

Whole-body cortisol concentrations and ontogeny of aggressive behavior in yellowtail (*Seriola quinqueradiata* Temminck & Schlegel; Carangidae).

Yoshitaka Sakakura*, Masatomo Tagawa and Katsumi Tsukamoto

Ocean Research Institute, The University of Tokyo
Minamidai 1-15-1, Nakano, Tokyo 164, Japan.

* Present address: Department of Zoology, University of Guelph
Guelph, Ontario N1G 2W1, Canada

Short Title ; CORTISOL AND AGGRESSIVE BEHAVIOR

ABSTRACT

Ontogenetic changes in whole-body immunoreactive cortisol concentrations (IRC) and aggressive behavior were examined in yellowtail *Seriola quinqueradiata* (Temminck & Schlegel; Carangidae). Baseline IRC significantly increased during the transition from larval to juvenile stage, and was correlated with the onset of aggressive behavior. Handled fish (13.1 ± 2.6 ng/g tissue) showed a IRC level about three times higher than unhandled fish (4.7 ± 1.4 ng/g tissue), indicating that whole body immunoreactive cortisol level may be used an indicator of stress in juvenile yellowtails. Behaviorally subordinate fish (8.6 ± 1.6 ng/g tissue, $n=4$) showed IRC levels significantly higher than dominant fish (0.6 ± 0.3 ng/g tissue, $n=4$). Whole-body immunoreactive cortisol levels may thus reflect stress and social status in juvenile yellowtails, and the inverse relationship between social rank and IRC may result from agonistic interactions.

In agonistic interactions, subordinate individuals appear physiologically more stressed (Schreck, 1981). In many animals, including teleosts, the pituitary-interrenal axis is activated by stress (Donaldson, 1981). Plasma cortisol concentrations and interrenal cell activity are often used as stress indicators in teleost fishes. Interrenal cell dimensions were greater in subordinate rainbow trout *Oncorhynchus mykiss* (Noakes and Leatherland, 1977), and plasma cortisol levels higher in subordinate coho salmon *O. kisutch* (Ejike and Schreck, 1980) and European eel *Anguilla anguilla* (Peters *et al.*, 1980).

Recently, whole-body immunoreactive cortisol concentrations (IRC) during early development were reported for Japanese flounder *Paralichthys olivaceus* (de Jesus *et al.*, 1991), chum salmon *O. keta* (de Jesus *et al.*, 1991) and tilapia *Oreochromis mossambicus* (Hwang *et al.*, 1992), focusing on the role of cortisol in development in relation to thyroid hormones, and in seawater adaptation. Moreover, IRC may indicate physiological stress in the early development of rainbow (Pottinger and Mosuwe, 1994; Barry *et al.*, 1995) and brown trout *Salmo trutta* (Pottinger and Mosuwe, 1994).

In the yellowtail *Seriola quinqueradiata* (Temminck & Schlegel; Carangidae), a migratory marine species whose juveniles show typical schooling behavior associated with drifting seaweeds (Uchida *et al.*, 1958; Anraku and Azeta, 1965), aggressive behavior appears just after transition from larva to juvenile (Sakakura and Tsukamoto, 1996), and social rank is established in the schools (Sakakura and Tsukamoto, 1997a). Moreover, tolerance of handling stress (netting and air exposure) in yellowtail rapidly decreases just after the transition to juvenile stage (Shiozawa, 1997).

However, the interrenal response to stress and social interactions, such as plasma cortisol levels, has not been examined in larval and juvenile yellowtails, partly because blood collection from the larval and juvenile yellowtails (about 4 to 12 mm in total length) is difficult. The ontogenetic changes of baseline IRC and aggressive behavior have been examined during the development of physiological stress responses, and the IRC levels have been related to the social interaction caused by aggressive behavior in juvenile yellowtails.

MATERIALS AND METHODS

Fish

Three batches of larval and juvenile yellowtail were used in this study during 1993 (Lot 1) and 1994 (2 batches: Lots 2 and 3). Yellowtail matured artificially using human chorionic gonadotropin injections were allowed to spawn naturally in a 90-m³ indoor tank at the Goto station of the Japan Sea Farming Association (JASFA) in Nagasaki Prefecture. Fertilized eggs were maintained in a 0.5-m³ tank, and after 2 days, approximately 1,000,000 larvae were obtained (Lot 1: April 29, Lot 2: April 24, Lot 3: May 28). Two days after hatching (day 2), larvae were transferred to an outdoor concrete rearing pond (90 m³). Larvae were fed with the rotifer *Brachionus plicatilis* between days 3 and 20, with *Artemia salina nauplii* between days 7 and 24, and with dry pellets (C-400 and 1000; Kyowa, Tokyo) from day 22 until the end of the experiment. Water temperature ranged from 22 to 24°C under natural daylight.

Ontogenetic Changes in Behavior and Whole-body Immunoreactive Cortisol Concentrations (IRC)

Fish used for behavioral observations and baseline IRC measurement were randomly sampled from the rearing pond with 13-l buckets at night (2200-2400). Night time was chosen

for sampling, because it is easy to collect sample with minimal stress, as juvenile yellowtails cease swimming and drift at surface to make dense patchiness (mass of aggregated juveniles in the current rip) at night time (Sakakura and Tsukamoto, 1997a,b).

Fish for IRC measurement were immediately anesthetized with an overdose of MS222 and stored frozen at -85°C until analysis. Total lengths (TL; mm) and wet body weight (BW; mg) of samples at each age before IRC measurement were measured individually. The developmental stage was determined according to Fukuhara *et al.* (1986). Then samples were pooled into three to five groups of ca.100 mg in wet weight (about 5 to 50 fish for one group) at each age until day 20. From day 20 to day 39, fish were measured individually. Samples were then homogenized in three volumes of phosphate-buffered saline (PBS: 10 mM phosphate buffer, pH 7.3, containing 140 mM NaCl).

For the behavioral study, thirty fish were observed at each age. Three groups, each consisting of 10 fish (2 fish l^{-1}), selected from the bucket sample to minimize size variation, were introduced into experimental tanks (30 cm in diameter, 5-l) in a water bath at 22°C . Fish were kept in the experimental tanks overnight, then fed *Artemia* 4 hours before observation. Following Sakakura and Tsukamoto (1996), aggressive behaviour was divided into the following 3 phases; (1) Aim: a dominant holds position towards a subordinate hovering for a short period (ca. 1 s) (2) Chase: a dominant bursts towards and follows a subordinate for 1 to 10 seconds (3) Nip: a dominant attacks and bites at the tail or body of a subordinate. These 3 phases were observed in a fixed order. Abandonment by a dominant during Chase behavior caused failure to complete an entire aggressive behavior sequence. However, Chase behavior was always observed in every instance of aggressive behavior. Since Chase behavior was also easy to observe and record, the frequency of Chase behavior was used as an index of aggressive behaviour. "Chase behavior" was recorded for 5 minutes in each tank. Experiments were conducted during the day (1000-1200) at intervals of 3 to 5 days from days 7 to 40. Following behavioral observations, all fish were immediately anesthetized with MS222 and TL of each fish was measured with a micrometer or calipers. The developmental stage was defined according to Fukuhara *et al.* (1986).

IRC Radioimmunoassay

The extraction method for IRC was followed Hiroi *et al.* (1997). In brief, a 250- μl aliquot of PBS-homogenate was extracted twice with 3 ml diethyl ether. Combined extracts were dried and resuspended in 250- μl of carbon tetrachloride, followed by a second extraction with 250- μl of Gel-PBS (PBS containing 0.1% gelatin). One hundred μl Gel-PBS phase was used for IRC-RIA (de Jesus *et al.*, 1991). The efficiency of extraction of standard cortisol added to PBS-homogenate was $83.0 \pm 14.8\%$ ($n=4$), and the displacement curve of the extracted sample was parallel to the standard curve (data not shown).

Stress and IRC

Three groups of 20 fish (Lot 2; 27 mm TL, day 40) were introduced into 3 experimental tanks (55 cm in diameter containing 100 l seawater at 22°C), and acclimated for 4 hours (0800-1200). All fish in the first tank were sampled as the non-treatment group ("Control"). Fish in the second tank were vigorously mishandled by chasing them with a hand-net for 3 minutes until the fish did not respond further to the hand-net ("Handling").

In the third tank, fish were classified into three categories based on 10 minutes of behavioral observation as follows: when fish A was chasing fish B, fish A was classified as

"Dominant" and fish B as "Subordinate"; a fish showing no agonistic interactions within 10 minutes was classified as a "Non-responder". Four pairs of "Dominant" and "Subordinate" were easily discriminated and scooped immediately, because these pairs frequently swam away from the school. Following this treatment "Non-responders" (n=12), which made one synchronized school, were immediately scooped.

All fish were anesthetized with an overdose of MS222, and then stored at -85°C until used for TL measurements and IRC-RIA.

Statistical analysis

Statistical analysis of baseline IRC 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 groups. On the other hand, when significant differences existed among variances ($p < 0.05$ by Bartlett's test), the Kruskal-Wallis test for multiple groups was applied to determine differences among the medians. Significant differences among medians were further examined by the Mann-Whitney U test against the previous age group.

The median frequency of "Chase" among 3 tanks was used as the representative value of an age group, and differences in the frequency of "Chase" among age groups were statistically examined by the Mann-Whitney U-test.

In the stress experiment, statistical analysis of TLs and IRC were done using the Student's t-test between the groups "Control" and "Handling", and using Duncan's new multiple range test after ANOVA treatment among "Dominant", "Subordinate" and "Non-responder", after Bartlett's test for comparison of variances ($p < 0.05$).

RESULTS

Ontogenetic Changes in Behavior and IRC

In all lots, the transition from post-larvae to juveniles occurred around day 20 at a mean TL of 10 mm with the full complement of fin rays (Fig. 1). Fish grew relatively slowly throughout the larval interval, but more rapidly after metamorphosis (Fig. 1). In Lots 1 and 3, aggressive behavior first occurred when the transition from larval to juvenile stage was finished at day 23 and 25 (about TL 12 mm), and increased thereafter (Fig. 2) as well as in the rearing pond. In Lot 2, aggressive behavior was not recorded until day 30 (TL 15.9 mm, Fig. 2), and not in the rearing pond. In comparison with the results from all lots in this study and the result from Sakakura and Tsukamoto (1996), fish of Lot 2 appeared to be behaviorally unusual because of the delay of the onset of aggressive behavior, although the growth pattern was similar to other lots (Fig. 1).

Baseline IRC increased at day 7 in all lots, when the fish reached about TL 5 mm (Fig. 2; U-test, $p < 0.05$). After this peak, IRC remained at a low level until day 18 (ca. TL 9 mm), and it significantly increased at day 20 (over TL 9 mm, Fig. 2; Duncan's new multiple range test, $p < 0.05$) and increased further thereafter.

Stress and IRC

The stressed group ("Handling": 13.1 ± 2.6 ng/g tissue) showed about 3 times higher IRC

level than the unstressed group ("Control": 4.7 ± 1.4 ng/g tissue; T-test, $P < 0.05$; Fig. 3a). There was no significant difference in total length between control (27.0 ± 1.8 mm) and handled (27.6 ± 3.1 mm) groups (T-test, $p > 0.1$).

The "Subordinate" group (8.6 ± 1.6 ng/g tissue, $n=4$) showed the highest IRC level (Duncan's new multiple range test, $p < 0.05$; Fig. 3b). The "Dominant" group was the lowest (0.6 ng/g tissue, $n=4$; Duncan's new multiple range test, $p < 0.05$). The "Non-responders" showed an intermediate level (3.5 ± 1.7 ng/g tissue, $n=12$). There was no significant difference in the total lengths of the groups (dominant, 27.3 ± 1.8 mm; subordinate, 25.2 ± 3.0 mm; non-responder, 27.7 ± 3.1 mm: ANOVA, $p > 0.1$).

DISCUSSION

After the transition from larval to juvenile stage, baseline IRC levels of juvenile yellowtails increased concomitantly with the onset and development of aggressive behavior. Stress tolerance in the early life stages of yellowtail, examined from the survival ratio from air exposure for 10 seconds by netting, was around 100 % in the larval and transitional stage, and decreased from the juvenile stage from day 23 (TL 10 mm) and was at low levels thereafter (Shiozawa, 1997). In rainbow trout, whole-body corticosteroids did not increase in response to acute stress until 2 weeks after hatching, although immunoreactive ACTH in the pituitary (Saga *et al.*, 1993) and interrenal cells capable of corticosteroid biosynthesis (Hwang *et al.*, 1992; Barry *et al.*, 1995) were already present prior to this time. Barry *et al.* (1995) postulated that this lack of a stress response reflected an immaturity of the higher brain and hypothalamic centers regulating stress response, and that the adrenocorticotrophs and interrenal cells are not fully mature until two weeks after hatching in rainbow trout. In the yellowtail, the interrenal first appears histologically on day 7 (about TL 5 mm, data not shown; Chantanachookhin *et al.*, 1991). Together these observations may suggest that the pituitary-interrenal axis and stress response of yellowtail are not functional during larval stages and becomes activated from the juvenile stage; histological observation were not made on the pituitary, however.

Significant standing pool of IRC corresponded to transition from larva to juvenile in yellowtail from day 18 (TL 9 mm), whereas tissue thyroid hormones increased just before the beginning of transition from larval to juvenile stage, where typical behavior at this period named 'J-posture' appears (Sakakura and Tsukamoto, 1996), and calcification of bones and fin-ray formation occurs and is regarded equivalent as metamorphosis (Sakakura & Tsukamoto, 1997c). In metamorphosing Japanese flounder from larva to juvenile, increased tissue cortisol levels herald increased thyroid hormones (de Jesus *et al.*, 1991) and cortisol accelerates dorsal fin-ray resorption in combination with thyroid hormones *in vitro* (de Jesus *et al.*, 1990). Cortisol may have a role in metamorphosis of yellowtail as well as Japanese flounder, although there are differences in IRC patterns during metamorphosis between the two species (this study; Tanaka *et al.*, 1995). Since metamorphosis involves morphological, physiological, and biochemical, as well as behavioral changes that are often associated with increased energy demands (Youson, 1988), metabolic effects of cortisol, such as gluconeogenic action and lipid and protein metabolism, may be important, in view of development of significant swimming activity (Sakakura and Tsukamoto, 1996) and calcification of bones and fin-ray formation (Sakakura and Tsukamoto, 1997c) during metamorphosis.

A significant peak of IRC occurs on day 7 (about TL 5 mm), when the interrenal first appears histologically (data not shown; Chantanachookhin *et al.*, 1991) and high mortality

occurs in the rearing pond as a result of failure to ingest food properly (Shiozawa, 1997). This so-called critical period (point of no return) by the starvation in the larval stages of fishes (May, 1974), may occur around day 7 (TL 5 mm) and possibly lead to gluconeogenesis from muscle resulting by a failure to feed in larval yellowtails.

Handled juvenile yellowtails showed significantly higher IRC levels than control, indicating that acute stress elevated the IRC levels in this species. As Pottinger and Mosuwe (1994) pointed out, the IRC must be interpreted with caution. Because clearance of cortisol from the body involves metabolism and conjugation, and possibly accumulation within liver or bile (Pottinger *et al.*, 1992), the dynamics of IRC levels during stress are likely to differ in the blood (Pottinger and Mosuwe, 1994). However, in the relatively short period after stress (4 to 8 hours), plasma cortisol and IRC in rainbow trout showed similar pattern of change (Pottinger and Mosuwe, 1994). Given this evidence, whole-body immunoreactive cortisol concentration may be a reasonable stress indicator in the early life stages of this species for a short-term period after stress, as in juvenile brown (Pottinger and Mosuwe, 1994) and rainbow trout (Pottinger and Mosuwe, 1994; Barry *et al.*, 1995). Although blood samples could not be collected from the juvenile yellowtails due to the small body size, whole-body immunoreactive corticosteroids is applicable as a stress indicator in terms of blood samples for the small juvenile fishes. Thus, the positive correlation between whole-body immunoreactive cortisol and aggressive behavior in juvenile yellowtails may reflect not only the development of stress response but also the development of social rank.

In the past, fish schools were thought to be egalitarian and leaderless societies (Breder, 1954; Shaw, 1962). However, in schools of yellowtail juveniles, individual differences emerge as a consequence of agonistic interactions and social rank (Sakakura and Tsukamoto, 1997a). "Subordinate" yellowtail in a school showed significantly higher IRC levels than "Dominant" fish. It is suggested that "Subordinate" yellowtail juveniles are physiologically stressed by their social rank in a school in experimental tanks, akin to the effects of social rank in rainbow trout (Noakes and Leatherland, 1977) and in coho salmon (Ejike and Schreck, 1980). It seems the majority of the school is stressed for a long period, but the social rank of this species is not stabilized strictly and has some flexibility to experience a rank reversal within in long-term period (about 1 week; Sakakura and Tsukamoto, 1997c). Therefore, the balance of stress in school members can be mediated during the juvenile stage.

The present study demonstrates for the first time a positive correlation between ontogenetic changes in tissue cortisol concentration and aggressive behavior. A direct relation between the activity of the pituitary-interrenal axis and the onset of individual aggressive behavior has not yet been demonstrated, and the ability of endocrine factors including cortisol to regulate aggressive behavior require examination.

ACKNOWLEDGMENTS

We are grateful to Mr. S. Shiozawa, K. Maruyama, Y. Mizuta and T. Furusawa of JASFA for support and help, without which the study could not have been made. Thanks are also due Prof. T. Hirano, Ocean Research Institute, the University of Tokyo, for his discussions and suggestions during this study. We are grateful to Prof. David L.G. Noakes, University of Guelph, and Prof. Howard A. Bern, University of California at Berkeley, and three anonymous reviewers for their critical reviews of the earlier version of the manuscript. Y.S. was supported by the Fellowship for Japanese Junior Scientists and the Postdoctoral Fellowships for Research Abroad of the Japan Society for the Promotion of Science.

REFERENCES

- Anraku, M. and Azeta, M. (1965). The feeding habits of larval and juveniles of yellowtail, *Seriola quinqueradiata* Temminck et Schlegel, associated with floating seaweeds. *Bull. Seikai Reg. Fish. Res. Lab.* **33**, 14-15.
- Barry, T.P., Malison, J.A., Held, J.A. and Parrish, J.J. (1995). Ontogeny of the cortisol stress response in larval rainbow trout. *Gen. Comp. Endocrinol.* **97**, 57-65.
- Breder, C.M. (1954). Equations descriptive of fish schools and other animal aggregations. *Ecology* **35**, 361-370.
- Chantanachookhin, C., Seikai, T. and Tanaka, M. (1991). Comparative study of the ontogeny of the lymphoid organs in three species of marine fish. *Aquaculture* **99**, 143-155.
- Donaldson, E.M. (1981). The pituitary-interrenal axis as an indicator of stress in fish. In "Stress and Fish" (A.D. Pickering, Ed.). pp. 11-48. Academic Press, London.
- Ejike, C. and Schreck, C.B. (1980). Stress and social hierarchy rank in coho salmon. *Trans. Am. Fish. Soc.* **109**, 423-426.
- Fukuhara, O., Nakagawa, T. and Fukunaga, T. (1986). Larval and juvenile development of yellowtail reared in the laboratory. *Nippon Suisan Gakkaishi* **52**, 2091-2098.
- Hwang, P.-P., Wu, S.-M., Lin, J.-H. and Wu, L.-S. (1992). Cortisol content of eggs and larvae of teleosts. *Gen. Comp. Endocrinol.* **86**, 189-196.
- de Jesus, E.G., Hirano, T. and Inui, Y. (1991). Changes in cortisol and thyroid hormone concentrations during early development and metamorphosis in the Japanese flounder, *Paralichthys olivaceus*. *Gen. Comp. Endocrinol.* **82**, 369-376.
- de Jesus, E.G. and Hirano, T. (1992). Changes in whole body concentrations of cortisol, thyroid hormones, and sex steroids during early development of chum salmon, *Oncorhynchus keta*. *Gen. Comp. Endocrinol.* **85**, 55-61.
- de Jesus, E.G., Inui, Y. and Hirano, T. (1990). Cortisol enhances the stimulating action of thyroid hormones on dorsal fin-ray resorption of flounder larvae *in vitro*. *Gen. Comp. Endocrinol.* **79**, 167-173.
- Hiroi, J., Sakakura, Y., Tagawa, M., Seikai, T. and Tanaka, M. (1997). Developmental changes in low-salinity tolerance and responses of prolactin, cortisol and thyroid hormones to low-salinity environment in larvae and juveniles of Japanese flounder, *Paralichthys olivaceus*. *Zool. Sci.* (Tokyo) **14**, in press.
- May, R.C. (1974). Larval mortality in marine fishes and the critical period concept. In "The Early Life History of Fish" (J.H.S. Blaxter Ed.). pp. 3-19. Springer-Verlag, Berlin.
- Noakes, D.L.G. and Leatherland, J.F. (1977). Social dominance and interrenal cell activity in rainbow trout, *Salmo gairdneri* (Pisces, Salmonidae). *Env. Biol. Fish.* **2**, 131-136.
- Peters, G., Deleventhal, H. and Klinger, H. (1980). Physiological and morphological effects of social stress in the eel, (*Anguilla anguilla* L.). *Arch. Fischereiwiss.* **30**, 157-180.
- Pottinger, T.G., Moran, T. A., and Cranwell, P. A. (1992). The biliary accumulation of corticosteroids in rainbow trout, *Oncorhynchus mykiss*, during acute chronic stress. *Fish Physiol. Biochem.* **10**, 55-66.
- Pottinger, T.G. and Mosuwe, E. (1994). The corticosteroidogenic response of brown and rainbow trout alevins and fry to environmental stress during a "critical period". *Gen. Com. Endocrin.* **95**, 350-362.
- Saga, T., Oota, Y., Nazaki, M., and Swanson, P. (1993). Salmonid pituitary gonadotrophs. III. Chronological appearance of GTH I and other adenyhypophysial hormones in the pituitary of the developing rainbow trout (*Oncorhynchus mykiss irideus*). *Gen. Com.*

- Endocrin.* **92**, 233-241.
- Sakakura, Y. and Tsukamoto, K. (1996). Onset and development of cannibalistic behaviour in early life stage of yellowtail. *J. Fish Biol.* **48**, 16-29.
- Sakakura, Y. and Tsukamoto, K. (1997a). Effects of water temperature and light intensity on the aggressive behavior in the juvenile yellowtails. *Fish. Sci* (Tokyo). **63**, 42-45.
- Sakakura, Y. and Tsukamoto, K. (1997b). Age composition in the schools of juvenile yellowtail, *Seriola quinqueradiata*, associated with drifting seaweeds in the East China Sea. *Fish. Sci.* (Tokyo) **63**: 37-41.
- Sakakura, Y. and Tsukamoto, K. (1997c). Ontogeny of aggressive behaviour in schools of the migratory marine fish, yellowtail *Seriola quinqueradiata*. In "Proceedings from the First International Workshop of the Fish Ontogeny Network of Europe (FONE)" (G.H. Copp and V. Kovac, Eds). pp. 16. FONE, Bratislava.
- Schreck, C.B. (1981). Stress and compensation in teleostean fishes: response to social and physical factors. In "Stress and Fish" (A.D. Pickering, Ed.), pp. 295-321. Academic Press, London.
- Shaw, E. (1962). The schooling of fishes. *Scient. Am.* **66**, 166-175.
- Shiozawa, S. (1997). Seedling production of yellowtail at Goto station. *Annual Report of Japan Sea Farming Association.* **H7**, 157-159. (in Japanese)
- Tanaka, M., Tanagonan, J.B., Tagawa, M., de Jesus, E.G., Nishida, H., Isaka, M., Kimura, R. and Hirano, T. (1995). Development of the pituitary, thyroid and interrenal glands and applications of endocrinology to the improved rearing of marine fish larvae. *Aquaculture.* **135**, 111-126.
- Uchida, K., Dotu, Y., Mito, S. and Nakahara, K. (1958). The spawning and early life history of "Buri", Japanese yellow tail, *Seriola quinqueradiata* Temminck et Schlegel. *Sci. Bull. Fac. Agr., Kyushu Univ.* **16**, 329-342.
- Youson, J. H. (1988). First metamorphosis. In "Fish Physiology" (W. S. Hoar and D. J. Randall, Eds.), Vol. 11, pp. 135-196. Academic Press, San Diego.

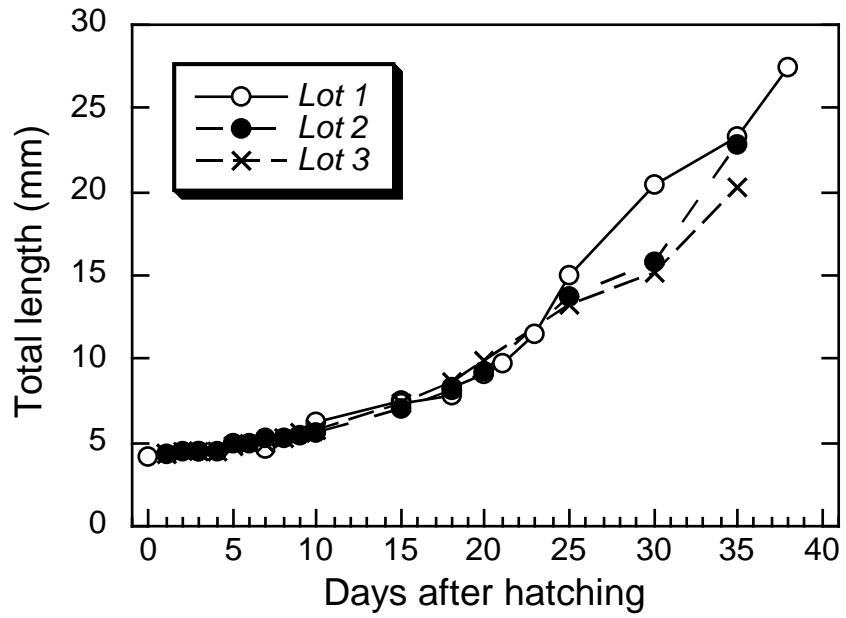


FIG. 1. Growth pattern of the yellowtail. Each point represents the mean total length of 30 fish (open circle, Lot 1, year 1993; closed circle, Lot 2, the first lot of year 1994; cross, Lot 3, the second lot of year 1994).

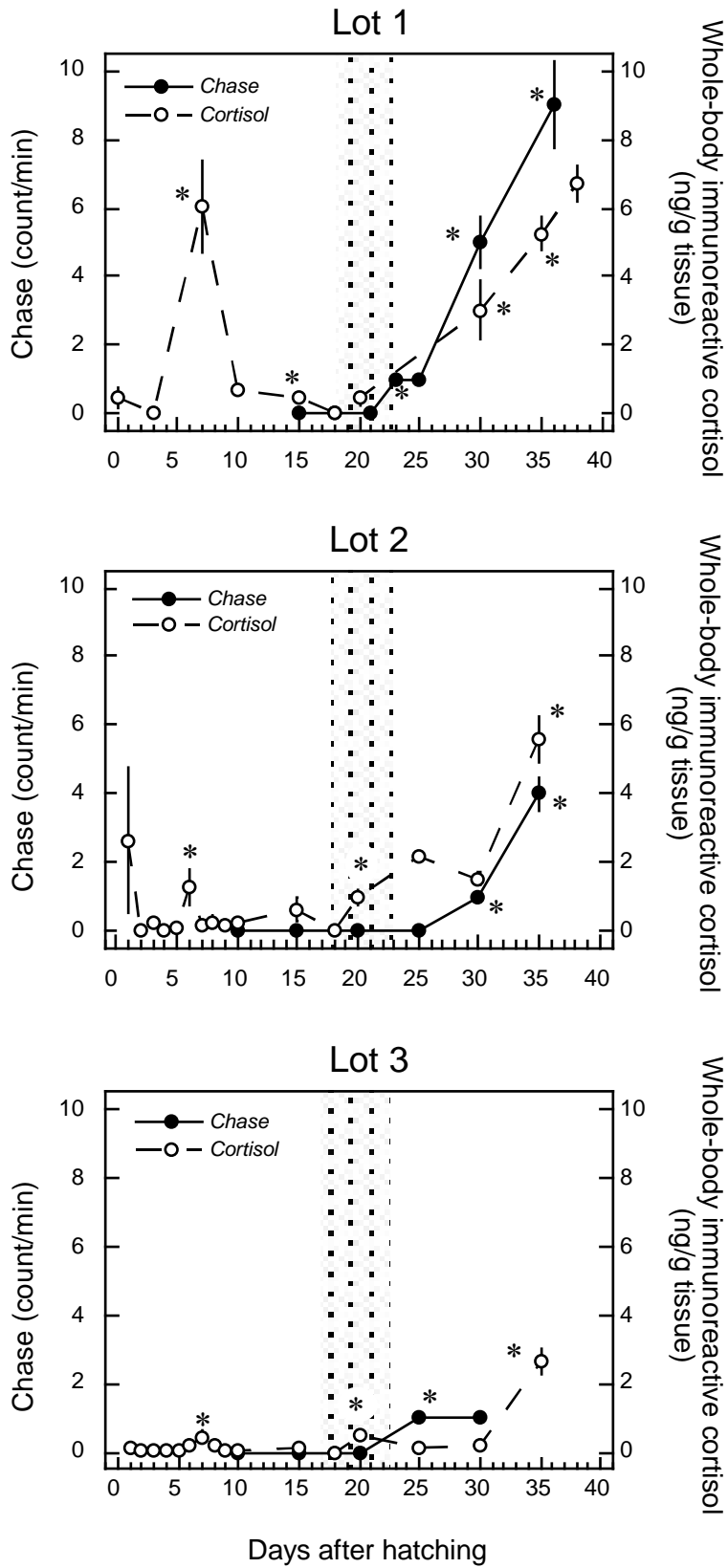


FIG. 2. Changes in frequency of chase behavior (closed circles) and whole-body immunoreactive cortisol concentrations (open circles) in early life stages of yellowtail (upper, Lot 1, year 1993; middle, Lot 2, the first lot of year 1994; bottom, Lot 3, the second lot of year 1994). Vertical bars represent quartile deviation of medians for closed circles ($n = 3$), and standard errors of the means for open circles ($n = 3 < \text{day } 10$; $n = 5 < \text{day } 20$; $n = 20 \geq \text{day } 20$). An asterisk indicates a significant difference ($p < 0.05$) from the previous point by the Mann-Whitney U test or Duncan's new multiple range test. Shaded areas represent time of metamorphosis (transition from larva to juvenile).

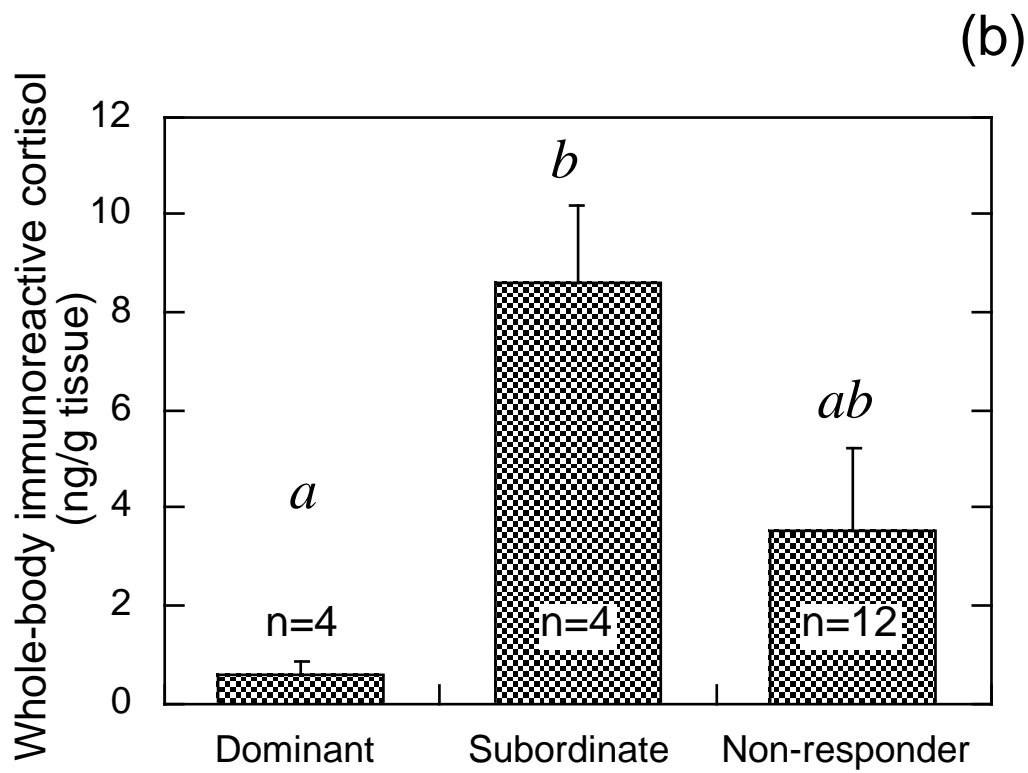
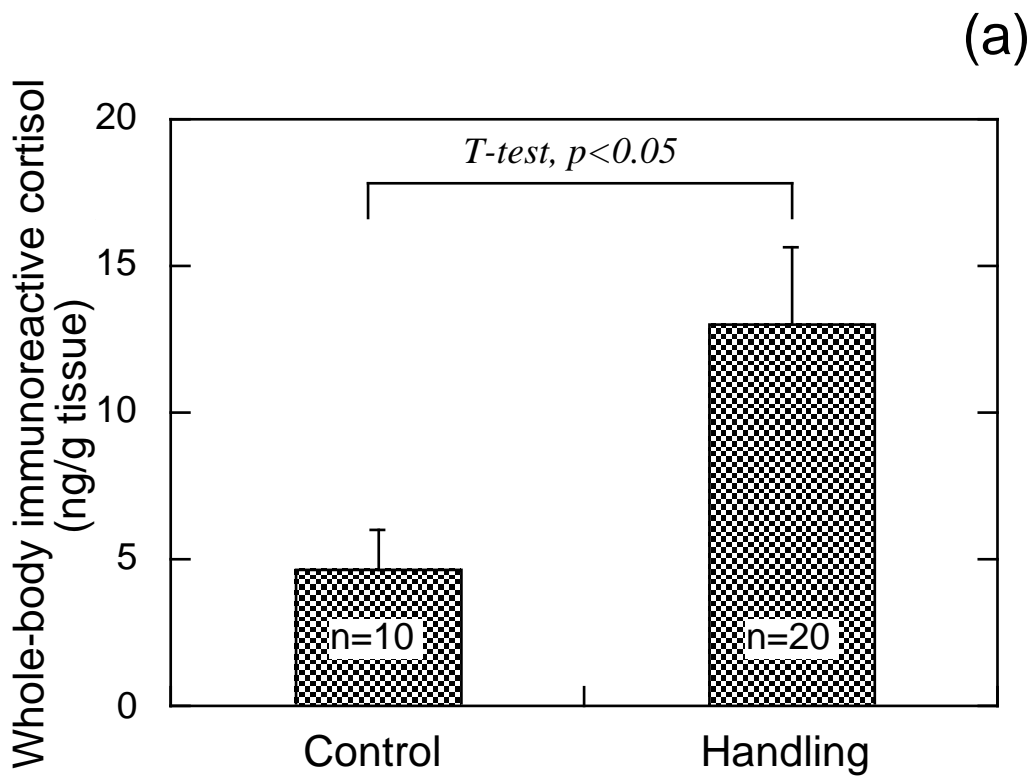


FIG. 3. (a) Whole-body immunoreactive cortisol (IRC) levels before ("Control") and after handling stress ("Handling"). (b) IRC levels in relation to social rank; "Dominant" = aggressive fish, "Subordinate" = attacked by aggressive one and "Non-responder" = no agonistic interactions. Vertical bars represent standard errors of means, and different letters indicate significant differences ($p < 0.05$; Duncan's new multiple range test).