Effects of density, starvation, and size difference on aggressive behaviour in juvenile yellowtails
(Seriola quinqueradiata)

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Summary
Effects of density, starvation, and size difference on aggressive behaviour in juvenile yellowtails
Seriola quinqueradiata (Temminck and Schlegel) were investigated. When fish were
acclimated to densities at 1, 2, 4, 8, and 16 fish L⁻¹, frequencies of aggressive behaviour per fish
decreased significantly. Under the starvation of 0, 4, 8, 12 and 24 hours, the highest aggression
was observed at 24 hours, while aggression showed same levels between 0 to 8 hours.
According to pair match test among three groups of different size from one batch (TL mm;
16.6±2.0, 21.1±2.1, and 29.7±4.9), the highest aggression was observed in the most size different
group, whereas others showed the same levels. Therefore, in view of the three investigated
parameters, aggressive behaviour was concluded to be starvation- and size-dependent but
negatively density-dependent in the yellowtail. Synthesizing all relevant results, including
abiotic factors such as light condition, a practical method to reduce the mortality caused by
aggressive behaviour in the yellowtail culture is presented.
INTRODUCTION
Biotic factors such as fish density, food availability, and size difference of members in a school can affect not only fish growth (Brett 1979), but also fish behaviours including aggressive behaviour and cannibalism. Since cannibalism and aggressive behaviour cause economic losses in aquaculture (Smith and Reay 1991; Ruzzante 1994), there are numerous studies of biotic factors on cannibalism and cannibalistic behaviour in experimental conditions (reviewed by Smith and Reay 1991; Hecht and Pienaar 1993; Folkvord 1997).

In the yellowtail *Seriola quinqueradiata* (Temminck and Schlegel; Carangidae), which is a highly migratory species and important commercial fish for aquaculture in Japan, cannibalism (Mizuta 1981; Imaizumi 1993; Sakakura and Tsukamoto 1996) and aggressive behaviour by a social rank (Sakakura and Tsukamoto 1997a,b; Sakakura et al. 1997) in a rearing pond for restocking, have been one of the major causes of high mortality at early juvenile stage. Previous study (Sakakura and Tsukamoto 1996) showed that aggressive behaviour in yellowtail onsets just after the transition from larva to juvenile stage and it is observed only in juvenile stage. Aggressive behaviour is accelerated by abiotic factors, such as high water temperature and low light intensity (Sakakura and Tsukamoto 1997a). Aggressive behaviour and cannibalism are also recognized among wild juvenile school, in which the members are uniformed both in size and age (Sakakura and Tsukamoto 1996,1997c). In the previous study (Sakakura and Tsukamoto 1996), we suggested that cannibalism of this species is interpreted as a climax phase of aggressive behaviour, and a cannibalistic juvenile regards its conspecific not as a food but as a counterpart to which it feels aggression and presents aggressive behaviour.

However, little is known about the effects of biotic factors on aggressive behaviour of this species. Thus, here, we firstly aim to determine the effects of fish density, starvation, and size difference of members in a school on aggressive behaviour in the juvenile yellowtails. Secondly, we discuss possible some recommendations for seedling production of this species.

MATERIALS AND METHODS
Experimental fish
 Four batches of juvenile yellowtails were used in this study during year 1993 (Batch 1), 1994 (Batch 2 and Batch 3), and 1995 (Batch 4). Yellowtail matured artificially by human chorionic gonadotropin injection were allowed to spawn naturally in a 90 m$^3$ indoor tank at the Goto station of the Japan Sea Farming Association (JASFA) in Nagasaki Prefecture, except for Batch 4 by wet method of insemination. Fertilized eggs were maintained in a 0.5 m$^3$ tank, and after 2 days, approximately 500,000 to 1,000,000 larvae were obtained (Batch 1: April 29, 1993; Batch 2: April 24, 1994; Batch 3: May 28, 1994; Batch 4: May 10, 1995). Two days after hatching (day 2), larvae were transferred to a concrete rearing pond (90 m$^3$). Larvae were fed with the rotifer *Brachionus plicatilis* (O. F. Müller) cultivated with *Nannochrolopsis* sp. between days 3 and 20, with newly hatched *Artemia salina* (Leach) nauplii enriched with feed oil (Riken feed oil, Riken Co.) between days 7 and 24, and with dry pellets (C 400-1000, Kyowa Co.) from day 22 until the end of the experiment. Water temperature ranged from 22 to 24°C under natural light conditions.

Fish-density experiment
Fifteen white plastic tanks (30 cm in diameter) containing 10-L of filtered seawater at 22 °C (14 cm in depth) were placed in a water bath kept at 22 °C, as experimental tanks. These experimental tanks were divided into 5 groups with 3 tanks for each. Fish used for behavioral observations were randomly sampled from the rearing pond with 13-L buckets. thirty fish were observed at each age. A total of 930 fish were selected from the bucket sample to minimize size
variation by a hand net were introduced into these 5 groups for each experimental tank in following numbers; 10, 20, 40, 80 and 160, respectively (1, 2, 4, 8, and 16 fish L⁻¹ in fish density, respectively). Experiments were repeated 4 times using different total length (TL mm) and age (day) groups; 15.9±1.1 mm, day 30 (Batch 2); 24.4±1.7 mm, day 35 (Batch 2); 12.3±1.5 mm, day 25 (Batch 3); 17.4±1.5 mm, day 30 (Batch 3). The density of 1 fish L⁻¹ corresponded to a ordinary rearing density of this species (Sakakura and Tsukamoto 1996).

Fish were acclimated in the experimental tanks for 4-8 hours after introducing. They were fed *Artemia* until satiation 2 hours before observation, following replacement of half the volume of seawater by fresh-seawater after feeding. Frequency of chasing behaviour (CHS; count min⁻¹) in each tank, which is found to be available as an index to estimate the magnitude of aggressive behaviour in the yellowtail (Sakakura and Tsukamoto 1996), was counted from above for the duration of 5 min after 2 hours acclimation from feeding. Mean values of CHS (count min⁻¹.fish⁻¹) were obtained at each experimental tank.

**Starvation experiment**

Fifteen white plastic tanks (30 cm in diameter) containing 10-L of filtered sea water at 22 °C (14 cm in depth) were placed in a water bath kept at 22 °C. These experimental tanks were divided into 5 groups with 3 tanks for each. Ten fish of comparable size sorted as well as the density experiment from Batch 1 (150 fish in total; TL 21.4±1.7 mm, day 28) were introduced into each experimental tank and 2 hours after fed with *Artemia* until satiation following half volume of seawater exchange (first treatment) after feeding. In five experimental groups, control groups were fed *Artemia* sufficiently with 2 hours intervals for 24 hours after the first treatment. The others were kept under starvation before observation with following intervals; 4 hr, 8 hr, 12 hr and 24 hr respectively, so that the observation could be conducted at the same time with control group.

24 hours after the first treatment, CHS was counted in each experimental tank for 5 min. Additional observation (group Fed) was conducted on 24 hr group, in which fish were fed sufficient *Artemia* after the experiment. Data were pooled, and mean values of CHS were obtained at each experimental group (three tanks).

**Size-difference experiment**

Fish used for this experiment were sampled randomly from the rearing pond (Batch 4, day 34) and were divided into three different body size groups: small fish, S (TL 16.6±2.0 mm); medium fish, M (TL 21.1±2.1 mm); large fish, L (TL 29.7±4.9 mm), respectively (ANOVA, df=2, F-value=483.492, p<0.01). Five experimental groups of different combination of size groups were set, which consisted of of three same size groups (S-S, M-M, L-L) and of two different two size groups (S-M, S-L). A total of 10 fish were introduced into each experimental tank, in which 5 fish from same size group were introduced.

Experimental tanks, conditions, and observations were the same to those of the fish density experiment. Experiments were repeated 5 times (10 times for the combination of S-S) using different individuals (300 fish in total). Data were pooled, and mean values of CHS were obtained for each experimental group.

**Statistical analysis**

For the fish density experiment, logistic curve fitting were applied. Pearson's correlation coefficient was applied against the r-value to determine the significant correlation between fish density and CHS.

For the starvation- and size-difference experiments, statistical analysis of CHS was done
using Bartlett's test for comparison of variances. A one-way analysis of variance (ANOVA) was applied when there was no significant difference between the variances of the different groups (p>0.05 by Bartlett's test). In cases where significant differences were found among the means by ANOVA (p<0.05), Duncan's new multiple range test was applied for comparison among experimental groups.

RESULTS

Fish density
The CHS value per fish (Fig. 1) decreased significantly with the increase in fish density in each size group (Pearson's correlation coefficient, n=15, p<0.05). Relationship between fish density (x fish·L^{-1}) and CHS (y count·min^{-1}·fish^{-1}) obtained from the logistic curve fitting were described as following equations: y = -0.78\log(x) + 0.88 (r=0.92) for the group of TL 12.3 mm; y = -0.76\log(x) + 0.88 (r=0.92) for the group of TL 15.9 mm; y = -1.29\log(x) + 1.33 (r=0.85) for the group of TL 17.4 mm; y = -2.08\log(x) + 2.48 (r=0.82) for the group of TL 24.4 mm, respectively.

Starvation
In each experimental group, 2-7 CHS (count min^{-1}) were observed. According to the increase of starved time over 8 hr, CHS increased significantly at 12 hr and 24 hr (Duncan's new multiple range test, df=5, F-value=12.2, p<0.05; Fig. 2), while CHS showed same levels among control, 4hr and 8 hr (Fig. 2). Moreover, aggression of the Fed group, which fed after 24 hours starvation, decreased at the same level of control (Fig. 2).

Size difference
Aggressive behaviour was observed in all combinations. Frequency of CHS was the highest at S-L combination (2.6±1.4 count min^{-1}, Fig.3), of which size variance is the highest (Duncan's new multiple range test, df=4, F-value=6.505, p<0.05; Fig.3). Aggressive individual from 4 of 5 tanks at S-L combination and all S-M combination belonged to large fish group (L). However, in one tank of S-L combination, one small fish (S) chased after the larger (L) fish solely and there was no aggressive behaviour among other larger fish in that tank. This phenomenon was observed at least for 2 hours additional observation. There were no significant difference in CHS among the same size combinations (S-S, M-M, and L-L) and small size difference combinations (S-M). S-S combination showed the lowest CHS value (0.5±0.3 count min^{-1}; Fig. 3).

DISCUSSION

Density-effect
Aggressive behaviour in juvenile yellowtails was negatively density-dependent according to the increase of density. Density-dependent mortality by cannibalism are reported in many larval and juvenile fishes in the experimental conditions, such as sea bass *Dicentrachus labrax* (Katavic *et al.* 1989), carp *Cyprinus carpio* (Damme *et al.* 1989; Hecht and Pienaar 1993) and pike *Esox lucius* (Giles *et al.* 1986; Wright and Giles 1987). On the other hand, mortality from cannibalism were density-independent in catfish *Clarias gariepinus* (Hecht and Pienaar 1993) and walleye *Stizostedion vitreum* (Moore *et al.* 1994). Hecht and Pienaar (1993) pointed out that aggressive behaviour by territoriality decreased with the increase of fish density, whereas the rate of cannibalism increased. Since fish were sorted as comparable size in this study, negatively density-dependent aggressive behaviour in yellowtail reflects the aggressive behaviour observed in this experiment is from social rank and cannibalism rate is expected to increase
according to increase of fish density as well as in catfish. Our results also indicate the presence of thresholding density (4 fish L$^{-1}$) of aggressive behaviour in yellowtail.

In schools of juvenile yellowtail, there are differences in aggressive tendency among individuals (Sakakura and Tsukamoto 1996), and social rank with dominance hierarchy (Sakakura and Tsukamoto 1997b; Sakakura et al. 1997). In this social rank, about 20% fish appear as dominant individuals, and environmental factors suppose to only accelerate the aggressive tendency of these dominants (Sakakura and Tsukamoto 1997b). In this study, CHS per fish decreased according to the increase of fish density. In case the density was between 1 and 4 fish L$^{-1}$, aggressive tendency of only dominant fish increase according to the increase of fish density and total CHS increased. Corresponding to this increase of CHS in the dominants of top hierarchy, aggressive tendency of the other fish (about 80%) belonging to lower hierarchies in a tank are strongly suppressed. As a sum of CHS values of all fish in a tank, CHS per fish may possibly decrease according to the increase in fish density. If the fish density exceeded over 4 fish L$^{-1}$, the fish density seemed to be too high to keep their ordinary swimming behaviour and avoiding physical contact, potentially suppressing their aggressive tendency.

**Starvation-effect**

Starvation period between 12 and 24 hours enhanced aggressive behaviour significantly. A threshold of starved time of aggressive behaviour may be about 8 hours, until which aggression is maintained at the same level.

Positive effects of starvation on cannibalism are reported in sea bass *Dicentrachus labrax* (Katavic et al. 1989), Atlantic cod *Gadus morhua* (Folkvord 1991), catfish *Clarias gariepinus* (Hecht and Appelbaum 1988), and European eel *Anguilla anguilla* (Degani and Levanon 1983). In the yellowtail, since aggressive behaviour was also affected by starvation, aggressive behaviour appeared to be based on the motivation for feeding. However, aggressive behaviour was observed at a same level, even when fish are well fed both in experimental and wild conditions (Sakakura and Tsukamoto 1996, 1997a). Smaller individuals sometimes showed aggressive behaviour as a dominant toward larger fish (in size-difference experiment; Sakakura and Tsukamoto 1996), apparently indicating the presence of a motivational system directly related to aggression, which is different from the motivation for feeding or predatory behaviour, as reported in the sunfish *Lepomis macrochirus* (Poulsen and Chiszar 1975), although variation in hunger has complex effects on different components of aggressive behaviour (Poulsen 1977).

**Size-effect**

Dominance in fish is generally related to larger body size (Francis 1983). In this study, the highest aggression was observed in S-L combination, indicating the presence of general size-advantage in social interaction. However, since we observed in one case of S-L combination that one small fish (S) chased after the larger fish solely, the result suggests that difference in individual aggressive behaviour may depend on not only size-advantage but also individual aggressive tendency, or the individual motivation.

Relatively less aggression observed in group S-S, indicates that smaller fish may be less aggressive than larger fish among same batch. Abbot *et al.* (1985) reported that no subordinate rainbow trout *Oncorhynchus mykiss* became dominant despite being given extra food, which must be caused by learning position in a hierarchy. In European eel, learning position in a hierarchy was also reported (Knights 1987). It is assumed that fish belonging to a small group had learned their social position through the interaction with larger agonistic encounters in the rearing pond, and therefore their aggression had been suppressed before experiment.

In yellowtail, social rank and aggressive behaviour are regarded to be functional to make
the body size of school members uniformed for predator avoidance (Sakakura and Tsukamoto 1996, 1997b). A direct relation between individual aggressive behaviour and growth in the territoriality are reported in salmonids (Yamagishi 1962; Abbot and Dill 1989). However, in highly migratory fish such as yellowtail, it has not yet been demonstrated.

Some recommendation on yellowtail production
Cannibalism is observed among the schools associated with drifting seaweeds in the field (Sakakura and Tsukamoto 1996), and it is estimated that school members are formed from same batch by age determination (Sakakura and Tsukamoto 1997c). It is easily presumed that not only cannibalism but also aggressive behaviour as the precursor of cannibalism would occur frequently in the field. Aggressive behaviour is observed even in a low density, under a well fed condition, and among individuals of the same size (This study; Sakakura and Tsukamoto 1996, 1997a). It is considered that aggressive behaviour of this species may not be an abnormal behaviour under extraordinary environmental conditions but a normal behaviour of healthy specimen.

Since no agonistic interaction is observed in dark conditions (Sakakura and Tsukamoto 1997a), aggressive behaviour in yellowtail can be regarded as a response to a visual stimuli (counterpart), and aggressive individuals may not be able to distinguish which fish is a specific subordinate, suggesting the stimuli may be always at a same level in an experimental tank. Therefore, juvenile yellowtails are able to show aggressive behaviour as far as they can recognize another conspecific by vision. In order to prevent cannibalism in the amberjack *Seriola lalandi* juveniles, which is the same genus species of yellowtail, Ebisu and Tachihara (1993) covered the rearing tank with dark sheet to keep the tank at low light condition, but they could not find significant effect. As the critical threshold of light intensity of aggressive behavior in juvenile yellowtails is between $10^{-2}$ lx and $10^{-3}$ lxt as well as the threshold of schooling and feeding behaviour (Sakakura and Tsukamoto 1997a), it seems that control of light condition is not applicable in order to prevent cannibalism of this species.

One approach to control cannibalism in seedling production is providing adequate food of adequate size (Folkvord and Otterå 1993) and frequent feeding (Katavic et al. 1989). In the seedling production of the yellowtail, feeding is kept to be satiated at least three times a day in every production (Shiozawa 1996, 1997). Thus, yellowtails are not starved for 8 hours during day time, which enhances aggressive behaviour (this study). However, fish are kept starved in the night time, because yellowtails do not feed in the night time (Sakakura and Tsukamoto 1997a). Therefore, frequent feeding at the dawn and dusk can be one of the controls for cannibalism.

Controlling fish density is also effective to reduce the loss from cannibalism (e.g. Degani and Levanon 1983; Giles et al. 1986; Damme et al. 1989; Hecht and Pienaar 1993). In our study, the density 1-2 fish L$^{-1}$ showed highest aggressive behaviour and CHS increased according to fish growth from TL 17.4 mm. Since cannibalism rate is expected to increase when fish density exceeds thresholding density of aggressive behaviour (4 fish L$^{-1}$) and high density may cause reduced growth and increased infection, optimal fish density is estimated as 3 fish L$^{-1}$.

Size-grading can be effective to reduce cannibalism in many species of aquaculture (e.g. Knights 1987; Parazo et al. 1991; Folkvord and Otterå 1993; Hecht and Pienaar 1993), whereas in case of juvenile yellowtails, it has not been practical because swimming activity is high (Sakakura and Tsukamoto 1996) and juveniles are very sensitive to handling stress at day time (Shiozawa 1997). However, at night time, juvenile yellowtails cease swimming and drift at surface to make dense patchiness in the rearing pond (Sakakura and Tsukamoto 1997a, c), and are easy to handle. Shiozawa (1996) reported that preliminary trial of size-grading at TL 15 mm at
night time raised total production of juvenile yellowtails up to 1.5 to three times higher than usual productions. As indicated in the density experiment, juvenile yellowtails become more aggressive when they reached ca. TL 16 mm. Therefore, size-grading at TL 12-15 mm can be most practical and effective method in order to reduce the loss by cannibalism and aggressive behaviour in the seedling production of this species.

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Fig. 1 Frequency of chasing behaviour (CHS) per fish in juvenile yellowtails at various fish densities (n=15) and fish sizes. Lines indicate the logistic-curve fitting.
Fig. 2  Mean frequency of chasing behaviour (CHS) of juvenile yellowtails (TL 21.4±1.7 mm) at various starved time (n=3). In group Fed, fish were fed sufficiently after 24 hours starvation. Vertical bars indicate standard deviations and letters give significance level (a<b<c; Duncan's new multiple range test, p<0.05).

Fig. 3  Mean frequency of chasing behaviour (CHS) of the yellowtail at various fish size. S (TL 16.6±2.0 mm), M (TL 21.1±2.1 mm) and L (TL 29.7±4.9 mm) show the fish groups graded in body size. Vertical bars represent standard deviations. Alphabets indicate significant difference (a<b; Duncan's new multiple range test, p<0.05).