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**염분의 급변이 점농어(*Lateolabrax* sp.) 산소소비
생체리듬에 미치는 영향**

**Effects of sudden changes salinity on endogenous
rhythm of the spotted sea bass *Lateolabrax* sp.**

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제 출 문

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본 보고서를 '염분의 급변이 점농어(*Lateolabrax* sp.) 산소소비 생체리듬에 미치는 영향' 의 보고서로 제출합니다.

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요 약 문

I. 제 목

염분의 급변이 점농어(*Lateolabrax sp.*) 산소소비 생체리듬에 미치는 영향

II. 연구개발의 목적 및 중요성

- 최근 양식기술의 발달로 인하여 고급 어종의 소비가 늘고 있으며, 농어도 고급어종으로 각광을 받고 있다. 그러나 초기의 기형개체 출현과 대량폐사는 아직까지도 해결되지 않고 있다. 본 연구는 농어의 생리적 현상을 좀더 심도 있게 연구하여 앞으로 이러한 문제를 해결하는데 기초자료로 활용하고, 양식업에도 중요한 자료를 제공하는데 있다.

III. 연구개발의 내용 및 범위

- 담수어를 갑자기 해수에 노출하게 되면, 체내의 물질대사를 조절하는 여러 기관들이 손상을 입게 되어 심지어 사망에 이르게 된다. 마찬가지로 해산 어류들도 갑자기 담수에 노출 되면 체내의 이온과 가스교환이 불균형을 이루게 되어 사망에 이르게 된다. 그러나 농어와 같은 몇몇 해산어류들은 강에서 산란을 하고 바다에서 주로 일생을 보낸다. 바다에서 서식하고 있던 어류가 산란을 위하여 일시적으로 강으로 소상할 때 생리적으로 많은 변화가 있을 것으로 추정된다. 따라서 본 연구는 갑작스런 주위 환경변화가 호흡생리활성에 어느정도 영향을 끼치게 되는지 정성적으로 관찰하는데 있다.

IV. 연구개발의 결과 및 활용에 대한 건의

- 농어의 호흡에 의한 내부생체리듬을 명확히 관찰하였다. 이러한 자료는 양식업에서 먹이 공급에 관한 정보를 제공할 뿐만 아니라, 다른 어류들의 생태생리연구에도 중요한 정보가 된다.
- 농어는 갑자기 담수에 노출시켜도 생존에는 커다란 영향을 끼치지 않는다. 따라서 이러한 결과는 육상 양식업의 가능성에도 중요한 자료를 제공하리라 믿는다.
- 농어가 기생충이나 바이러스에 감염되었을 때 항생제를 사용하지 않고, 염분을 갑자기 낮추어서 치료 할 수 있는지 더 많은 연구가 요구 된다.

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Effects of sudden changes in salinity on endogenous rhythm of the spotted sea bass *Lateolabrax* sp.

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Abstract Endogenous rhythm in oxygen consumption of juvenile spotted sea bass *Lateolabrax* sp. was measured to test the effects of sudden changes in salinity (SCS) on the metabolic activity. Mean oxygen consumption rates of this euryhaline fish decreased by 13.5 - 16.0 % and 25.3 - 36.4 % when they were transferred from 31.5 to 15 ‰ seawater and fresh water (0 ‰), respectively. The maximum rate of oxygen consumption was observed at 18:00 to 19:00 p.m. in local time, 1 to 2 h before sunset even though they were kept in constant darkness. The peaks of oxygen consumption occurred at 23.2 and 23.3 h intervals, which correspond with a circadian rhythm, as revealed by maximum entropy spectral analysis (MESA). A markedly weekend rhythm in the oxygen consumption occurred from 8 to 10 d after onset of the experiments. This study indicates that spotted sea bass can withstand sudden drops in salinity from 31.5 ‰ to fresh water, and yet maintain a regular though somewhat dampened endogenous rhythm of oxygen consumption.

Introduction

Exposure of aquatic organisms to sudden changes in environmental conditions or to toxic chemicals may cause a severe impact on the physiological processes. For example, rhythmicity of oxygen consumption and swimming activity breakdown when fish are exposed to the higher toxic chemicals (Kim et al. 1996a) and antibiotic concentrations (Harries and Morgan 1984).

Salinity affects various physiological processes in aquatic animals such as metabolism, osmoregulation and biorhythm. This is particularly significant for euryhaline, migratory fishes which experience both fresh water and seawater in their life span. Osmotic and ionic regulation in marine fish has been intensively reviewed by Alderdice (1988) and Evans (1993). The previous studies on the effects of sudden changes in salinity (SCS) have been focused on the acclimation of gametes (Hintz and Lawrence 1994), balance of blood constituents (Hwang et al. 1989; Roche et al. 1990; Mancera et al. 1993; Chen and Lin 1994; Munro et al. 1994), muscle electrolyte content (Engel et al. 1987; Chernitsky et al. 1993), and oxygen consumption (Oertzen 1984; Barton and Barton 1987; Moser and Gerry 1989; Aarset and Aunaas 1990; Moser and Miller 1994; Cheung and Lam 1995; Gaumet et al. 1995; Lucu and Pavicic 1995). Variation in salinity affected the circatidal locomotor activity of the shore crab, *Carcinus maenas* (Reid and Naylor 1989). Little data, however, are available on the effects of SCS on the endogenous rhythm of oxygen consumption in fish.

Sea bass *Lateolabrax japonicus*, a euryhaline fish, spend early life cycle in the low salinity waters of estuaries and in rivers (Chyung 1977). The sea bass

Dicentrarchus labrax are known to have a remarkable ability to regulate plasma electrolytes over wide range of salinities (Carrillo et al. 1986). It is assumed, therefore, that there should be variations in metabolic activities of sea bass when they are adapted to different levels of salinity.

The purpose of this work is to study the effects of sudden changes in salinity on the metabolic activity in terms of oxygen consumption of the spotted sea bass.

Materials and methods

Experimental fish

The spotted sea bass *Lateolabrax* sp.* used in this study were 3 to 12 mo old juveniles reared in a culture tank (100 l) in the laboratory after artificial propagation at the Korea Ocean Research and Development Institute (KORDI). Test fish [n = 4, mean total length = 14.1 ± 0.3 cm ($X \pm SE$), 5.7 ± 0.4 g dry wt (DW), 1995 year class; n = 42, mean total length = 9.1 ± 0.5 cm, 1.6 ± 0.4 g DW ind⁻¹ - 1996 year class] were held in 31.5 ‰ and acclimated to 14 °C for 4 weeks. In 16 experiments, oxygen consumption of the 46 animals (2 fish group; N = 2, 3 fish group; N = 14) was monitored with 2 to 3 fish being put in one chamber at a time (Tables 1, 2). Controls were held in 31.5 ‰ throughout the experiment. Feeding of fish was stopped 48 h before their introduction into the test chamber and they were not fed throughout the experiments. At the end of the tests, individuals were removed and weighed. Dry weight (DW in g) was calculated from regressions

relating body dry weight to total length (TL < 15 cm) and was expressed as: $DW = 0.00135 \cdot TL^{3.182}$ (n = 40, $r^2 = 0.97$, $p < 0.01$).

Experimental design

The test fish were kept in the experimental chamber for 72 to 119 h for adaptation and then exposed to 15 ‰ or fresh water (FW). Measurements of the oxygen consumption were conducted over 96.1 h to 427.3 h. Human intervention and disturbance were minimized throughout the experiments. The oxygen consumption rate was measured by using an automatic intermittent-flow-respirometer (AIFR). The AIFR has a greater flexibility in determining the oxygen consumption at various intervals and oxygen tensions than other types of respirometers (closed and flow-through). No measurements were made while exchanging the experimental water between 31.5 and 0 ‰. It took about 20 seconds to exchange water in the test chamber.

~ Two forms of the sea bass *Lateolabrax japonicus* have been recognized in the northeast Asian waters: sea bass with black dots on the lateral body region (spotted) and without dots. Although these two forms are not yet differentiated taxonomically, the spotted sea bass appears to be a distinct species from the non spotted one *L. japonicus*, as there exist considerable morphological and genetic differences between the two (Yokogawa and Seki 1995; Park et al 1996).

The probe voltage for polarization was examined for 20 min in order to ascertain whether it had deviated from the gauge voltage at the beginning of the experiment. Experimental waters were filtered free of bacteria through sterile membrane filters (with two Sartorius Capsule Filters, input 0.2 μm and output 0.07 μm). Oxygen levels in the plexiglass experimental chamber (1.4 l) was maintained between 85 (lowest) and 95 % (highest) of saturation. Measurements were conducted in a constantly darkened incubator (RI-50-1060, REVCO, USA) with constant temperature of 13.8 to 14.9 °C. The magnetic drive gear pump (MS-Z, Ismatec Sa, Switzerland) produced horizontal water flow rates of 690 ml min⁻¹. During the experiments, water temperature and air pressure were continuously monitored using a thermometry sensor (Pt-100, Farnell, Germany) and a barometric pressure sensor (Sensym Hs 20, Farnell), respectively. Before and after the experiments, salinity (‰) was measured with a salinometer (LF 320, WTW, Germany). At every second the actual oxygen level was recorded by the digital controlling unit through a picoammeter. Mean oxygen consumption values for the test organisms were calculated every 90 s and displayed graphically at 90 s intervals. Data readings, including local and experimental time (seconds), temperature (°C), air pressure (hPa), oxygen consumption (ml O₂ h⁻¹) and oxygen levels (%), were stored directly into a hard disk drive for future analysis. More detailed descriptions of AIFR, including the location of the probe, calculation methods and a schematic of the apparatus are given in Kim et al. (1996a, 1997).

Analysis of oxygen consumption records

Rhythmicity was determined by a Maximum Entropy Spectral Analysis (MESA) program using raw data transformed into 30 min lag intervals. Time series were analysed for periodicity using MESA spectra following the procedures and algorithms described by Dowse and Ringo (1989). Statistical comparisons between experimental and control groups were made by the Student's *t* - test. The analysis of the rhythm of oxygen consumption was performed using the weighted smooth curve procedure of 2 % (KalcidaGraphy custom program for Macintosh, Synergy Software). To plot a best fit smooth curve, through the center of the data, the locally weighted least squared error method was used. The value of 2 %, obtained from the repeated tests showed a best fit curve. Statistical values were computed for each batch from the data points measured (Tables 1, 2).

Results

Endogenous rhythm in oxygen consumption of the spotted sea bass (control)

The instantaneous rates of oxygen consumption in the 2 fish (A) and 3 fish groups (B) exhibited a rhythmicity throughout the experiments (Fig. 1). The oxygen consumption rate was elevated in the first 3 to 4 h of experiment probably due to initial stress. Four cycles were observed with a period 96.1 and 96.9 h, respectively in the 2 fish groups (Fig. 1A) and in the 3 fish groups (Fig. 1B).

The juvenile spotted sea bass maintained relatively low uptake of oxygen during the daylight (in local time) which is referred to as the 'basal' rate in this study. Thereafter the rate of oxygen consumption drastically increased, reaching a peak around 6:00 to 7:00 p.m. (which correspond to sunset in natural condition) even though they were kept in constant darkness and constant temperature. The amplitude of the oxygen consumption rate ranged from 0.66 to 1.19, and from 0.46 to 1.70 ml O₂ g DW⁻¹ h⁻¹ in the 2 and 3 fish groups, respectively. The mean oxygen consumption rate, averaged over the entire duration of the experiment and over the entire range of oxygen levels (between 94.4 and 85.6 %), was 0.84 ± 0.001 and 0.85 ± 0.003 (X ± SE) ml O₂ g DW⁻¹ h⁻¹ in the 2 and 3 fish groups, respectively (Table 1).

MESA spectra of each of the data sets presented in Fig. 1 indicated that peaks of oxygen consumption mainly occurred at 23.3 and 23.2 h intervals, which corresponds to a circadian rhythm (Figs. 2A, 2B). The instantaneous rate of oxygen consumption by 2 fish group also showed minor peaks in the short periods of 11.7 h intervals (Fig. 2A).

Effects of sudden changes in salinity (SCS)

Exposure from 31.5 to 15 ‰ - Group I

Instantaneous rate of oxygen consumption in the 3 fish group was fitted to a weighted smooth curve of 2 ‰ (Fig. 3). The frequency of rhythmicity observed was 6 cycles over a 6 d period (144.1 h) at 31.5 ‰, indicating approximately 24 h circadian cycles. The juvenile spotted sea bass maintained the same rhythmicity of

oxygen consumption after the exposure at 15 ‰ (Fig. 3B). However, the mean oxygen consumption rates appeared to gradually decrease ($r^2 = 0.85$, $p < 0.05$) with time. After transfer to 15 ‰, the oxygen consumption rate of the fish (Group I) increased for a short period presumably due to stress, then decreased by 13.5 to 16.0 % compared to control at 31.5 ‰ (Table 2).

Exposure from 31.5 to 0 ‰ (fresh water - FW) - Group II

The instantaneous rate of oxygen consumption for groups of 3 fish was observed for 119.3 h (Fig. 4A) at 31.5 ‰ and for 308.1 h in FW (Fig. 4B). The instantaneous rate of oxygen consumption, therefore, was recorded for a period of 427.4 h and it was also fitted to a weighted smooth curve of 2 %. The rate of oxygen consumption of fish exposed to FW dropped sharply. In spite of a reduced rate, however, the oxygen consumption of fish maintained clear endogenous circadian rhythm. The mean oxygen consumption rates before and after FW exposure were significantly different ($p < 0.01$) indicating that the fish in FW (Group II) consumed about 25.3 to 36.4 % less oxygen than fish at 31.5 ‰ (Table 2). It was noted that a markedly weaker endogenous rhythm of oxygen consumption occurred during the 8th to 10th d (April 4 to 6, 1997) after the onset of experiment. After this period, oxygen consumption resumed a clear endogenous circadian rhythm. The mean oxygen consumption rate on the 8th to 10th d were transformed into 30 min lag intervals to dampen rhythms. The analysis revealed a significant difference ($t = 4.19$, $p < 0.001$) between the two periods and compared to the 13th to 15th d using a t -test.

Discussion

Transfer of fish from high to low salinity is accompanied by a series of physiological events described by an adaptive and a regulative phase (Houston 1959). It is known that the adaptation time for euryhaline fishes to fluctuating salinities varies between 5 ~ 12 hours (Oertzen 1984) and a few days (Parry 1966). In this study, the change of salinity resulted in 13.5 - 16.0 ‰ and 25.3 - 36.4 ‰ decreases in oxygen consumption rates by the juvenile spotted sea bass transferred from 31.5 ‰ to 15 and 0 ‰ (FW), respectively. Moser and Gerry (1989) found 20 and 59.6 ‰ reduced oxygen consumption by the juvenile croaker *Micropogonias undulatus* (8 - 10 cm in body length) when the fish were exposed to 15 and 0 ‰ respectively from 34.0 ‰ at 25 °C. These results may suggest that energy for the osmoregulatory adaptation to salinity change is lower for juvenile spotted sea bass than for juvenile croaker. It seems that the differences in oxygen consumption may be partly due to differences in the adaptation time by the different species. It also could be due to the fact that croaker have lower metabolic rates at low salinity regardless of adaptation time. More work is needed to elucidate the respiratory adaptation to salinity change.

Most marine organisms show an endogenous rhythm in their behaviour and physiological processes (Palmer 1995). Morgan and Iwama (1990) stated that changes in metabolic rate in relation to endogenous rhythm would be very difficult to measure accurately. However, the use of an automatic intermittent-flow-respirometer (AIFR) made it possible to observe the endogenous rhythm of oxygen consumption of river puffer fish *Takifugu obscurus* (Kim et al. 1996a, 1997) and

manila clam *Ruditapes philippinarum* (Kim et al. 1996b). Juvenile spotted sea bass showed a strong endogenous circadian rhythm of ca. 24 h of their instantaneous rate of oxygen consumption, which was similar to that observed in juvenile river puffer fish (Kim et al. 1996a, 1997). However, the rhythm patterns were different from the river puffer fish, even though these two species were reared in the same laboratory. The maximum rate of oxygen consumption in juvenile spotted sea bass was observed in the afternoon (6 ~ 7 p.m.), while juvenile river puffer fish showed the peak consumption at early morning, 6 ~ 7 a.m. in local time even though they were kept in constant darkness (Kim et al. 1997). The difference between rhythms of sea bass and puffer fish could probably be related to the feeding patterns of two species in the wild. However, the eco-physiological significance in their endogenous rhythms cannot be immediately explained, as their behaviors in the natural environments are not well documented yet.

The majority of juvenile sea bass are found within brackish and estuarine waters (Chyung 1977) or may be found around minor freshwater inputs and tidal lagoons on the open coast (Dando and Demir 1985; Kelley 1986, 1988; Pickett and Pawson 1994). It is generally known that estuarine waters provide better nursery ground for juvenile bass, because they offer good feeding conditions and contain relatively few predators (Jennings et al. 1991; Pickett and Pawson 1994). The results of this study indicate that juvenile spotted sea bass can withstand rapid salinity changes from 31.5 ‰ to fresh water and yet maintain an endogenous rhythm of oxygen consumption without breakdown. While it appears that spotted sea bass adapt quickly to an euryhaline environment, a markedly reduced oxygen consumption

and weekend rhythm was observed during 8 to 10 d after the onset of the experiment. Information on the relationship between endogenous rhythm of oxygen consumption in aquatic organisms and intrinsic factors is very limited. Mehner and Wieser (1994) reported that the pattern of swimming activity of the starved perch *Perca fluviatilis* was changed when glycogen reserves in muscles and liver were exhausted due to starvation for 7 to 8 d. They noticed that the perch switched energy fuel around this time from carbohydrates to other sources. It can be assumed, therefore, that the weak rhythm of oxygen consumption we observed in the juvenile spotted sea bass was probably coincided with the switching of energy source after starvation period of 8 to 10 d.

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Table 1. *Lateolabrax* sp.. Experimental parameters and oxygen consumption rate of the spotted sea bass (control groups). Values are mean \pm SE.

	2 Fish	3 Fish
Mean total length (cm ind ⁻¹)	14.1	9.2
Mean dry weight (g DW ind ⁻¹)	6.13	1.57
Number of points measured	2711	2900
Flow rate (ml min ⁻¹)	690	690
Temperature (°C)	14.9 \pm 0.005	14.3 \pm 0.001
Levels of oxygen saturation (%)	94.2 - 85.7 (\pm 0.02)	94.4 - 85.6 (\pm 0.05)
Salinity (‰)	31.5	31.5
Duration (h) of the experiment	96.1	96.9
Mean oxygen consumption (ml O ₂ g DW ⁻¹ h ⁻¹)	0.84 \pm 0.001	0.85 \pm 0.003
Number of experiments	2	4
Total number of fish	4	12

Table 2. *Lateolabrax* sp.. Comparison of the oxygen consumption rates in spotted sea bass before and after sudden changes in salinity to 15 ‰ (I) and fresh water (II). Values are mean \pm SE.

Group	I	II
Number of fish in each experiment	3	3
Mean total length (cm ind ⁻¹)	9.1	9.4
Mean dry weight (g DW ind ⁻¹)	1.54	1.62
Number of points measured	1508 - 2266	6263 - 13788
Flow rate (ml min ⁻¹)	690	690
Temperature (°C)	14.4 - 14.6 (\pm 0.002 - 0.003)	13.8 - 14.1 (\pm 0.001 - 0.002)
Levels of oxygen saturation (%)	94.9 - 85.1 (\pm 0.002)	94.9 - 85.2 (\pm 0.03)
Salinity (‰)	31.5 - 15.0	31.5 - 0
Duration (h) of the experiment	98.3 - 144.1	153.1 - 427.3
Mean oxygen consumption (ml O ₂ g DW ⁻¹ h ⁻¹)		
Before exposure (A)	0.74 - 0.81 (\pm 0.001 - 0.002)	0.87 - 0.99 (\pm 0.002 - 0.003)
After exposure (B)	0.64 - 0.68 (\pm 0.001 - 0.002)	0.63 - 0.65 (\pm 0.0001 - 0.002)
Decrease in the rate of oxygen consumption (%) = (B-A)/A (ml O ₂ g DW ⁻¹ h ⁻¹)	\downarrow 13.5 - 16.0	\downarrow 25.3 - 36.4
Number of experiments	4	6
Total number of fish	12	18

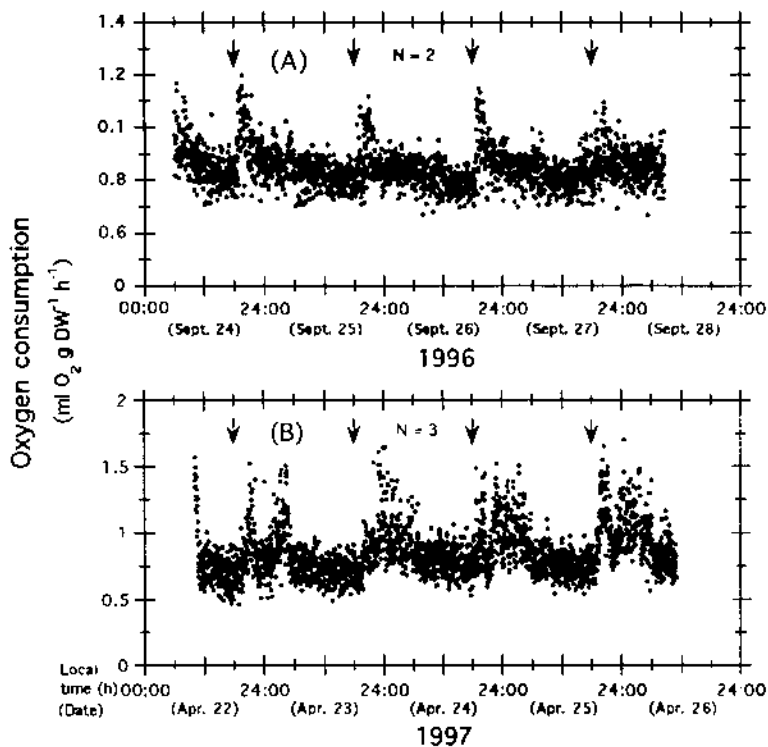


Fig. 1. *Lateolabrax* sp.. The time series of the oxygen consumption rate (ml O₂ g DW⁻¹ h⁻¹) by 2 spotted sea bass, during about 4 d (A) and by 3 fish during 4 d (B). The fish were kept under at 14.3 and 14.9 °C and oxygen levels of 85.6 - 94.4 %. (o) Mean oxygen consumption rate during 90 s. Arrows indicate peaks of oxygen consumption at around 18:00 p.m..

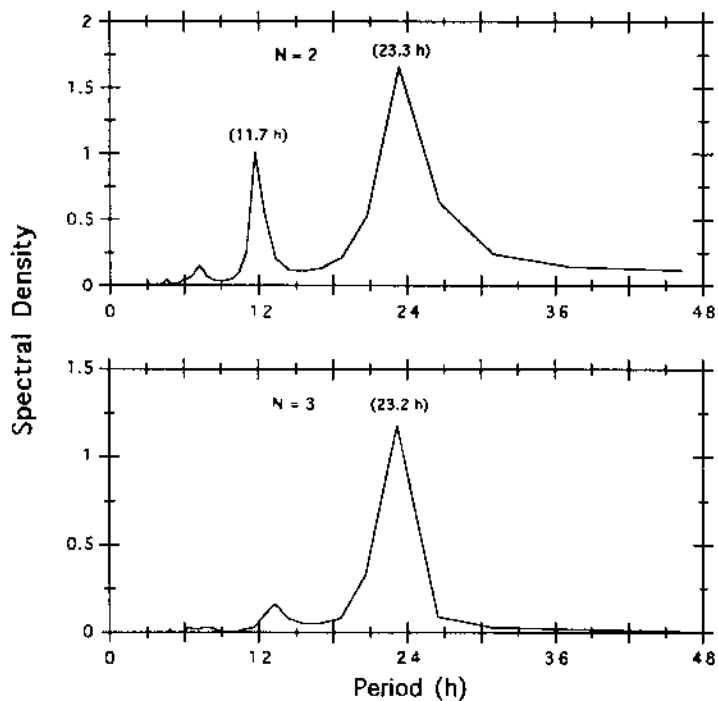


Fig. 2. *Lateolabrax* sp.. Maximum Entropy Spectral Analysis (MESA) spectra for 2 (2A) and 3 (2B) spotted sea bass data presented in Figs. 1A and 1B. Period length (h) corresponding to the dominant peaks in the MESA plots are given in parentheses.

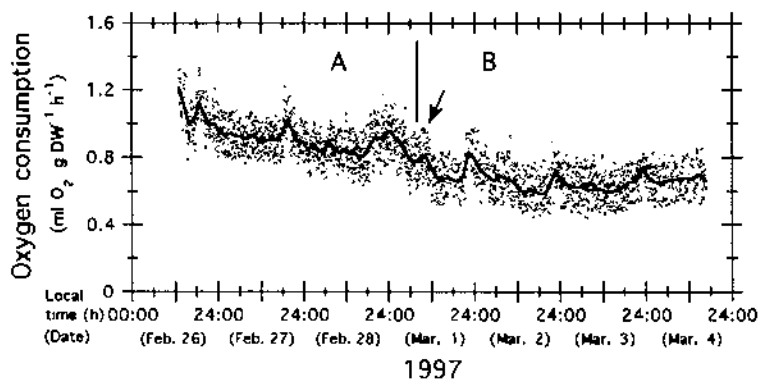


Fig. 3. *Lateolabrax* sp.. Patterns of the oxygen consumption rate of 3 groups of juvenile spotted sea bass before (31.5 ‰) and after transfer to 15 ‰ (salinity change indicated by vertical bar). The arrow indicates that the oxygen consumption rate increased for a short time after transfer to 15 ‰. A dot point represents the mean oxygen consumption rate during 90 s intervals.

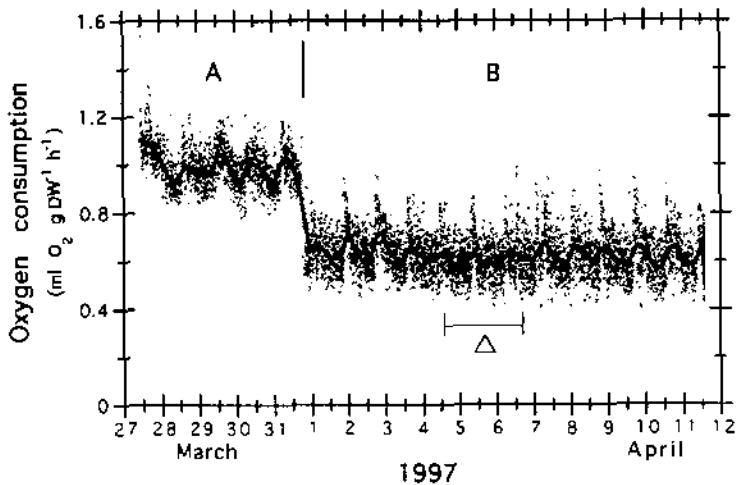


Fig. 4. *Lateolabrax* sp.. Patterns of the oxygen consumption rate of groups of 3 juvenile spotted sea bass for 427.3 h before (31.5 ‰) and after transferred to fresh water (0 ‰) as indicated by the vertical bar. The triangle indicates a period of weekend endogenous rhythm. A dot point represents mean oxygen consumption rate during 90 s intervals.

