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Changes in Oxygen Consumption Rate during Development of Larval Japanese Whiting, *Sillago japonica*

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Abstract Oxygen consumption rates of larval Japanese whiting, *Sillago japonica*, were examined at various developmental stages from hatching (Day 0) to just before the juvenile stage (Day 28). The oxygen consumption rate per larva increased exponentially with larval age throughout development. The weight-specific oxygen consumption rate increased with age from hatching to Day 13, subsequently decreasing to Day 28, being subject to diurnal variation throughout, with higher rates during daytime. The latter were more variable than nighttime rates, which were almost equal to the values observed at hatching.

Oxygen consumption rates of fishes have been regarded as an important physiological indicator of metabolic activity (Fry, 1957; Rombough, 1988). The oxygen consumption rates of fish larvae vary according to developmental stage (Lasker and Theilacker, 1962; Holliday et al., 1964; De Silva and Tytler, 1973; Houde and Schekter, 1983; Morioka, 1985; Oikawa and Itazawa, 1985, 1992, 1993; De Silva et al., 1986; Higano and Yasunaga, 1986; Oikawa et al., 1991), most of the above studies having focused on the egg to yolk absorption stage (Lasker and Theilacker, 1962; Holliday et al., 1964; Houde and Schekter, 1983), or from the post-larval to fry stage (Morioka, 1985; Higano and Yasunaga, 1986). Although marked morphological changes occur during the larval period, little is known about ontogenic changes in the oxygen consumption rate (Oikawa and Itazawa, 1984, 1985, 1992, 1993; Oikawa et al., 1991). Oikawa et al. (1991) presented a triphasic relationship between oxygen consumption of an individual fish and body weight, using *Pagrus major* larvae. However, they also mentioned the possibility of species-dependent relationships of metabolism and size in the larval stages of different fish species (Oikawa et al., 1991).

Marine fish larvae are visual predators that actively search for prey during the daytime. Because both feeding and swimming behavior vary in intensity from hatching to the juvenile period, diel variation in the oxygen consumption rate should be considered relative to such activities. Few studies have examined diel patterns in the oxygen consumption rate in marine fish larvae (Holliday et al., 1964; De Silva and Tytler, 1973), whereas diel variation has been reported in detail in freshwater fish fry (Brett and Zala, 1975).

In this paper, the oxygen consumption rates of larval Japanese whiting, *Sillago japonica* (Temminck and Schlegel), were determined in relation to development from hatching to just before the juvenile stage. Diurnal variations in consumption rate were also examined.

Materials and Methods

Measurements of oxygen consumption rate were conducted in August and September 1985, at the Fisheries Laboratory, University of Tokyo. Naturally-spawned eggs of the Japanese whiting, *Sillago japonica*, were collected from brood stock tanks (Oozeki and Hirano, 1985), and larvae reared in a 1,000*l* polycarbonate tank at 25.1±1.0°C, being fed with a small-type rotifer, *Brachionus plicatilis* (S-type; Fu et al., 1991a, b), from 2 days post-hatching (Oozeki et al., 1992).

Oxygen consumption rates were determined under conditions of darkness throughout, using a differential gas-volumeter (Yokohama and Ichimura, 1969) at 26.0±0.5°C. Oxygen-saturated, filtered sea water

(mesh size: 1 μm) was used as an incubation medium (4 m*l* per 10 m*l* flask), the flasks being periodically agitated at 0.3 cycles per second during the measurements. Larvae were carefully transferred from a rearing tank to a 100 m*l* beaker filled with oxygen-saturated filtered sea water maintained at 26°C, and left to acclimatize for 30 minutes. All measurements of grouped larvae were duplicated, the volumeter being read every 20 minutes for two hours following acclimatization. Numbers of larvae ranged from two to 40, depending on larval size (0.8–8.1 mg in dry weight). All measurements were conducted using a flask of oxygen-saturated, filtered sea water as a control, following Yokohama and Ichimura (1969). Oxygen consumption rates were calculated from the linear regression of gas-volumeter readings, which corresponded to the decrease in dissolved oxygen volume over time. Daytime oxygen consumption rates were determined on 0, 5, 9, 13, 16, 21, 26, and 27-day old larvae, taken from the rearing tank just before measurement. Rates were measured every four hours on 9, 16, and 26-day old, fed larvae and on 27-day old larvae isolated without food for 12 hours in filtered sea water (mesh size: 1 μm) prior to measurement. After the measurements, the larvae were fixed in 2.5% glutaraldehyde, dried at 60°C for 24 hours, and weighed to the nearest 0.1 μg on an ultra-micro balance (Inaba Co., Ltd., M-1A). Development of the gill lamellae on the fixed specimens was noted, using a dissecting microscope. All statistical analyses were conducted using SYSTAT version 5.1 (Wilkinson, 1989).

Results

All larvae were alive and active at the end of the experimental procedures. Linear regressions of the gas-volumeter readings against time were significant in all measurements ($p<0.01$).

The oxygen consumption rate increased exponentially with larval age (Fig. 1), the relationship being expressed by;

$$Y=0.0532\,e^{0.212X} \quad (n=36,\ r^2=0.974,\ p<0.001) \quad (1)$$

where X is days after hatching, and Y, oxygen consumption rate per larva (μl O_2 individual^{-1} h^{-1}).

Weight-specific oxygen consumption rate (μl O_2 mg dry weight^{-1} h^{-1}) varied as a function of larval age and size. The rate increased with time from hatching to Day 13, and decreased from Day 16 to

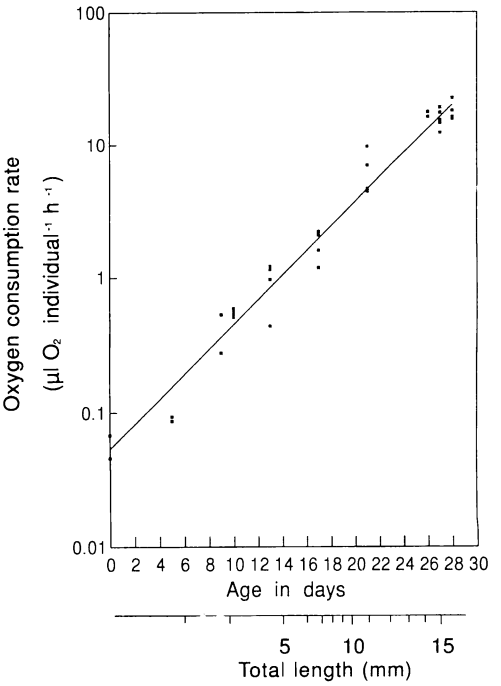

Fig. 1. Relationship between age in days and oxygen consumption rate of larvae (μl O_2 individual^{-1} hour^{-1}) of *Sillago japonica*.

Day 28 (Fig. 2), the relationships being expressed by the following equations;

$$\text{For 0 to 13-day old larvae:} \quad Y=5.06\,e^{0.058X} \quad (n=14,\ r^2=0.516,\ p<0.01) \quad (2a)$$

$$\text{For 16 to 28-day old larvae:} \quad Y=28.5\,e^{-0.059X} \quad (n=22,\ r^2=0.821,\ p<0.001) \quad (2b)$$

where X is days after hatching and Y, weight-specific oxygen consumption rate (μl O_2 mg dry weight^{-1} h^{-1}). The two regressions had significantly different intercepts and regression coefficients (ANCOVA; $p<0.001$). The weight-specific oxygen consumption rate decreased for larvae greater than 0.15 mg dry weight (Fig. 3), the average dry weight of 15-day old larvae (Oozeki et al., 1992). The relationships between weight-specific oxygen consumption rate and larval dry weight for the two size classes were expressed as:

$$\text{0.01–0.15 mg:} \quad Y=18.0X^{0.239} \quad (n=15,\ r^2=0.344,\ p<0.05) \quad (3a)$$

$$\text{0.15–3.70 mg:} \quad Y=7.3\,X^{-0.238} \quad (n=21,\ r^2=0.880,\ p<0.001) \quad (3b)$$

where X is dry weight (mg) and Y, weight-specific

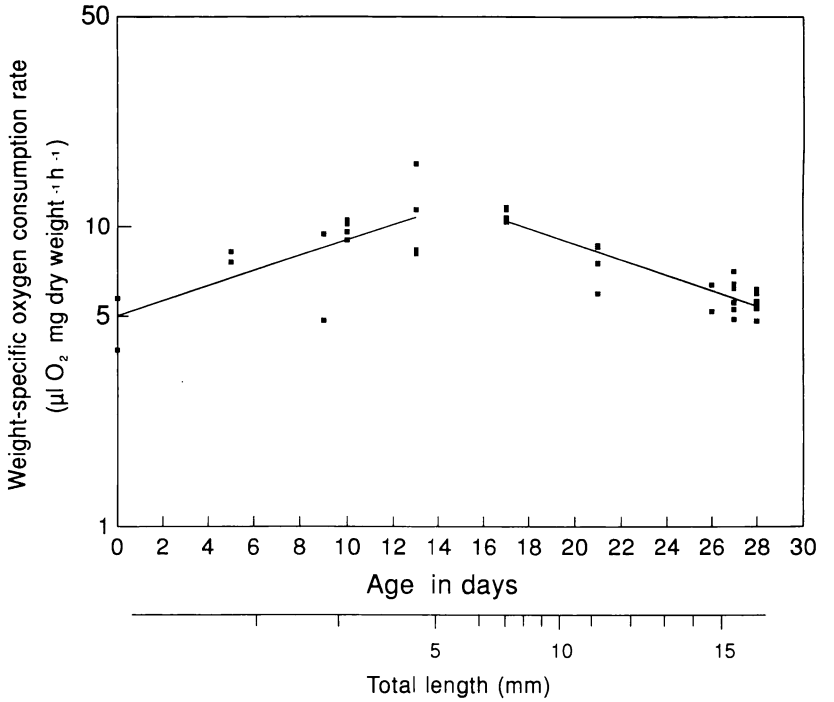

Fig. 2. Relationship between age in days and weight-specific oxygen consumption rate of larvae (μl O_2 mg dry weight^{-1} hour^{-1}) of *Sillago japonica.*

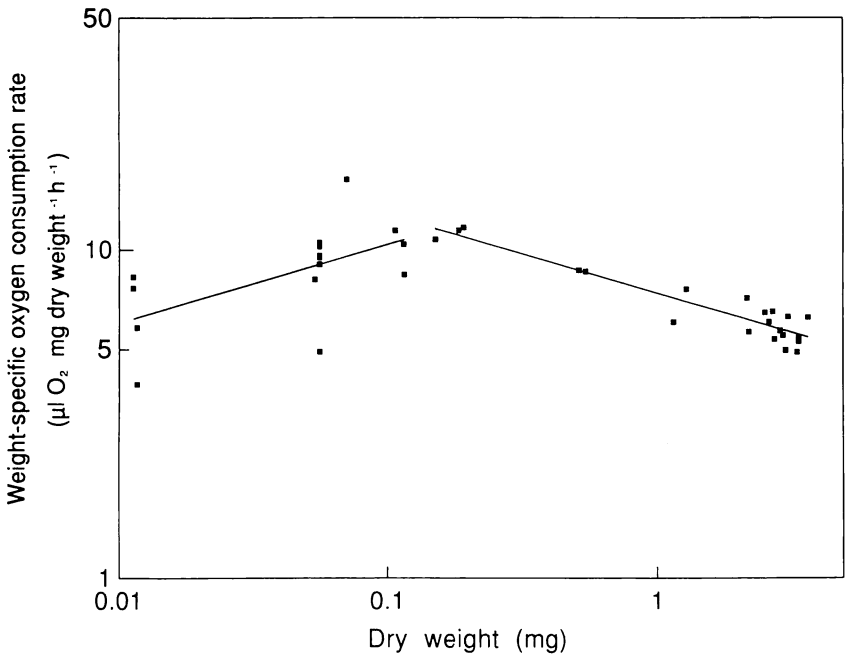

Fig. 3. Relationship between dry weight (mg) and weight-specific oxygen consumption rate of larvae (μl O_2 mg dry weight^{-1} hour^{-1}) of *Sillago japonica.*

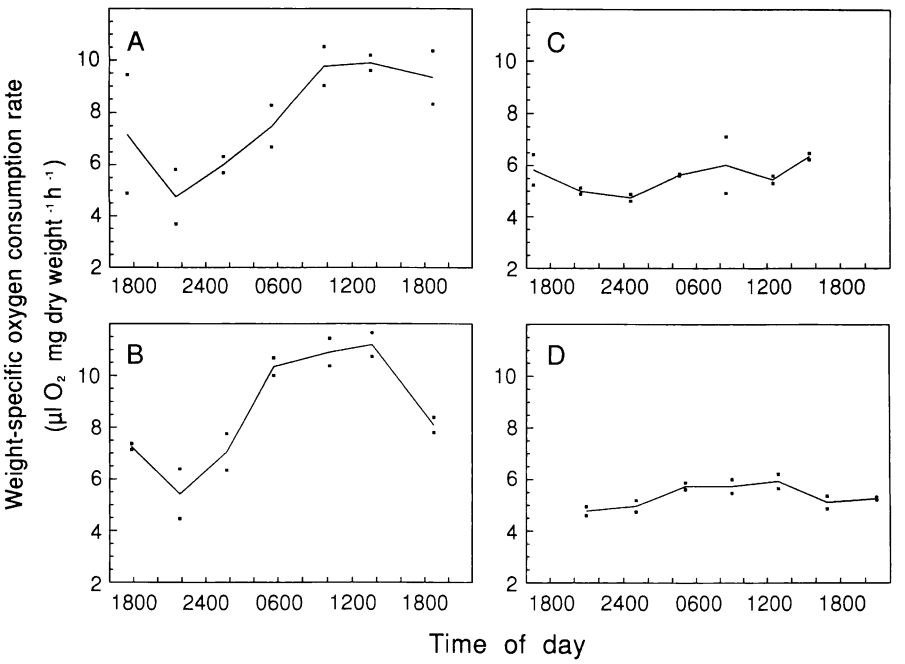

Fig. 4. Diel changes in weight-specific oxygen consumption rate in larvae of *Sillago japonica*. A) 9–10 days old; B) 16–17 days old; C) 26–27 days old; D) 27–28 days old. A) B) and C) fed, D) unfed condition.

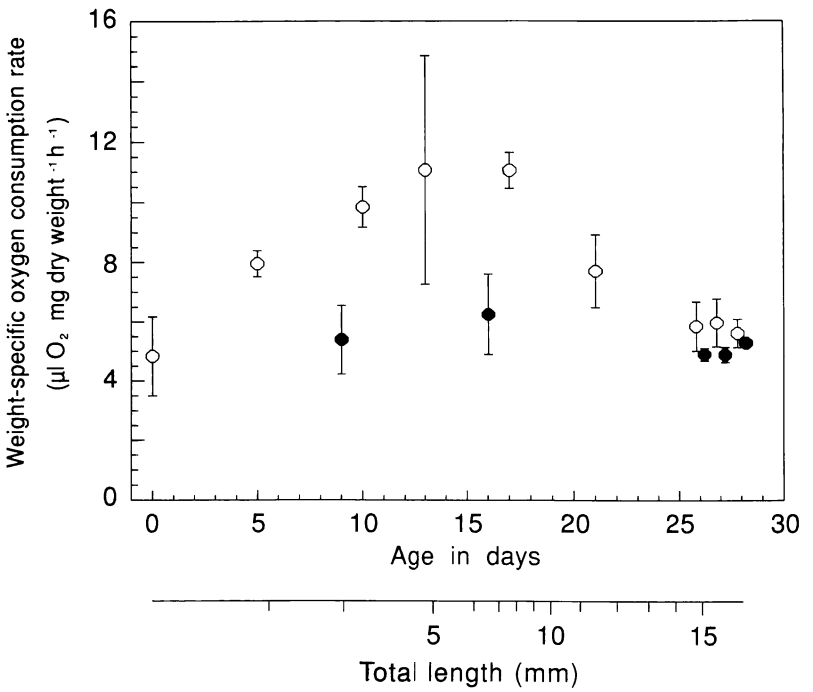

Fig. 5. Relationships between diel changes of weight-specific oxygen consumption rate and age in days in larvae of *Sillago japonica* (○: mean daytime values [06:30–17:30]; ●: mean nighttime values [19:30–04:30]). Vertical lines indicate standard deviations. Values measured at dusk and dawn are omitted.

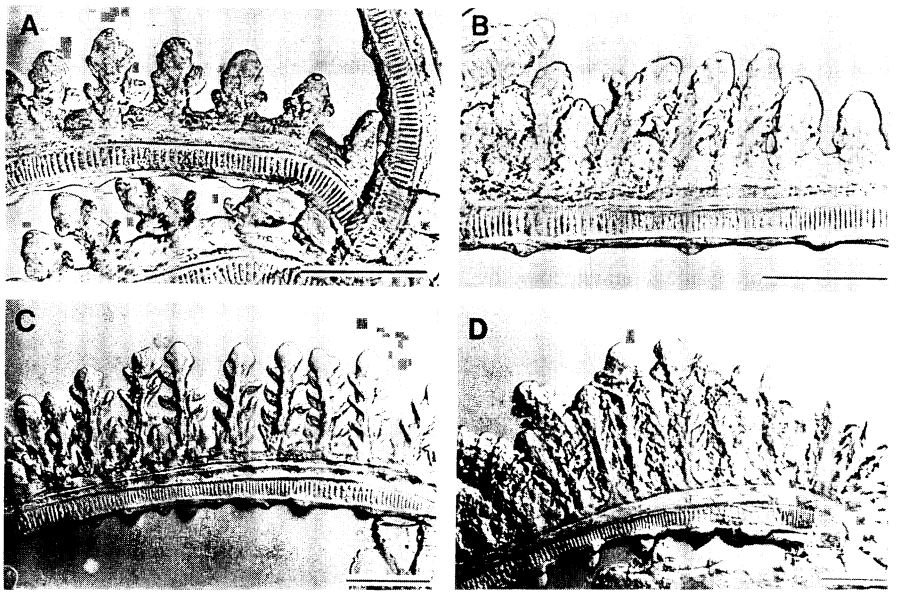

Fig. 6. Development of gill lamellae in larval *Sillago japonica*. A) 11 days old; B) 13 days old; C) 15 days old; D) 18 days old. Scale bars: 100 μm.

oxygen consumption (μl O_2 mg dry weight^{-1} h^{-1}). The two regressions had significantly different intercepts and regression coefficients (ANCOVA; $p<0.001$).

The diel pattern of weight-specific oxygen consumption was observed to change with larval age and feeding status (Fig. 4). Daytime weight-specific oxygen consumption rates (06:30–17:30) were approximately twice as high as those at nighttime (19:30–04:30) on Days 9 and 16 (ANOVA; $p<0.01$, Fig. 4A, B). A similar trend, but of lesser magnitude, was observed for 26-day old fed larvae (Fig. 4C). Weight-specific oxygen consumption did not vary significantly between fed and starved larvae of similar age (ANOVA; $p>0.05$, Fig. 4C, D), although the differences between daytime and nighttime oxygen consumption of unfed larvae (0.84 μl O_2 mg dry weight^{-1} h^{-1} in average) were smaller in magnitude than those of fed larvae (1.04 μl O_2 mg dry weight^{-1} h^{-1}).

Weight-specific oxygen consumption during the daytime increased from Day 0 to Day 13, subsequently decreasing to almost the same values observed at hatching (Fig. 5). However, nighttime values of weight-specific oxygen consumption showed little change throughout larval development. Day and nighttime values on Days 26–28 did not differ significantly from those values observed on Day 0 ($p>0.05$).

Although the mouth opened on Day 2, rudiments of the gill lamellae were not observed until Day 7, with gill lamellae beginning to form on Day 11 (Fig. 6). Rapid development of gill lamellae was observed between Days 13 to 15 (Fig. 6B, C).

Discussion

The results of this study confirmed an exponential relationship between oxygen consumption rate and larval age. Morioka (1985) reported similar results for the Pacific flounder, *Paralichthys olivaceus*. The exponential increase in the oxygen consumption rate is believed to have resulted from a rapidly enlarging gas-exchange surface, the body surface being the main site for gas exchange until Day 11 owing to the poor state of development of the gill lamellae up to that time (the gills having little role in gas exchange despite the mouth opening on Day 2). The subsequent, rapid formation of gill lamellae may be responsible for the exponential increase in the oxygen consumption rate after Day 13. Iwai and Huges (1977) found similarly that the formation of gill lamellae in black sea bream, *Acanthopagrus schlegeli*, occurred between 8 to 12 days after hatching at 17.8–21.1°C, and suggested that they began to play

an important role in gas exchange from that time.

The weight-specific oxygen consumption rate changed abruptly during larval development, being best described by two regressions with an inflection point between Days 13 and 16. Such an inflection point is well explained by the hypothesis proposed by Itazawa and Oikawa (1983), on the basis of their tissue respiration studies of carp, *Cyprinus carpio*. They proposed that the decline in weight-specific, standard metabolic rate of an intact animal could be explained if tissues with low metabolic rates increased in weight relative to whole-body weight during development. Organ development and changes in weight-specific metabolic rate in larval Japanese whiting agree well with this hypothesis. Oozeki et al.(1992) noted the rapid development of digestive organs from hatching to Day 10 (total length 4.0 mm), and the development of locomotor and respiratory organs from Day 11 to Day 23 (TL 12.0 mm). In addition, Itazawa and Oikawa (1983) reported that the QO_2 (μl O_2 g wet weight^{-1} h^{-1}) of brain and digestive organs were significantly higher than those of respiratory and locomotor organs in carp. If the QO_2 of these organs in Japanese whiting follow a similar trend, the positive relationship (2a) between weight-specific oxygen consumption rate and larval age may be ascribed to the rapid growth of the digestive organs. On the contrary, the negative relationship (2b) may be explained by the development of the respiratory and locomotor organs. Oikawa et al. (1991) reported a similar relationship between weight-specific oxygen consumption rate and age in larval red sea bream (*Pagrus major*), the former increasing linearly with age from hatching to Day 8, and subsequently remaining almost constant in 8 to 27-day old fish.

A similar transitional phenomenon was observed in the relationship between weight-specific oxygen consumption rate and larval dry weight, somewhat contrary to the results of Oikawa et al. (1991), who found no clear relationship between hatching and Day 6, but an almost constant relationship (regression coefficient $=-0.051$) from Day 8 to Day 25. The differences between the studies may be due to differences between the species and in the experimental methods, as the weight-specific oxygen consumption rates reported by Oikawa et al. (1991) were based on larval wet weight, a more variable measure of biomass.

The above results indicated that oxygen consumption rates in larval Japanese whiting were lower at night than during the day, even though all measurements were taken under conditions of darkness. Diel variation in oxygen consumption rates has been reported for larvae of many fish species (Holliday et al., 1964; De Silva and Tytler, 1973; Geffen, 1983; De Silva et al., 1986). Daily respiration patterns of herring larvae agree with the results of the present experiments, although the decrease in oxygen consumption rate in the former after sunset (Holliday et al., 1964) was not observed here. Similar patterns of decreasing oxygen consumption rates at night were reported by De Silva and Tytler (1973), De Silva et al. (1986) and Geffen (1983), although the mechanisms for diel changes in respiration rate were not discussed.

The diel pattern observed in larval Japanese whiting may be explained by differences in metabolic activity levels. Following Yamashita and Bailey (1989), oxygen consumption rates were measured at three different metabolic activity levels. Nighttime values were considered to represent resting (basal) metabolism (M_{re}), because larvae had no food in the gut at that time and were measured in complete darkness. Daytime values were considered to represent feeding metabolism (M_{fn}), which included Mre plus additional costs for specific dynamic action (SDA; Jobling, 1985). Accordingly, the difference between day and nighttime values might be ascribed to the activity level of SDA. Although SDA was formerly thought of as the cost of digestion, deamination, and urea-synthesis (Jobling, 1985; Kiørboe et al., 1987), it is now generally taken as representing the cost of biosynthesis (Kiørboe et al., 1987). The former definition of SDA is followed for the purpose of this discussion. Values of larvae starved in daytime were also considered to be $(M_{fn}-SDA)=M_{re}$, although some of the difference between day and nighttime values was thought to reflect SDA in part, owing to the detection of a daily pattern in the oxygen consumption rate in unfed larvae. A similar phenomenon was reported for nitrogen excretion in fingerling sockeye salmon, *Oncorhynchus nerka* (Brett and Zala, 1975). Although a peak of daytime ammonia excretion disappeared in starved salmon, an oxygen consumption peak persisted (Brett and Zala, 1975). Love (1980) suggested that at least part of the oxygen consumption peak in the latter, reflected physiological movements rather than energy needed for digesting food. Hence, the diel pattern observed in fed larvae may be the sum of the costs of digestion, deamination and urea-synthesis, with some

part of these costs possibly being detectable under starved conditions.

Daytime rates of oxygen consumption increased from Day 0 to Day 13, and subsequently decreased. Nighttime rates of weight-specific oxygen consumption, however, showed little change during development and were not significantly different from those at hatching (5 μl O_2 mg dry weight^{-1} h^{-1}). The weight-specific rates at night, considered to be the resting or basal metabolic rate, were stable during the larval stage.

Information obtained in this study may be useful for ecological studies of marine fish larvae. An approach combining respiration measurements with an enzymatic study of the Electron Transport System (ETS) may be more promising for metabolic studies of wild-caught larvae.

Acknowledgments

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シロギスの初期生活史における酸素消費量の変化

大関芳沖・平野禮次郎

シロギス (*Sillago japonica*) の受精卵を自然採卵法により採卵・孵化させた後，シオミズツボワムシを与えて 25.1±1.0°C で飼育した。仔稚魚の酸素消費量を孵化直後から変態期 (28 日齢) に至るまで，3–5 日おきに暗黒条件下で測定した。昼間に測定された個体当たり酸素消費量は孵化後日齢に対して指数関数的に増加した。乾重量当たり酸素消費量は孵化後 13 日齢に達するまでは増加し，その後は減少する傾向にあった。乾重量当たり酸素消費量の日周変化は仔魚期全体を通して観察され，昼間の計測値は夜間の値よりも高かった。昼間の計測値は成長過程を通して変化したが，夜間の値には変化は認められなかった。

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