

The use of scale increment as a means of indicating fish growth and growth impairment

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Abstract

Growth impairments were studied for three juvenile fish species: the red drum (*Sciaenops ocellatus*), the orange-spotted grouper (*Epinephelus coioides*) and the marine medaka (*Oryzias latipes*). Fish were subjected to long-term (i) food deprivation, (ii) exposure to benzo[*a*]pyrene, (iii) hypoxia, respectively. Fish growth (fork length and body weight) and scale increments (circulus number and radius length) were measured. Results of Pearson correlation analysis showed a significant positive correlation between scale increment and growth for the red drum (fast growing) and the marine medaka (small sized) ($r=0.741-0.882$, $p<0.001$). For the slow growing orange-spotted grouper, a significant correlation was found only between body weight and circulus number ($r=0.406$, $p<0.05$). Overall, scale circulus number is more consistently linked to fish growth than that of radius length. Importantly, our findings demonstrate that daily circulus deposition on scales of fast-growing red drum is feasible to indicate daily growth of the fish. Moreover, scale increment exhibited the strongest correlation with fish growth as compared to that of serum T₃ level and muscle RNA:DNA ratio. Our data support the use of scale increment as a means of indicating fish growth and growth impairment, particularly for the fast growing fish.

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1. Introduction

A major challenge in current fisheries and ecotoxicological research is to identify a suitable biological response (biomarker) for indicating fish growth, which should be sensitive, specific, reproducible, non-lethal,

easy to determine and cost-effective. Changes of body weight and body length are the direct means of indicating actual fish growth. However, despite being non-lethal, simple and inexpensive to measure, the use of biomass change as an endpoint often requires a relatively long experimental period (Wo et al., 1999). Conversely, biochemical changes such as muscle RNA:DNA ratio (Buckley, 1984; Bulow, 1987; Clemmesen, 1996) and serum 3,5,3'-triiodothyronine (T₃) hormone level (May, 1978; Klandorf and Harvey, 1985; Scanes

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and Griminger, 1990) provide relative simple, indirect means for estimating recent growth in fish. T_3 has a stimulatory effect on the somatotrophic axis (Wong et al., 2006) and has been shown more sensitive in response to food deprivation (Blake et al., 2006); it is often measured over growth hormone (GH) as the hormonal parameter for growth studies. However, given that fish sacrifice is inevitable, biochemical assays are not always suitable for studying growth of rare or endangered species, and not feasible for continued observation of fish growth under prolonged experimental conditions. Additionally, these biochemical methods are not sensitive enough to indicate daily growth of fish.

Fish scales are bony structures that grow shingle-like from pockets within the skin (Schneider et al., 2000). As fish grow the scales also increase in size, producing characteristic circuli (growth rings) at the scale margin, which can be examined and quantified directly using a light or scanning electron microscope. Once deposited on the scale, the circulus remains unchanged over the entire life span of the scale (Sire and Arnulf, 2000; Sire and Akimenko, 2004). Scales of wild fishes have been explored for age determination and back-calculation of growth history (Carlander, 1987; Schneider et al., 2000) and scale radius length found to be linearly proportional to the body length of *Pomacanthus imperator* (Chung and Woo, 1999). The quantitative relationship between scale circulus deposition and fish growth remains to be corroborated, and there remains a paucity of information on the relationship between abnormal scale increments and growth impairment in stressed fish (Kobayashi et al., 1972; Jones, 1986; Fisher and Pearcy, 2005). Daily deposition of scale circulus has only been reported for the juvenile *Chrysophrys auratus* (Kingsford and Atkinson, 1994). It remains uncertain whether daily circulus deposition is common for scales of other fish species or if it can provide a feasible means of indicating daily growth patterns or impairment.

Food deprivation, toxic polycyclic aromatic hydrocarbons (PAHs) and hypoxia are environmental problems worldwide and have been shown to impair growth for several fish species including the red drum, *Sciaenops ocellatus* (Smith and Fuiman, 2004), the orange-spotted grouper, *Epinephelus coioides* (Heintz et al., 2000; Yuen and Au, 2006), and the marine medaka, *Oryzias melastigma* (Pichavant et al., 2000; Yu et al., 2006). In the present study, red drum, orange-spotted grouper and marine medaka of different body size, growth rate and scale type were subjected to these three chronic stress conditions: (i) food deprivation, (ii) benzo [*a*]pyrene (a model PAH compound), or (iii) hypoxia, respectively. Changes of body growth parameters (fork

length and body weight) were measured and correlated with scale increment (circulus number and radius length). The sensitivity of these scale parameters for indicating fish growth was compared with muscle RNA: DNA ratio and serum T_3 hormone level for the red drum. The use of scale circulus deposition will be a highly promising indicator of fish growth if scale circulus deposition can be quantitatively linked to daily growth and growth impairment of the fish, regardless of fish species and stress conditions.

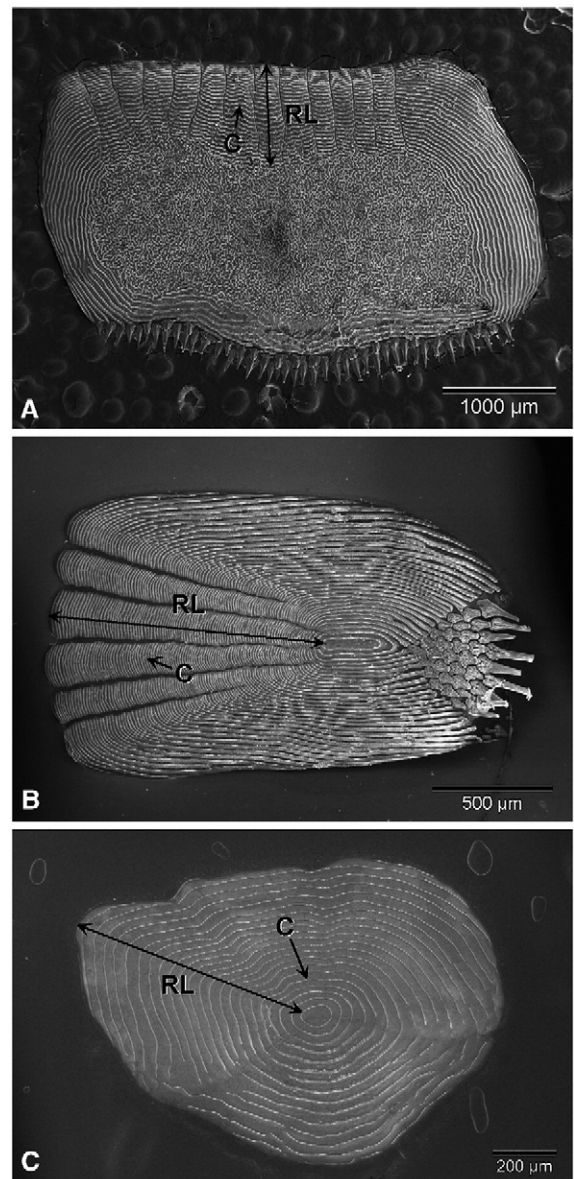


Fig. 1. Ctenoid scales of (A) red drum *Sciaenops ocellatus* and (B) orange-spotted grouper *Epinephelus coioides*. Cycloid scale of (C) marine medaka *Oryzias melastigma*. RL = radius length; C = circulus.

2. Materials and methods

2.1. Chronic food deprivation experiment

Fifty juvenile red drum *S. ocellatus* (mean fork length: 20.2 ± 1.9 cm; mean wet weight: 92.5 ± 17.3 g) were obtained from a commercial hatchery and subjected to normal feeding or food deprivation for 5 weeks at a fish farm in the pristine Kat O Fisheries Substation of the Agriculture, Fisheries and Conservation Department (AFCD), Hong Kong. Fish were acclimated for 7 days prior to experimentation. During acclimation, fish were fed to satiation daily with moist pellet feeds. According to and provided by AFCD, the use of moist pellet feed (comprised of trash fish, vitamins and binders with 40% protein and 20% lipid) can significantly improve both feed efficiency as well as reduce pollution. Fish were randomly divided into the fed and unfed groups using two separated net cages ($1.5 \text{ m} \times 1.5 \text{ m} \times 1.5 \text{ m}$). Fish in the fed group were fed to satiation with moist pellets while no food was given to the unfed group. Throughout the experimental period, seawater temperature ranged between 28 and 30 °C with pH 7.8.

Five to ten fish were sampled at week 0, 1, 2, 3, 4, 5 for growth and scale measurements. A total of 91 fish samplings were performed ($n=10$ at week 0; $n=43$ and $n=38$ for the fed and unfed groups, respectively). For each fish collected, fork length and wet weight were measured (to the nearest 0.1 cm and 0.1 g, respectively). Scales were collected simultaneously from below the lateral line and from the middle of spiny dorsal fin, placed into an Eppendorf containing distilled water and stored at 4 °C.

For scale examination, sticky scales were separated under a dissection microscope (Carl Zeiss Stemi SV 11, Germany) prior to ultrasonic cleaning (twice for 4 min each) to remove mucus and epidermal tissues covering

the surface. Cleaned scales were air-dried, mounted on microscopic slides and examined under an Axioplan 2 Imaging Compound Microscope (Carl Zeiss, Germany). For each fish scale, the middle radii were captured at 400× magnification. The average circulus number and average radius length (μm) were measured using the AnalySIS 3.2 software program (Soft Imaging System, Germany). Since scales of red drum, grouper and medaka are different in types and shape, the definition of radius length measurement varied for each scale type and is indicated in Fig. 1. Each scale datum of individual fish was an average of 10 scales.

At week 0, 2 and 5, four to five fish were scarified from each group for RNA:DNA ratio and T_3 analyses. For RNA:DNA ratio analyses, white muscle was dissected from the dorsal-anterior of the fish, immediately frozen in liquid nitrogen and stored at -80 °C prior to analysis ($n=22$). Measurement of RNA:DNA ratio followed the method of Foss and Forbes (1997) and details of procedures were described in Wu et al. (2003). For T_3 analyses, blood was collected from the caudal vein using a hematocrit capillary tube and centrifuged for 10 min at $3000 \times g$ at 4 °C ($n=17$, insufficient blood sample obtained from 5 fish). Serum was collected and stored at -80 °C prior to T_3 hormone assay using the Monobind competitive enzyme immunoassay kit (catalog number 1325-300, Costa Mesa, CA, USA).

2.2. Chronic foodborne exposure to B[a]P

Thirty juvenile orange-spotted grouper, *E. coioides*, (mean fork length: 12.2 ± 1.0 cm; mean wet weight: 28.5 ± 3.6 g) were collected from a local mariculture farm. Fish were randomly divided into two groups. Fish in both groups were force-fed daily by introducing the AFCD moist pellet feed (diameter ca. 5 mm equivalent to 2% of body weight) directly into the pharynx through the sphincter of the esophagus using a glass rod. In this study,

Table 1

Daily change of (a) fork length, (b) body weight, (c) circulus number and (d) radius length in red drum *Sciaenops ocellatus*, orange-spotted grouper *Epinephelus coioides* and marine medaka *Oryzias latipes* under normal and stress condition

Treatments		Fish species					
		<i>Sciaenops ocellatus</i> (S.o.)		<i>Epinephelus coioides</i> (E.c.)		<i>Oryzias melastigma</i> (O.m.)	
		Fed	Unfed	Control corn oil	B[a]P	Normoxia	Hypoxia
Fish Growth Rate	Fork length (mm/day)	1.57	0.10***	0.06	0.01	0.25	0.22
	Body weight (mg/day)	3708.57	325.86***	385.57	0.00	4.72	3.50***
Scale increment growth rate	Average circulus number (unit/day)	1.01	0.26***	0.19	0.00	0.15	0.10*
	Average radius length ($\mu\text{m}/\text{day}$)	33.53	2.75***	0.00	0.00	7.02	5.88

Data made with asterisk show significant different between treatment groups (* $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$).

fish were exposed to an environmentally realistic dose of a carcinogenic PAH, benzo[*a*]pyrene (B[*a*]P) for 4 weeks using the diet as the delivery mechanism. For the B[*a*]P treatment group, each pellet was injected with 250 µg B[*a*]P in corn oil so that the pellet was designed to provide 12.5 µg B[*a*]P/fish g of group per day (Wu et al., 2003; Au et al., 2004). For the control group, pellets were injected with corn oil only. The concentrations of B[*a*]P in the pellets were measured using the

method described by Xu et al. (1999). These were 2.78 ± 0.51 µg and 587.07 ± 13.14 µg B[*a*]P/pellet for corn oil control and B[*a*]P treatments, respectively. The grouper were reared in a 30 L plexiglass tank with 20 L of seawater at 20 ± 0.1 °C, $30 \pm 2\%$ salinity, pH 7.8 ± 0.5 , with full aeration. Eighty percent of seawater in aquaria was exchanged every 24 h to prevent the building up of wastes and ammonia. Five fish from each group were sampled at the end of exposure week 1 and 4 for fork

Sciaenops ocellatus
(S.o.)

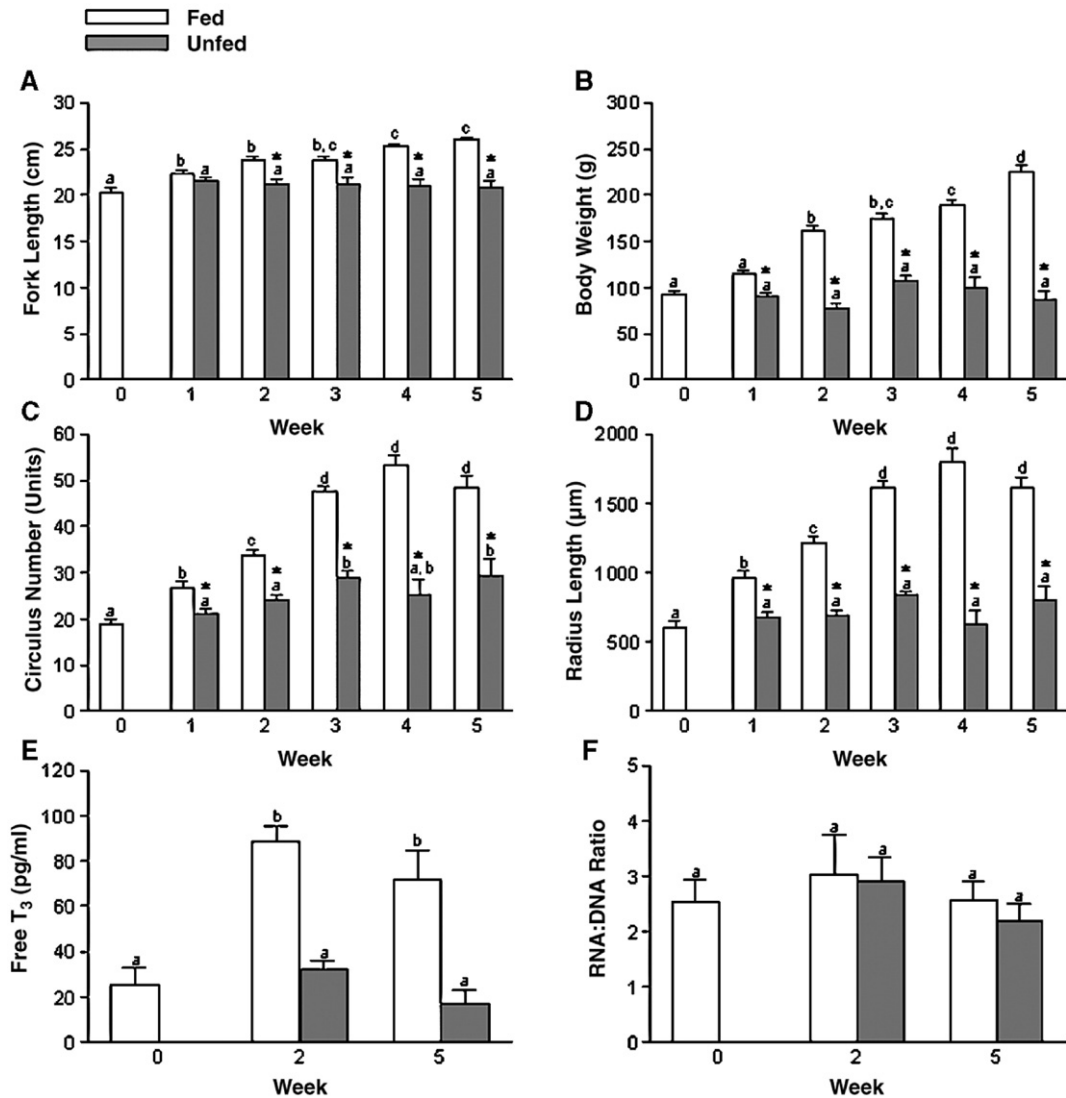


Fig. 2. Red drum *Sciaenops ocellatus*. Changes of (A) fork length, (B) body weight, (C) average circulus number, (D) average radius length, (E) serum free T₃ and (F) muscle RNA:DNA ratio in fish to normal or no feeding for up to 5 weeks. Data are expressed as the mean ± standard error of mean, $n = 10$ for (A) to (D); $n = 5$ for (E) and (F). Data made with same letter are not significantly different from each other whereas data made with an asterisk show significant differences within each time point by Tukey test ($p < 0.05$).

length, wet weight and scale measurements (as described in Section 2.1).

2.3. Long-term hypoxia experiment

In this study, 100 juvenile marine medaka *O. melastigma* (ca. 1.7 ± 0.2 cm) were divided into 2 groups. The first group was maintained in a hypoxic system at 1.8 ± 0.2 mg O₂ l⁻¹ (under 28 ± 0.5 °C; 14:10 h light dark cycle, pH 7–8, 7 mg O₂ l⁻¹, 30‰ salinity, 0.3 L water per fish). The control group was maintained in a normoxic system (7.0 ± 0.2 mg O₂ l⁻¹). Details of these systems are depicted in Zhou et al. (2001). Ten fish

were sampled from the normoxic and hypoxic groups after 4, 8 and 12 weeks to study changes in fork length and body weight. For the cycloid scale of marine medaka, circulus number was measured as described in Section 2.1, and the radius length was represented by scale length which is the longest distance measured from the centre of the scale to the margin.

2.4. Statistical analysis

Tests for homogeneity were first conducted using Bartlett's test, if necessary, data were normalized before analysis of variance. Two-way analysis of variance

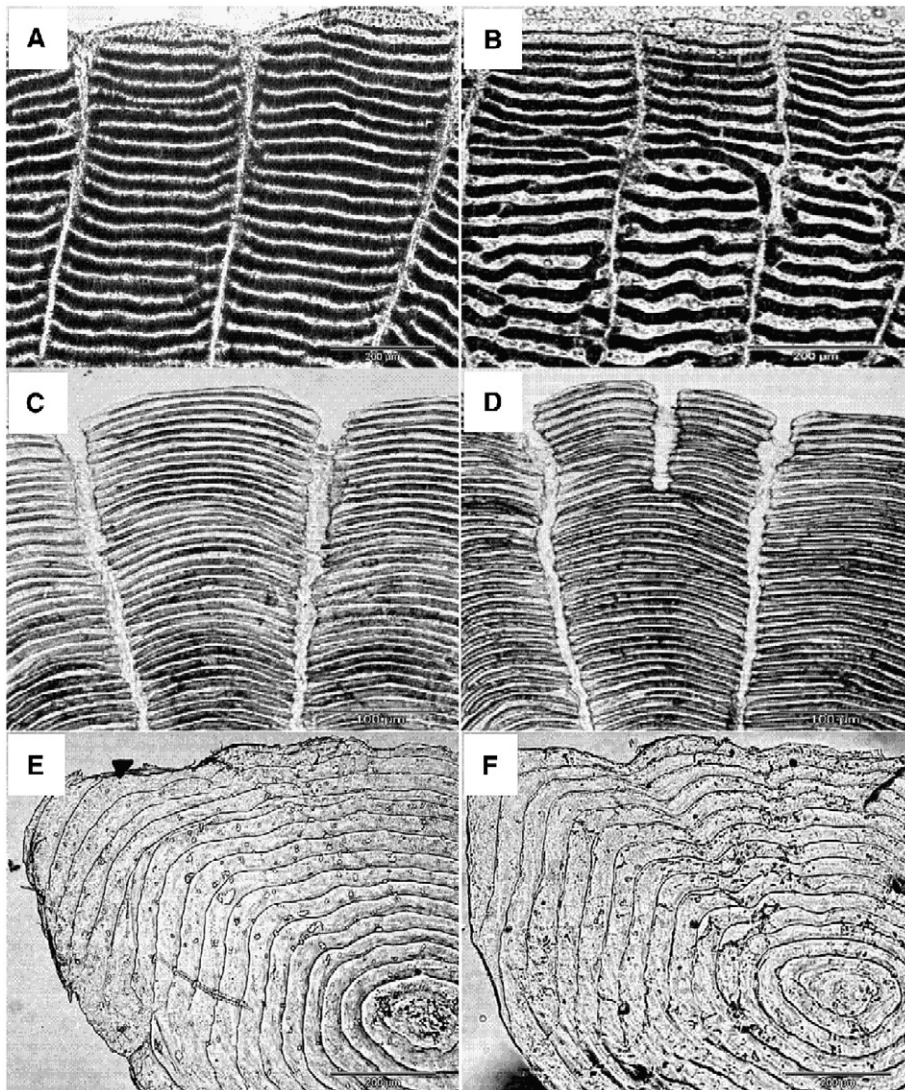


Fig. 3. Scale circulus pattern of red drum *Sciaenops ocellatus* subject to (A) normal feeding and (B) unfed for 4 weeks; orange-spotted grouper *Epinephelus coioides* upon exposure to (C) control corn oil and (D) B[a]P for 4 weeks; and *Oryzias melastigma* upon exposure to (E) normoxia and (F) hypoxia for 12 weeks.

(ANOVA) was used to test the effects of different treatments, duration of exposure and their interactions on different parameters measured: scale parameters (circulus number and radius length), physical parameters (fork length and body weight) and biochemical parameters (muscle RNA:DNA ratio and serum T₃). Where significant differences were identified among levels of the fixed-effects factors, *post-hoc* multiple comparisons between treatment groups/time intervals were carried out using a Tukey test. Pearson correlation analysis was used to determine the relationship between fork length and body weight with scale parameters (circulus number and radius length) as well as biochemical parameters (muscle RNA:DNA ratio and serum T₃). Significance level (α) was set at 0.05 level (Zar, 1996). Statistics were performed using the statistical software (Systat Sigma-Stat, 3.0.1, California, USA). Linear regression analysis was used to calculate the rate of change for each measured parameter over time.

3. Results

The majority of teleosts possess ctenoid and cycloid scales (Sire and Akimenko, 2004). The ctenoid scales in red drum and grouper are characterized by a serrated edge in the posterior margin (Fig. 1A–B). The cycloid scale in marine medaka is distinguished by a smooth posterior margin with circuli radiating from the central (Fig. 1C). Among the 3 juvenile fish species studied, body size in terms of fork length is the longest for red drum *S. ocellatus* (approx. 20 cm), followed by the orange-spotted grouper *E. coioides* (approx. 12 cm) and the marine medaka *O. melastigma* (approx. 1.7 cm). Under experimental control conditions the average growth rate (as represented by daily increase of fork length) is the fastest for the red drum (1.57 mm/day), followed by the small medaka (0.25 mm/day) and the slowest for the orange-spotted grouper (0.06 mm/day) (Table 1).

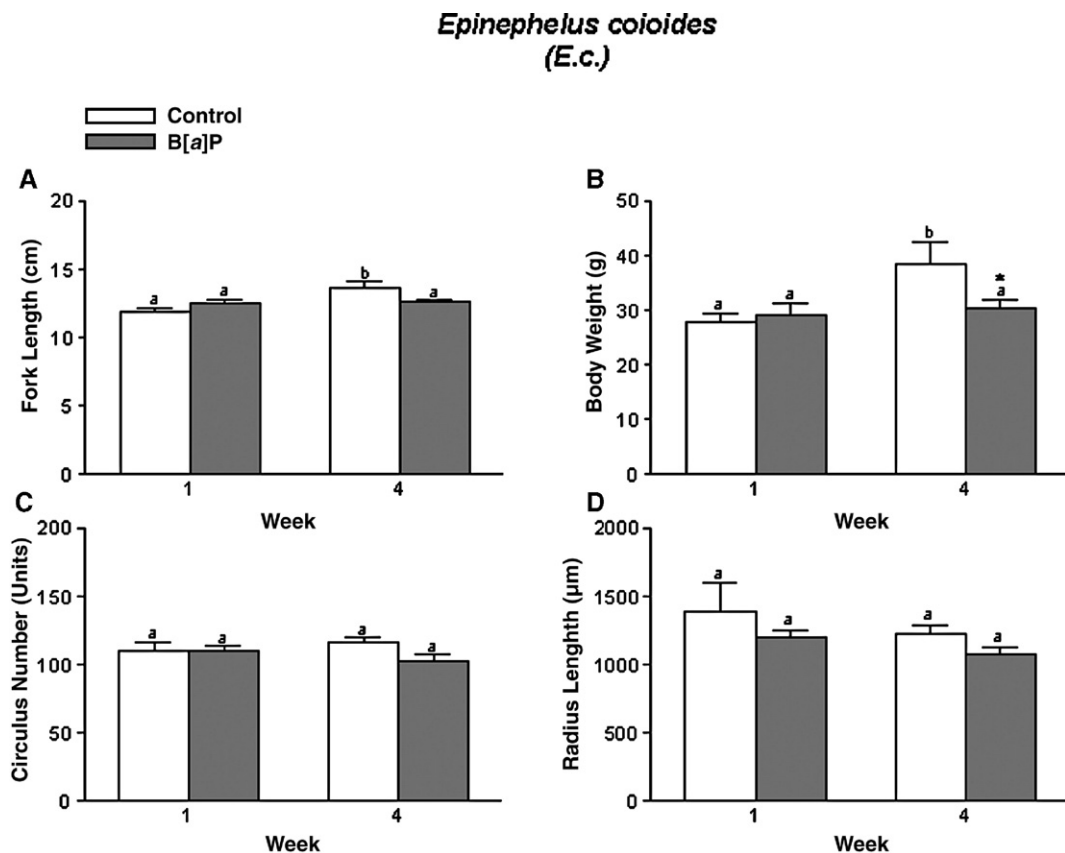


Fig. 4. Orange-spotted grouper *Epinephelus coioides*. Changes of (A) fork length, (B) body weight, (C) average circulus number and (D) average radius length in fish subject to control corn oil and B[a]P treatments for up to 4 weeks. Data are expressed as the mean \pm standard error of mean ($n=5$). Data made with same letter are not significantly different from each other whereas data made with an asterisk show significant differences within each time point by Tukey test ($p<0.05$).

3.1. Red drum subject to chronic food deprivation

In the fed group, fork length, body weight, circulus number and radius length were significantly increased over time (Fig. 2A–D). Food deprivation significantly impaired growth of red drum. No significant changes of fork length and body weight were detected in the unfed fish over the study period ($p < 0.05$) (Fig. 2A–B; Table 1). A daily deposition of circulus (1.01/day) and a concomitant increase of average radius length at a rate of 33.53 $\mu\text{m}/\text{day}$ were detected in the fed red drum (Table 1). The unfed red drum showed insignificant changes of radius length over the study period (Fig. 2D) and only a mild increase of scale circulus number was detected after week 3 (Fig. 2C). Significant differences between the fed and unfed fish were detected at week 1 for body weight, average circulus number and radius length, and at week 2 for fork length (Fig. 2A–D). Circulus deformations (e.g. incomplete and/or undulat-

ing circulus depositions) on radii were often observed on scales of food deprived red drum (Fig. 3A–B).

For biochemical parameters, serum T_3 in the fed red drum was significantly higher in the fed group at weeks 2 and 5 as compared to the unfed group (Fig. 2E). No significant change in RNA:DNA ratio was detected for both fed and unfed groups throughout the experimental period (Fig. 2F).

3.2. Orange-spotted grouper exposed to toxicant B[a]P

A mild but significant increase of fork length and body weight was detected in the control groupers over the study period (Fig. 4A–B). The B[a]P exposed groupers however showed no body growth as measured by fork length and body weight (Fig. 4A–B, Table 1). For the two scale parameters, no significant difference was detected between the control and B[a]P fish throughout the experimental period (Fig. 4C–D).

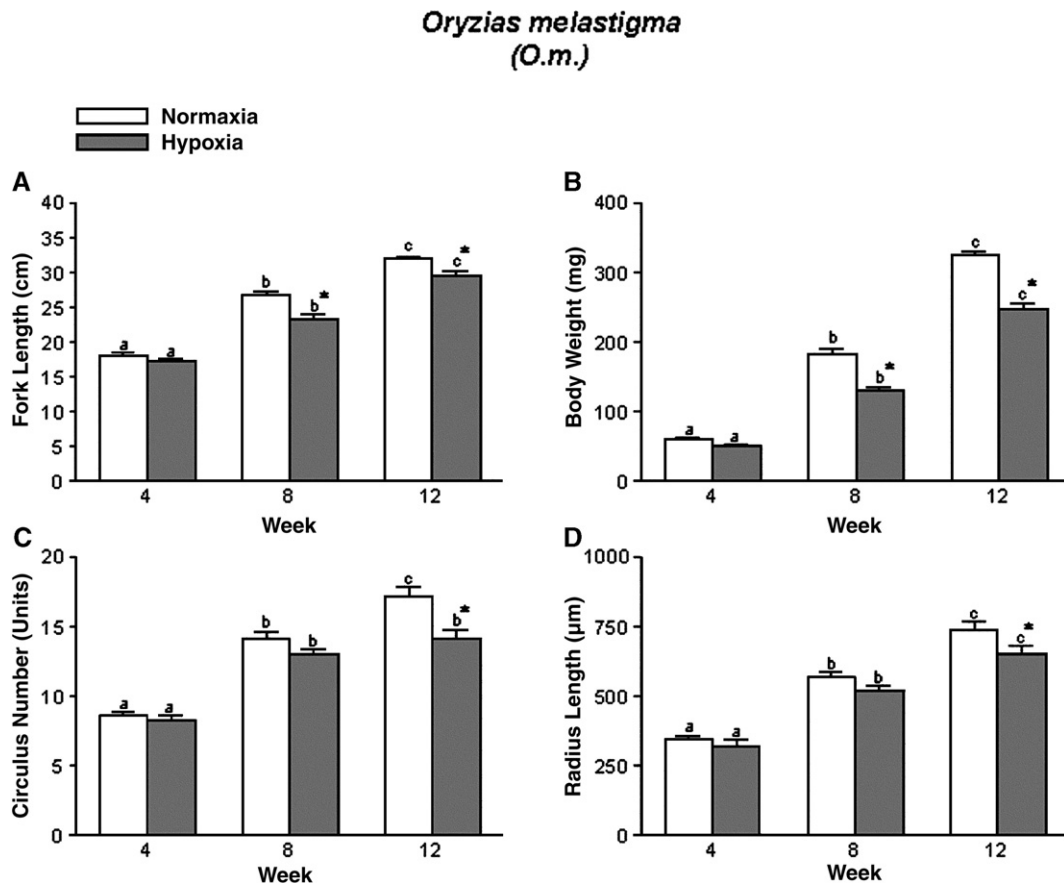


Fig. 5. Marine medaka *Oryzias melastigma*. Changes of (A) fork length, (B) body weight, (C) average circulus number and (D) average radius length in fish subject to normoxia and hypoxia for up to 12 weeks. Data are expressed as the mean \pm standard error of mean ($n = 10$). Data made with same letter are not significantly different from each other whereas data made with an asterisk shows significant differences within each time point by Tukey test ($p < 0.05$).

Table 2

Pearson correlation coefficient (r) between fish growth and scale increments and biochemical growth parameters for red drum *Sciaenops ocellatus* (S.o.); marine medaka *Oryzias melastigma* (O.m.) and orange-spotted grouper *Epinephelus coioides* (E.c.)

		Fish Species					
		<i>Sciaenops ocellatus</i> (S.o.)		<i>Epinephelus coioides</i> (E.c.)		<i>Oryzias melastigma</i> (O.m.)	
Fish growth		Fork length	Body weight	Fork length	Body weight	Fork length	Body weight
Scale increment parameters	Circulus number	0.741*** (n=91)	0.818*** (n=91)	0.340 (n=20)	0.406* (n=20)	0.767*** (n=60)	0.720*** (n=60)
	Radius length	0.794*** (n=91)	0.865*** (n=91)	0.0743 (n=20)	0.170 (n=20)	0.882*** (n=60)	0.855*** (n=60)
Biochemical parameters	Free T ₃	0.638*** (n=17)	0.768*** (n=17)	–	–	–	–
	RNA:DNA ratio	0.223 (n=22)	0.150 (n=22)	–	–	–	–

Significant correlations are indicated with asterisk (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Separation of radii was often found in the scales of B[a] P groupers (Fig. 3B, D).

3.3. Marine medaka under long-term hypoxic stress

The normoxic and hypoxic fish showed significant increases in fork length and body weight (Fig. 5A–B) as well as scale circulus number and scale length (Fig. 5C–D) over time. Rates of change of body weight and average circulus numbers in hypoxic medaka were significantly slower as compared to the normoxic fish (Table 1). We observed no major difference in circulus pattern between the normoxic or hypoxic fish scales (Fig. 3D, E).

3.4. Pearson correlation analysis of fish growth, scale increment and biochemical growth parameters

A positive significant correlation was found between fork length and body weight with circulus number and radius length ($r = 0.741–0.882$, $p < 0.001$) for both red drum and marine medaka. For the orange-spotted grouper, a significant correlation was found between circulus number and body weight ($r = 0.406$, $p < 0.05$). Circulus number was consistently correlated with body weight for all the 3 species used in this study, in the descending order: red drum > marine medaka > orange-spotted grouper ($r = 0.818–0.406$). For biochemical parameters, serum T₃ level, but not RNA:DNA ratio, was significantly correlated with fork length and body growth of red drum ($r = 0.638–0.768$, $p < 0.01$) (Table 2).

4. Discussion

The findings of this study demonstrate that daily circulus deposition in the fast-growing red drum is a suitable mechanism for assessing daily growth of the fish. Our results also show that reduction of scale

increment (circulus deposition and radius length) in unfed red drum were more responsive (detectable at week 1) than reduction of body length (detectable at week 2), suggesting that scale increment is highly sensitive for indicating growth impairment in fish. Indeed, a decline of growth rate in unfed red drum is coupled with a reduction of scale increment rate. Moreover, a significant positive correlation was consistently found between fish growth (fork length and body weight) of red drum and scale increment (circulus number and radius length) ($r = 0.741–0.865$, $p < 0.001$). Similar positive correlations have been reported for fast-growing salmonid species including sockeye salmon (*Oncorhynchus nerka*) (Bilton and Robins, 1971; Bilton, 1975; Fukuwaka and Kaeriyama, 1997), chum salmon (*Oncorhynchus keta*) (Healey, 1982) and coho salmon (*Oncorhynchus kisutch*) (Fisher and Percy, 1990, 2005). Positive correlations are not limited to salmonids and have been previously established in other fish with relatively high growth rates such as the walleye (Glenn and Mathias, 1985) and Tilapia (Talbot and Doyle, 1992). However, for the slow-growing orange-spotted grouper, such relationships were less promising with a positive correlation established only between body weight and average circulus number ($r = 0.406$, $p < 0.05$). For the small marine medaka, a significant positive relationship was generally established between fish growth and scale increment ($r = 0.767–0.882$, $p < 0.001$). These results suggest that there may be a potential limitation of using scale increment as an indicator of fish growth for slower growing species.

Between the two scale parameters studied, the rate of circulus deposition was significantly correlated with body weight for all the three fish studied (Table 1). In red sea bream and coho salmon, circulus deposition rate also exhibited a good correlation with growth rate (Arai and Sakamoto, 1995; Fisher and Percy, 2005). Taken together, circulus number may be more useful than the

radius length for indicating growth of fish. We observed circulus malformation (*e.g.* incomplete and/or undulating circulus deposition) on starved red drum and separation of scale radii in B[a]P fed orange-spotted grouper, which is in agreement with the findings of Schneider et al. (2000) reported for other starved fish. Further correlations between circulus formation and food availability have been observed in laboratory experiments on the cutthroat trout (*O. clarkia*) (Bigelow and White, 1996). If the pattern of circulus deformation is stress specific, more precise information on growth history of fish can be gained through examination of fish scale circulus patterns. This may confer additional advantages to studying growth and growth impairment of threatened and endangered fish species *in situ* and could provide valuable insights towards the identification of optimal conditions that could accelerate growth of selected fish species in culture.

Collection and counting of scale increment is fast and not technically difficult, precludes unnecessary fish kills and is highly cost-effective. Moreover, compared to other biochemical parameters for indirect measure of fish growth such as serum T₃ level and muscle RNA:DNA ratio, scale increment in red drum was found to be the most responsive and exhibited the strongest correlation with realized body growth. The results strongly support the use of scale increment as a biomarker for fish growth. Further studies on stress specific circuli pattern abnormalities and their quantitative relationship to growth impairment will be essential to ascertain the suitability of using circuli deposition as a growth biomarker to indicate the effects of environmental stress.

In conclusion, the findings of this study provide quantitative evidence to substantiate a significant positive correlation between scale increment and fish growth. The number of circulus is a promising indicator of fish growth. Scale increment exhibited a stronger correlation with fish growth than serum T₃ level and muscle RNA:DNA ratio and for the fast growing red drum, daily circuli deposition on fish scales was shown to be a feasible means of indicating daily growth and growth impairment.

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