This differs from yellowtail, (*Seriola quinquevadiata*) (Sakakura & Tsukamoto 1998) and greater amberjack (*Seriola dumerili*) (Miki et al. 2011), whose aggressive behaviour decreased as the stocking density increased. Even though PBT and yellowtail species are the same migratory fish, the trait of schooling behaviour is different, and it is considered that the influence of the density that causes the aggressive behaviour is reversed.

Stocking density affected not only the frequency of aggressive behaviour but also its quality. The frequency of biting under restricted feeding was significantly higher at high density (Fig. 2, Welch's test,  $p < 0.05$ ). Similarly, when fish size differences widened under restricted feeding, biting behaviour tended to be more frequent at high density (Fig. 2, Welch's test,  $p > 0.05$ ). Among the four types of aggressive behaviour, biting behaviour is a physical attack that is most likely to inflict deep damage on the opponent. In the case of PBT larvae with delicate and vulnerable epidermis, such an attack can be fatal. In fact, multiple PBT larvae that were bitten during the experiment were observed, and they weakened and died over time. It was speculated that when the stocking density was high, the distance between the individuals is small, and if there was a shortage of food, they instinctively took biting action.

## **Relationship between aggressive behaviour and survival or cannibalism**

The results of this experiment suggest that if the appropriate food is abundant and the fish size is uniform, the survival rate will not be affected by increasing the stocking density (Table 3, Fig. 3A). This is the same as the results of aggressive behaviour (Fig. 1) and also that of the behaviour of Japanese flounder (Dou et al. 2000). We speculated that PBT is less likely to cause cannibalism problems unless food shortages occur and fish size differences are widened. However, under restricted feeding and high-density we observed a significantly increased mortality rate (log-rank test,  $p < 0.05$ ; Fig. 3D). When the fish size difference widened, the mortality of small individuals increased significantly as the density increased (log-rank test,  $p < 0.05$ ; Fig. 3E). That is, it was shown that the aggressive behaviour of PBT larvae is mainly caused by lack of food, but cannibalistic mortality is more likely to occur in small individuals, and that it is significantly enhanced by high-density. It was also clarified that when the fish size difference in the tank was increased and a feeding shortage occurred, the aggressive behaviour and cannibalism increased sharply regardless of the stocking density, and a large number of small individuals died. This phenomenon is likely to occur even at seedling production sites when switching from hatched larvae to a formula diet (Ishibashi 2012). When acclimatising to the formula diet, the amount of hatched larvae is reduced, which may lead to a substantial shortage of feed and mass mortality of small individuals.

There were approximately 0–7 missing fish in each experimental group. However, no regularity with the set conditions of the group was confirmed (Table 2). No predated or swallowed fish were observed during the

experiment, so it was not possible to conclude that all missing fish were swallowed. It has been confirmed that PBT larvae peck dead fish that have sunk to the bottom and prey on their muscles and internal organs (Ishibashi 2012). Similarly in this experiment, cannibalism was thought to be due to various attacks and partial predation rather than depletion caused by swallowing small fish. As the frequency of aggressive behaviour (including all attack types) increases, the mortality rate increases ( $r= 0.87, p < 0.01$ ; Fig. 4). Therefore, not only biting (the physical attack that is most likely to injure the opponent), but also all aggressive behaviour is directly linked to mass death, and cannibalism will occur in the process and later. Aiming is the most observed low-attack behaviour, mainly aimed at the other individual from behind or from the side without changing the swimming speed or at rest, and it has a positive correlation with mortality ( $r= 0.85, p < 0.01$ ; Fig. 4). In other words, the trivial aggressive behaviour of PBT larvae may be a signal to prevent a decrease in production at the seedling production site.

## **Effect of stocking density on growth**

Under the conditions of this experiment, it was suggested that the stocking density did not directly affect the growth if the food was abundant and the spread of fish size differences was not remarkable (Table 3). Similar to PBT, yellowtail are carnivorous and highly migratory fish around Japan. It has been reported that their juveniles are usually produced at 1.0 fish / L, and if the juveniles are reared at a density higher than 4.0 fish / L, the frequency of aggressive behaviour increases and it adversely affects the growth (Sakakura & Tsukamoto 1998). The stocking density of PBT larvae and/or juveniles are generally considered to be lower than that of other fish species, and cannot be strictly compared between fish species. This is thought to be because, as mentioned above, there is a tendency for a shortage of appropriate feed to occur during the feed switching period from rotifers to hatched larvae or from hatched larvae to compound feed, and at that time, deaths due to aggressive behaviour increased.

Under the conditions of this experiment, the growth of PBT larvae was not affected by the stocking density. However, it was suggested that when the stocking density is low, the growth is not affected even if the fish body difference spreads under a lack of food, but it may affect the growth when the stocking density is high. PBT larvae violently attack small individuals when the fish body size difference is large if there is a lack of food. If the density is high, the distance to the neighbouring individual will be shortened, and its presence may make even large larvae susceptible to stress and affect their growth. The reason why PBT larvae have been empirically reared at low-densities so far is that when there is a lack of appropriate food or if fish are of radically different sizes, the adverse effects can be suppressed more effectively than when the fish are reared at high densities. On the contrary, it was suggested that PBT larvae could be produced at the density of at least 0.5 fish/L if appropriate food was sufficiently fed and the fish body size was made uniform. If the development of a formula diet to replace hatched larvae progresses in the future, it is thought that the problem of feed shortage due to feed switching will be solved and higher density rearing will be possible.

## **Abbreviations**

PBT, Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel); TL, total length; BW, body weight; C, control; F, restricted feeding; S, diverse size; FS, restricted feeding + diverse size; H, high-density; HF, high-density + restricted feeding; HS, high-density + diverse size; HFS, high-density + restricted feeding + diverse size conditions.

## **Declarations**

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### **Compliance with ethical standards**

This study followed the current laws in Japan and the “Ethical justification for the use and treatment of fishes in research: an update” (Metcalf and Craig 2011). This experiment was conducted in compliance with the ethical code of the Animal Experimentation Committee of the Faculty of Agriculture, Kindai University.

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### **Conflict of interest**

The authors have no relevant financial or non-financial interests to disclose.

### **Ethical approval**

All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors.

### **Author's contributions**

Takashi Takeda, Tokihiko Okada and Yasunori Ishibashi contributed to the study conception and design. Material preparation and data collection were performed by all authors. Takashi Takeda and Yasunori Ishibashi analyzed data and wrote the first draft of the manuscript. Then, all authors commented on previous versions of the manuscript, read and approved the final manuscript.

## Data availability statement

All data related to this study is contained in this published manuscript.

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## Tables

**Table 1** Experimental groups and rearing conditions

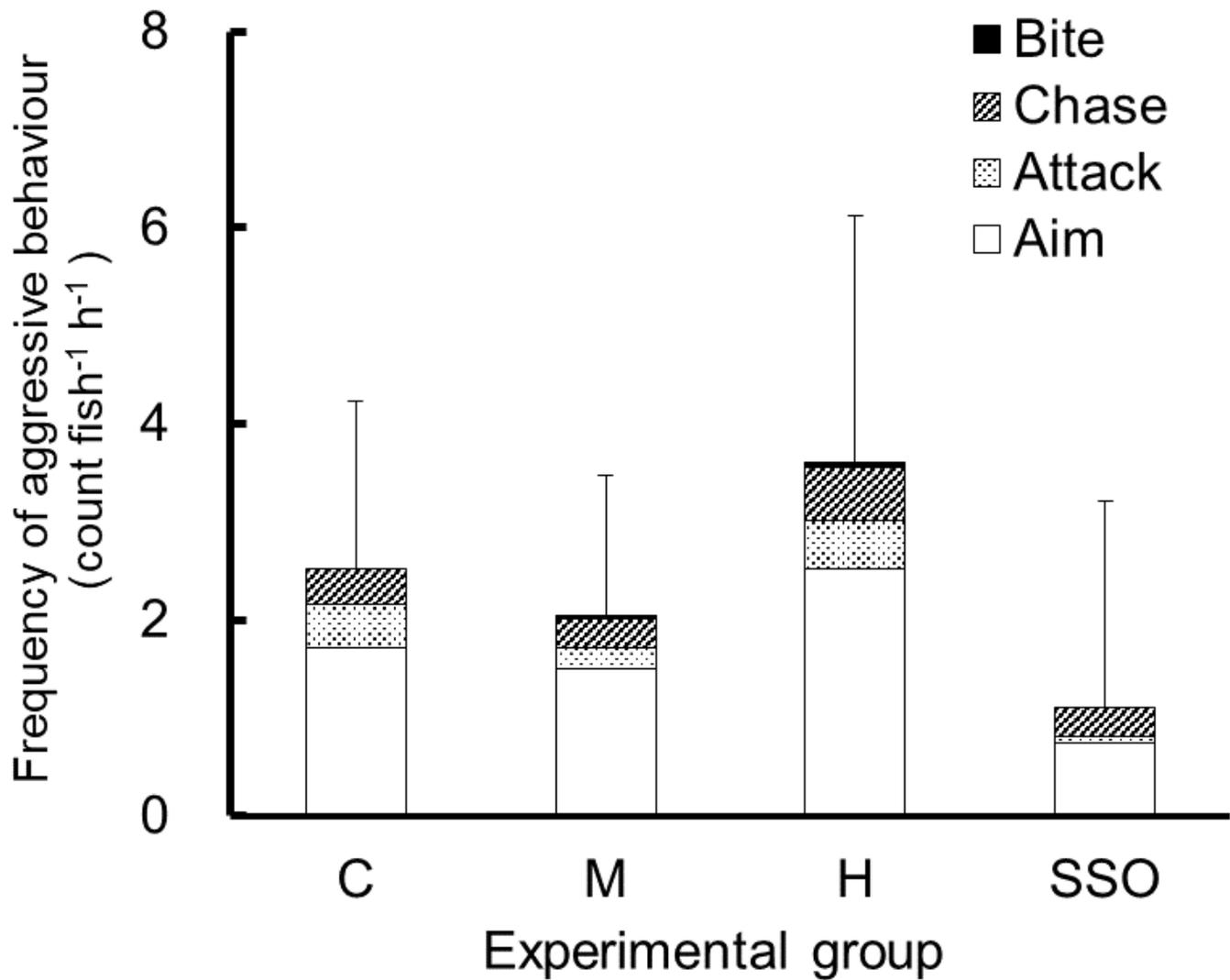
Experimental groups	Rearing conditions		
	Abbreviation: detail	Density: fish L <sup>-1</sup>	Feeding condition*
C: control	Low: 0.10	Satiation	Same: medium
F: restricted feeding	Low: 0.10	Restriction	Same: medium
S: diverse size	Low: 0.10	Satiation	Diverse: large and small
FS: restricted feeding and diverse size	Low: 0.10	Restriction	Diverse: large and small
M: medium density	Medium: 0.25	Satiation	Same: medium
H: high-density	High: 0.50	Satiation	Same: medium
HF: high-density and restricted feeding	High: 0.50	Restriction	Same: medium
HS: high-density and diverse size	High: 0.50	Satiation	Diverse: large and small
HFS: high-density, restricted feeding and diverse size	High: 0.50	Restriction	Diverse: large and small
SSO: small size only	Low: 0.10	Satiation	Same: small

\* Detailed conditions of feeding: fish were fed hatched larvae of the Japanese striped knifejaw *Oplegnathus fasciatus* three times per day in both the satiation and the restriction groups.

\*<sup>2</sup> Initial fish in the large size (TL  $17.4 \pm 1.1$  mm, BW  $47.3 \pm 7.8$  mg), medium size (TL  $13.7 \pm 1.1$  mm, BW  $23.0 \pm 8.1$  mg) and small size (TL  $10.8 \pm 1.0$  mm, BW  $11.7 \pm 3.1$  mg) were graded on TL (Games-Howell test, n = 9-13, P < 0.05).

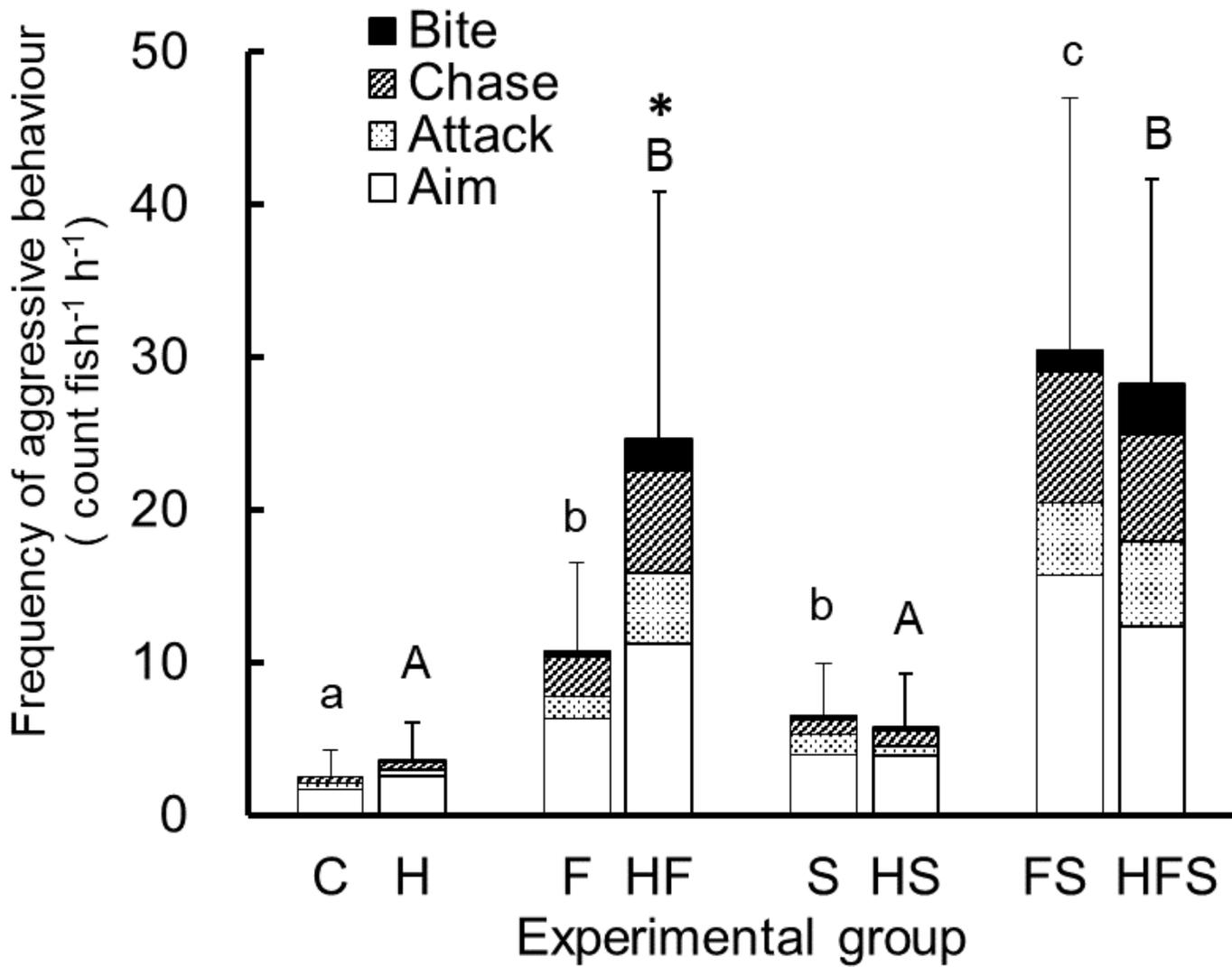
Table 2 is available in the Supplementary Files section.

## Figures



**Figure 1**

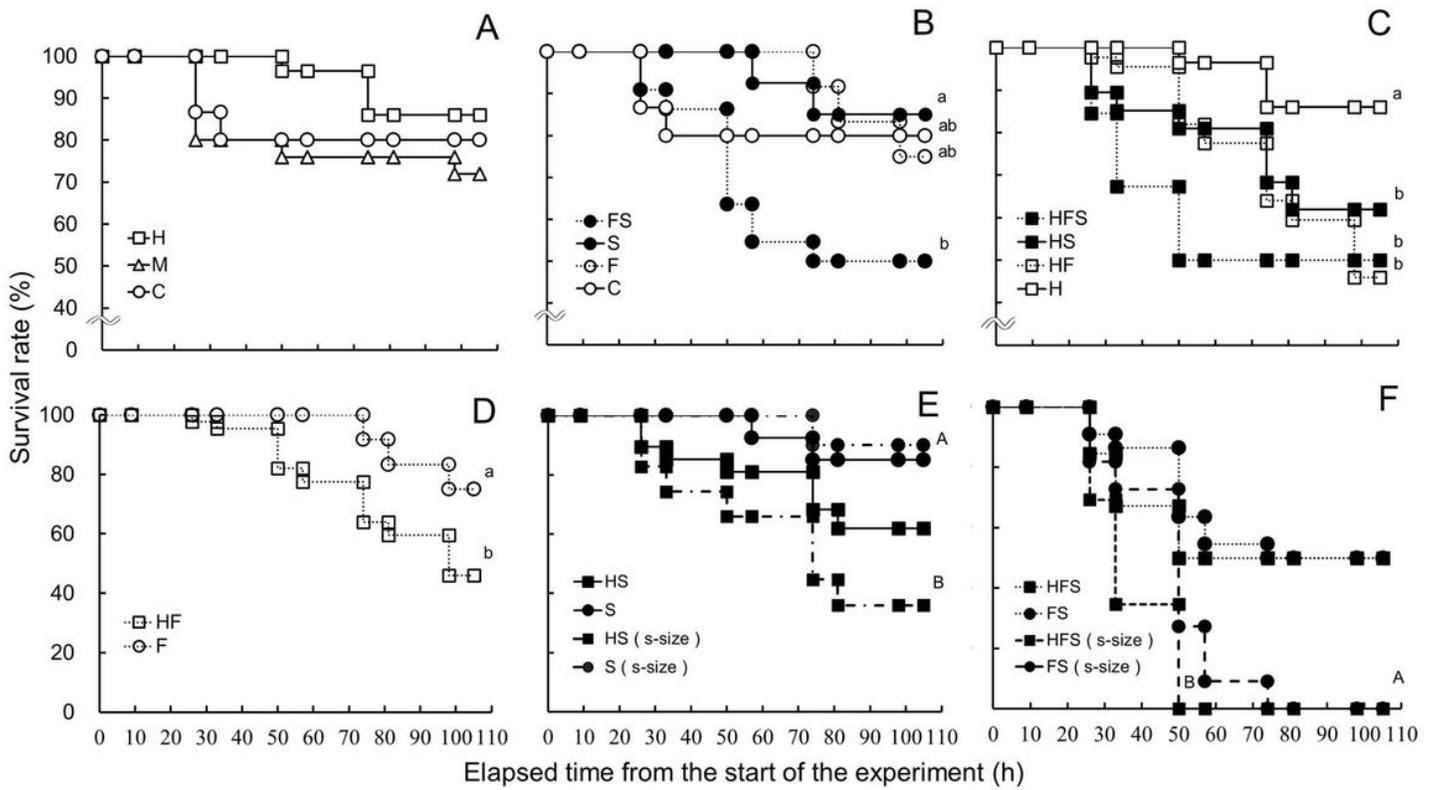
Effects of rearing conditions on frequency of aggressive behaviour in larval *Thunnus orientalis*. Experimental groups: C, control; M, medium-density; H, high-density conditions. Frequency of aggressive behaviour value represents mean  $\pm$  S.D (n = 9). There was no significant difference between the values (Games-Howell test, n= 9,  $p > 0.05$ ).



**Figure 2**

Effects of rearing conditions on frequency of aggressive behaviour in larval *Thunnus orientalis*.

Experimental groups: C, control; F, restricted feeding; S, diverse size; FS, restricted feeding + diverse size; H, high-density; HF, high-density + restricted feeding; HS, high-density + diverse size; HFS, high-density + restricted feeding + diverse size conditions. Frequency of aggressive behaviour value represents mean  $\pm$  S.D (n = 9). <sup>A-B, a-c</sup> Values with different letters in each high- and low-density section of aggressive behaviour are significantly different (Games-Howell test, n = 9,  $p < 0.05$ ). Values marked \* are significantly different from corresponding pairs under different stocking densities (Welch's test, n = 9,  $p < 0.05$ ).



**Figure 3**

Cumulative survival (Kaplan-Meier) of larval *Thunnus orientalis* under different stocking density subjected to single or multiple factors. Survival rate including all sizes:  $\square \bullet \square$ , C, control;  $\square \triangle \square$ , M, medium-density;  $\square \square \square$ , H, high-density; ...  $\bullet$  ..., F, restricted feeding;  $\square \bullet \square$ , S, diverse size; ...  $\bullet$  ..., FS, restricted feeding + diverse size;  $\square \square \square$ , H, high-density; ...  $\square \square$  ..., HF, high-density + restricted feeding;  $\square \blacksquare \square$ , HS, high-density + diverse size; ...  $\blacksquare$  ..., HFS, high-density + restricted feeding + diverse size. Survival rate only small size:  $\square \bullet \square$ , S;  $-\bullet-$ , FS;  $\square \blacksquare \square$ , HS;  $-\blacksquare-$ , HFS.

A Cumulative survival of different stocking densities (C,M and H).

B Cumulative survival of low stocking densities (C,F,S and FS).]

C Cumulative survival of high stocking densities (H,HF,HS and HFS).

D Cumulative survival of restricted feeding compared by density (F,HF).

E Cumulative survival of diverse size compared by density (S,HS).

F Cumulative survival of restricted feeding and diverse sizes compared to density (FS,HFS).

Values with different letters are significantly different (log-rank test;  $P < 0.05$ ,  $n = 20-50$ )

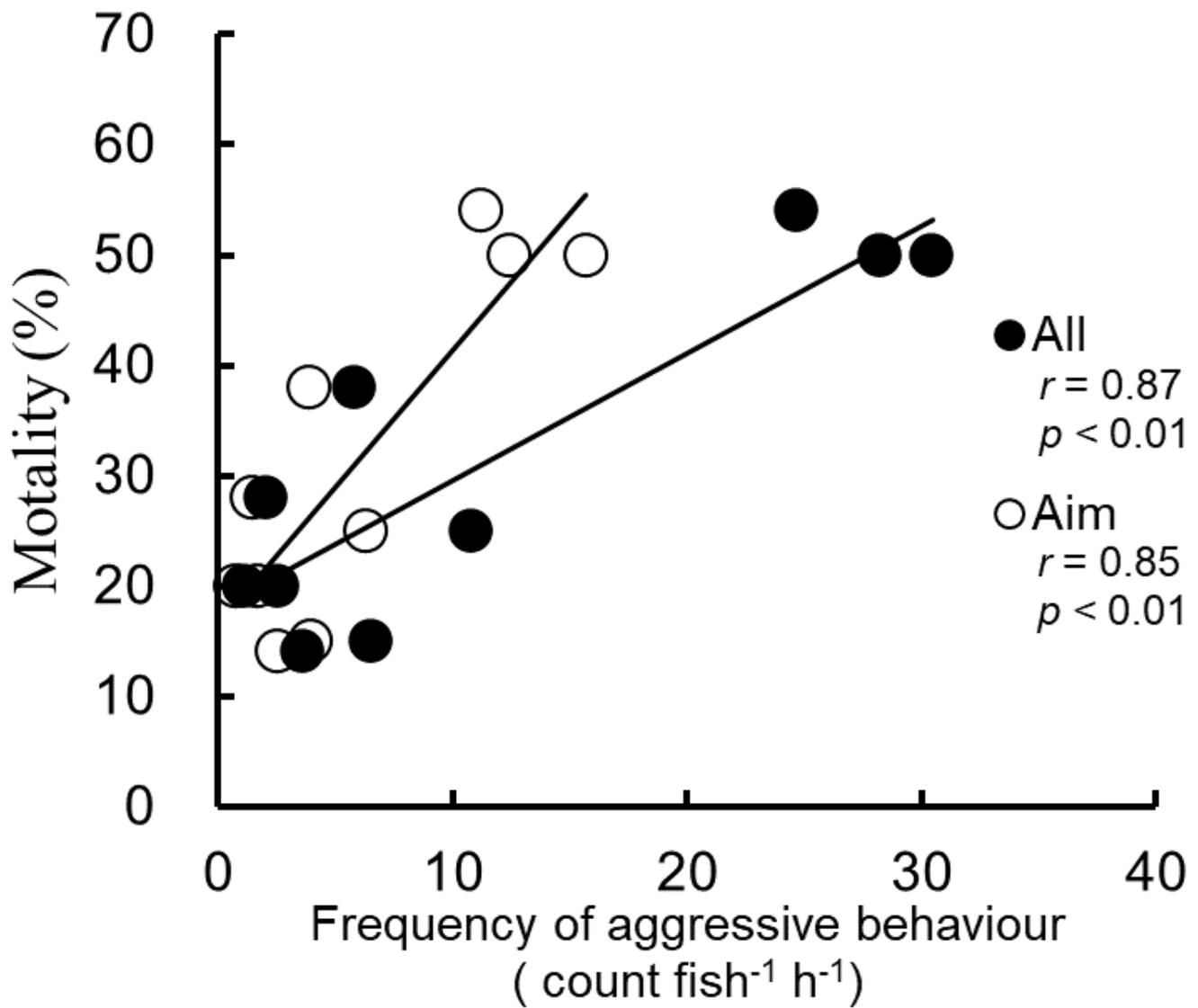


Figure 4

Relationship between mortality and attack frequency in *Thunnus orientalis* larvae exposed to different conditions.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table2.jpg](#)