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Zoological Studies

Effects of Linear Alkylbenzene Sulfonate (LAS) on the Respiratory Functions of Tigerperch (*Terapon jarbua*)

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Bao-Quey Huang and Dar-Yi Wang (1994) Effects of linear alkylbenzene sulfonate (LAS) on the respiratory functions of tigerperch (*Terapon jarbua*). *Zoological Studies* **33**(3): 205-210. The changes occurring in the respiratory functions of tigerperch (*Terapon jarbua*) were examined in order to evaluate the toxic effects of linear alkylbenzene sulfonate (LAS). Three approaches i.e. LC50, respiratory curve, and pathomorphological changes in gills were measured after exposure to sublethal concentrations of LAS.

The detergent treatment was observed to be accompanied by a significant decrease in respiratory rate from 0.018 ppm/min to 0.012 ppm/min, when LAS concentration changed from 3.5 ppm to 5.0 ppm. The destruction of secondary lamella in gill epithelium was found through means of non-parametric statistic method (McCormick 1989) to become significant when LAS concentration reached 2.5 ppm. The 24 hr LC50 value was 3.28 ppm as obtained by probability mortality method. The toxic effects of LAS on the respiratory functions have been reflected by the changes in both respiratory rates and the damage of gill epithelium. Thus, lethal was probably caused by the decreasing performance of the respiratory function.

Key words: LC50, Oxygen consumption curve, Histopathology, Estuarine fish.

Synthetic detergents are widely used in industry and at home. These chemicals are drained away through rivers to estuaries where they may become concentrated in brackish water and coastal fauna (Conti 1987, Marin et al. 1991, Chen et al. 1992). Linear Alkylbenzene Sulfonate (LAS) is an anionic surfactant which is one of the most toxic pollutants known (Hokanson 1971, Abel 1974). The concentrations of LAS are acutely toxic, at values of 0.4 ppm to 40 ppm (Abel 1974, Lewis and Suprenant 1983, Conti 1987). Surfactants are known to cause gill damage (Misra et al. 1985, Zaccone et al. 1985a). Fish gills are well known to be a multifunctional tissue involved in not only respiration, but also osmoregulation. The major histopathological destruction of the gill epithelium by LAS is: (1) edema or necrosis of secondary lamella (Fukuda 1983, Misra et al. 1985, Zaccone et al. 1985a), (2) the space between the secondary lamella being almost entirely filled with cell fragments (Fukuda 1983, Misra et al. 1985), (3) the significant acceleraton of mucous cell secretion and the subsequent decrease in oxygen permeability (Zaccone et al. 1985a, Roy 1988b). Gill damage is generally accepted as a cause of respiration difficulties and consequential death for many fishes (Zaccone et al. 1985a, Roy 1988b).

Tigerperch (*Terapon jarbua* Forskal) belongs to the family Teraponidae and is recognized as a prospective species in mariculture. The fish inhabit the estuarine waters of the Taiwan coast where their fry thrive. This species can therefore be a good indicator of LAS pollutant effects. Three approaches were employed for the sake of investigating the toxic effects of LAS on the respiratory function. An LC50 mortality probability value was first determined (Buikema et al. 1982, Roy 1988b), then the gill damage was diagnosed by the morphometry method (McCormick 1989). Oxygen consumption curve will finally be recorded for measuring the respiratory rate, lethal oxygen concentration and survival time. The 24 hr LC50 of LAS and the toxic effects of this detergent on both respiratory functions and consequential gill morphological damage will therefore be established here.

MATERIALS AND METHODS

Tigerperch (*Terapon jarbua*) obtained from the North-east coast of Taiwan, with a ranging total length from 37.4 mm to 56.2 mm and a body weight ranging from 0.67 g to 2.12 g, were acclimated with a natural photoperiod. Experimental fish were fed with commercial food once per day, and starved 24 hrs before the onset of the experiment. During the experiment, sea water was maintained at a pH of 7.91-7.99, a DO of 4.1-4.4 ppm, and a salinity of 35‰ temperature 27-28°C.

The oxygen consumption curve was recorded to determine the effects of LAS on respiratory functions by monitoring the sacrificing of an individual fish in a 300 ml bottle. The monitoring system was composed of a DO meter, stirrer, and pen recorder (Kipp and Zonen BD40). Concentrations of LAS (Linear Alkylbenzene Sulfonate) were prepared at 7.5, 5.0, 3.5, 3.0, 2.5 and 0 ppm as a control group. One individual fish was kept in each respiratory chamber. The survival time and lethal oxygen concentration were recorded and the average respiratory rate was then obtained by measuring the slope of the oxygen consumption curve. Triplicate examinations were done of each concentration. The Duncan Multiple Comparison was used for final analysis.

The 24 hr LC50 was determined by keeping 10 fish in each glass aquarium each of which contained 10 liters of an LAS solution with a concentration ranging from 2.5 to 7.5 ppm. During this experiment, fish which were exposed to 7.5 and 5.0 ppm LAS expired and fish which were exposed to 2.5 ppm and 0 ppm(control) LAS after 24 hr exposure LAS were immediately sacrificed and their gills were then fixed in Bouin solution. A routine paraffin section and Hematoxylin-Eosin staining was then performed. The necrosis and edema of the gill was then histologically studied by morphometric method (McCormick 1989). Severity was graded by the readers who had no previous knowledge regarding treatments. Four grades (0, 1, 2, 3) were assigned to rank the severity of gill damage, 0 was used for the control group. Each section was randomly read twice . The third reading was required only when the two scores were different. The Chi-square test was finally applied for statistical analysis of the differences between pathological damage occurring during treatment.

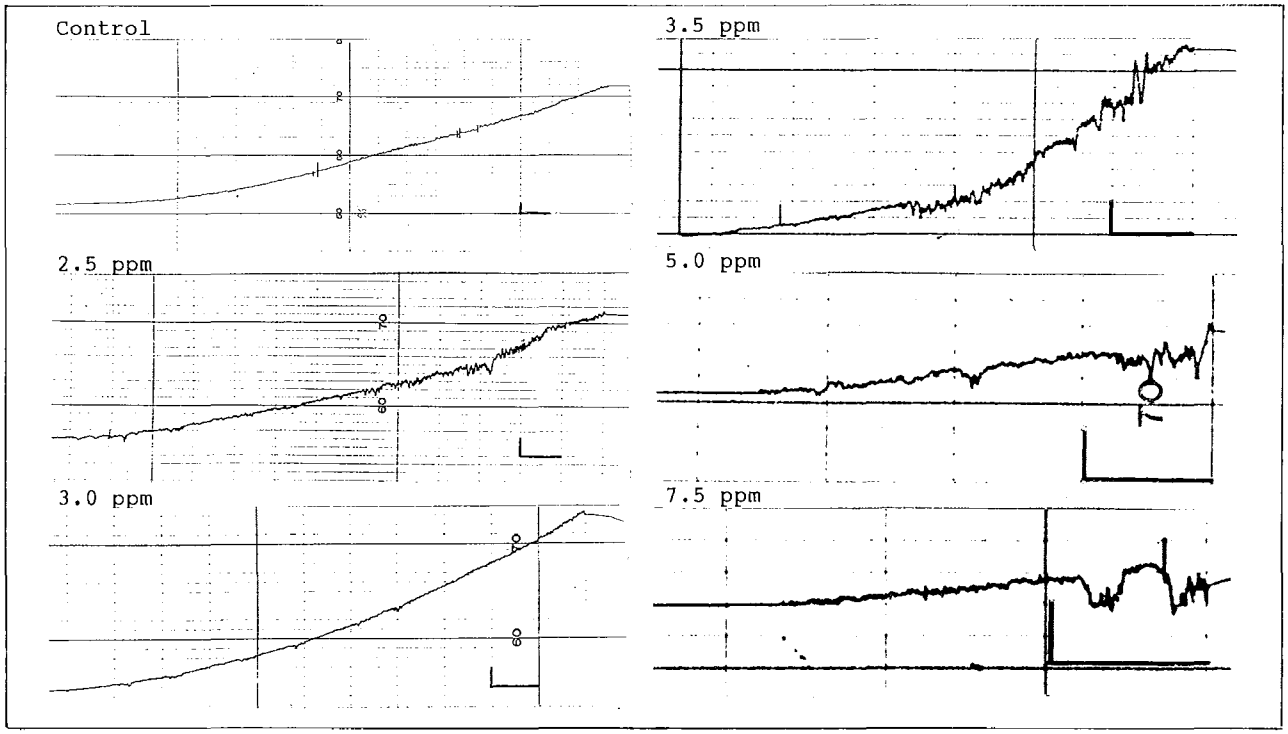

Fig. 1. Recording traces of the oxygen consumption curve after various LAS concentrations treatment. The curve should be read from the right (Time 0). The time period was defined as survival time and end point (the left of each graph) indicates the lethal oxygen concentration of each group. The slope of the curve was respiratory rate.
Calibration: 0.4 ppm, 10 min.

RESULTS

Some representative recording traces of when the fish were exposed to various concentrations of LAS. Survival time was sharply shortened when LAS concentration changed from 3.0 ppm to 3.5 ppm (Fig. 2C). Correspondingly, the lethal oxygen concentration also significantly shifted for these LAS concentrations (Fig. 2B). The average respiratory rates did not significantly change when LAS concentrations changed from 0 ppm (control) to 3.5 ppm. A sharp shifting was found only in the changing concentration of LAS from 3.5 ppm to 5.0 ppm (Fig. 2A). These results are summarized in Table 1.

The differences among each group are distinguishable as shown in Table 1. We may conclude here that LAS might have exhibited a critical effect on respiratory functions in young tigerperch.

For further modelling the lethal concentration of LAS, the mortality probability rate of tigerperch at different LAS concentrations was used in evaluating the 24 hr LC50 concentration (Buikema et al. 1982). 3.28 ppm LAS (upper limit 3.44 ppm, lower limit 3.13 ppm) was the value for young tigerperch (Fig. 3). During the first hour of the experiments, acute response (e.g. surfacing with gulping air, loss of equilibrium, erratic swimming) was observed in every group, particularly for LAS concentrations higher than 3.5 ppm. The results of the Duncan Test are revealed in Table 2 and show that the mortality rate is indicated changes sharply at 3.25 ppm. This accords well with the respiratory functions (above mentioned 3.5 ppm) and with LC50 (3.28 ppm).

Additionally, histological examinations found noticeable edema and hyperplasia (Plate 1B), exfoliation (Plate 1C), and necrosis (Plate 1D) in

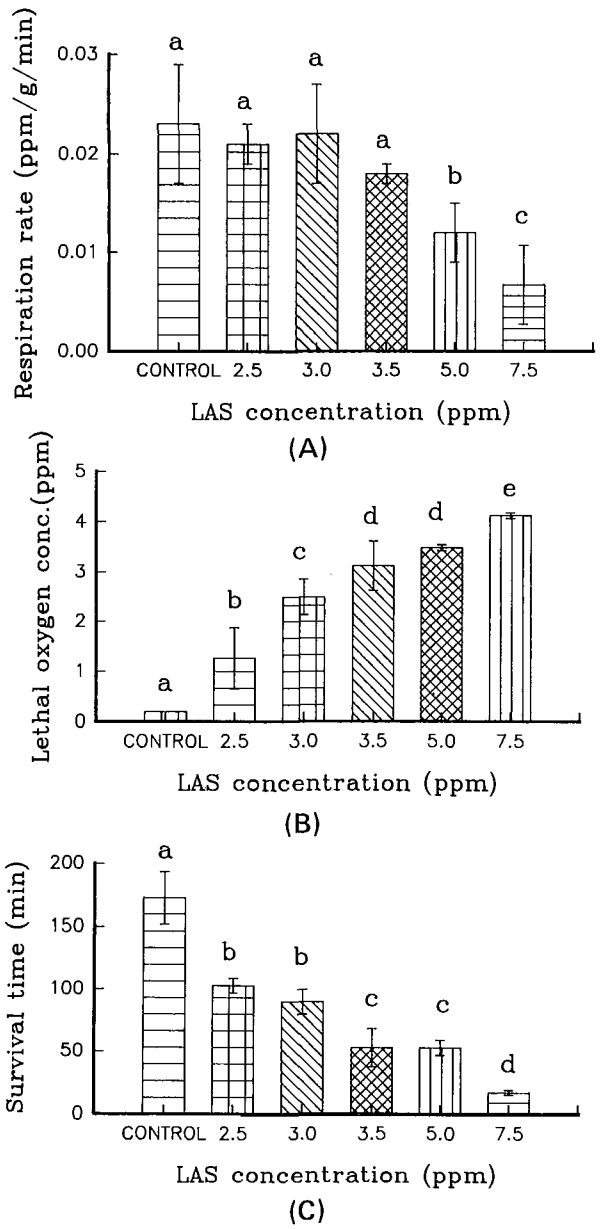

Fig. 2. Effects of LAS on respiratory functions in young tigerperch. The alphabets on the tops of each column illustrate the individual Duncan group.
A. Respiratory rate
B. Lethal oxygen concentrations
C. Survival time

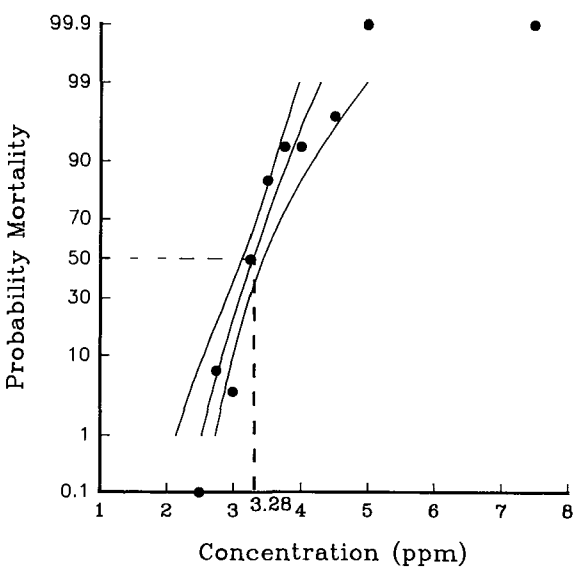

Fig. 3. 24 hr LC50 value of LAS to tigerperch determined by the probability mortality method (probit analysis, Buikema et al. 1982). The LC50 value corresponds to 3.28 ppm with a 95% confidence margin.

Table 1. Means and standard deviations of respiratory rate, lethal oxygen and survival time after treatment of various concentration of LAS. a, b, c, d, e, at the upper left corner of each value illustrates the Duncan group

Concentration	Control	2.5ppm	3.0ppm	3.5ppm	5.0ppm	7.5ppm
Respiratory rate (ppm/g/min)	a 0.023 ± 0.006	a 0.021 ± 0.002	a 0.022 ± 0.005	a 0.018 ± 0.001	b 0.012 ± 0.003	c 0.0067 ± 0.004
Lethal oxygen conc. (ppm)	a 0.20 ± 0	b 1.27 ± 0.61	c 2.50 ± 0.36	d 3.13 ± 0.50	d 3.50 ± 0.06	e 4.13 ± 0.06
Survival time (min)	a 173 ± 21	b 103 ± 6	b 90 ± 10	c 53 ± 15	c 53 ± 6	d 17 ± 2

Table 2. Means and standard deviations of mortality (%) at various concentrations of LAS. ANOVA and Duncan multiple comparison were used to group the toxic effects

LAS Concentration	24h Mortality (%)	Duncan group
control	0 ± 0	a
2.50	0 ± 0	a
2.75	10 ± 10	a
3.00	7 ± 5	a
3.25	48 ± 46	b*
3.50	83 ± 15	c
3.75	93 ± 11	c
4.00	93 ± 11	c
4.50	97 ± 5	c
5.00	100 ± 0	c
7.50	100 ± 0	c

Table 3. Damage to the secondary lamella after treatment with various concentrations of LAS. Grade 0 was used in control group while the severity was graded as 1, 2, 3. The distribution frequency was examined by Fisher's exact test

LAS Concentration (ppm)	Sample No. of each grade 0	1	2	3	Fisher's Exact Test
0.0	5	0	0	0	
1.0	0	3	2	0	a
2.5	0	5	0	0	a
5.0	0	0	2	3	b
7.5	0	0	1	4	b

the epithelium of the second lamella, the symptoms were more severe in the gills treated with higher LAS concentration (e.g. 5 ppm and 7.5 ppm) in which the space of the secondary lamella was completely filled with cell matter, the necrotic debris (plate 1D). The severity of gill damage under various LAS concentrations is shown in Table 3. Gill destruction is indicated in this table to have initiated at LAS concentrations higher than 2.5 ppm. There is a significant difference between the groups of 2.5 ppm and 5.0 ppm.

DISCUSSION

Gills are well known to be a critical respiratory organ and to be the organ most directly and seriously affected by aquatic toxins. Therefore, sublethal detergent concentrations are previously known to interfere with the respiratory functions (Rosas et al. 1988). Misra et al. (1985) explained that the enlarged pores of LAS treated gills provide a larger exposed mucosal surface trapping the needed amount of oxygen during dysfunction respiration. The effect of LAS on the activity of respiratory enzymes of the catfish gills (*Heteropneustes fossilis*) indicated that LAS has a high potential to interfere with the aerobic mechanism (Zaccone et al. 1985a). These parameters have not been investigated in an estuarine habitat of the present studied species. The results obtained from the present work did show the significant shifts of lethal oxygen concentration and survival time when LAS concentrations were changed from 3.0 ppm to 3.5 ppm. It seems probable that LAS has similar effects on the respiratory functions of tigerperch. The decreased activity of respiratory enzymes has been demonstrated by Zaccone et al. (1985b) to be important for metabolism of the epidermal cells in LAS-treated fish. This is consistent with the decrease of respiratory rates (rates of oxygen consumption) of the present experiments. The detergent, with regard to respiratory functions, notably

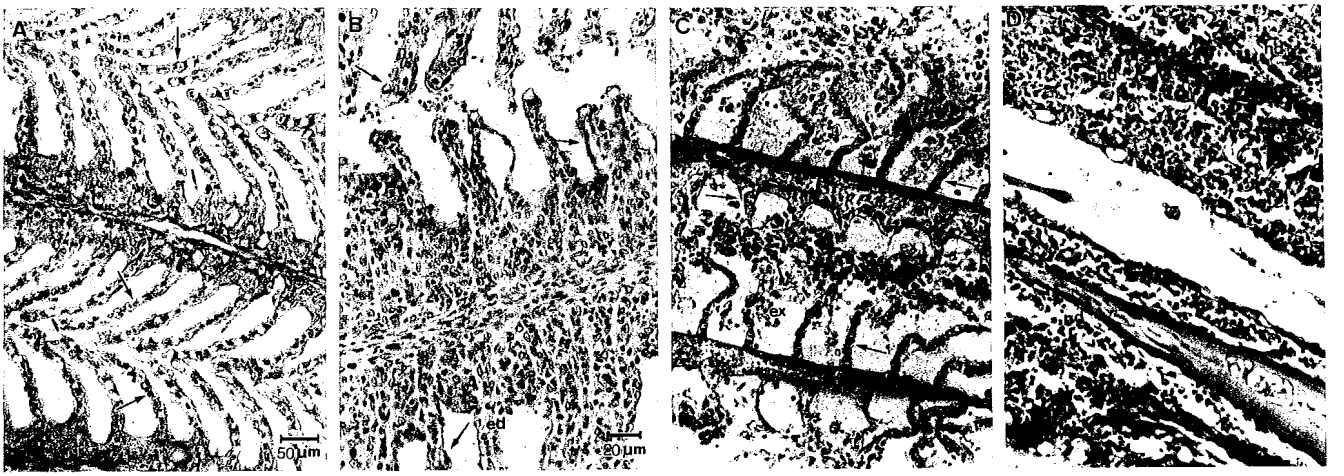

Plate 1. Morphopathologic effects of LAS on secondary lamella. Arrows show the severity of damage.
A. Control group to show the healthy second lamella (grade 0).
B. Effects of 2.5ppm LAS on gill showing a remarkable edema (ed) in the epithelium cells of secondary lamella (grade 1).
C. Effects of 5.0ppm LAS on gill showing exfoliation (ex) of epithelium cells. (grade 2)
D. Effects of 7.5ppm LAS on gill showing necrosis of epithelium cells, and the spaces between secondary lamellas were almost full with nectrotic debris (nd) (grade 3) .

interferred with respiratory structure and function and caused death among young tigerperch.

The activity of many metabolically important enzymes of the gill epithelium under LAS treatment has been observed to as becoming gradually or instantly inhibited (Roy 1989a). A sublethal effect of LAS has also been indicated by Zaccone et al. (1985b) to impair the activities of respiratory enzymes in the upper epidermis of the catfish (*Heteropneustes fossilis*). The wide spectrum of enzyme inhibitions of teleost (*Channa punctatus*) was further demonstrated by Gupta et al. (1989) to have reflected the effects of LAS and this surfactant was concluded to function as a "general" cell toxin rather than as a "specific" inhibitor. Histopathological examinations were done on gill epithelium in the present study since the gill tissue was directly exposed to the toxin, not because it is a "specific" tissue. In addition, the respiratory functions have been studied so as to correlate with the destruction of gill lamella.

The primary injury inflicted by LAS upon gill tissue was reported by Zaccone et al. (1985a) to be the progressive separation of the lamella from their vascular components. Destruction of gill epithelium has been evidenced by many reports which lead to the hypoxia (Roy 1988b, Zaccone et al. 1985b). The gill epithelium of tigerperch in the present study also appear to be affected by LAS. The most conspicuous injuries noticed in the gills exposed to sublethal concentrations of LAS (1.0 and 2.5 ppm) were the localized swellings and/or deformities of the gill lamellae. On the contrary, the presence of extensive deformity and even necrosis of the lamellae were evident after exposure in LAS concentrations higher than LC50 (5.0 and 7.5 ppm). The swelling edema, delamination and fusion of the lamella which occurred in the present study increased the diffusional distance and decreased the available surface area for gas exchange (Byrne et al. 1989). The death caused by LAS toxicity can therefore be hypothesized here to have occurred as an interference with respiratory gas exchange with consequentially resulting hypoxia.

By using a series of histochemical techniques, effect of the detergent on the gill epithelium was observed by Roy (1988b) to be instantaneous. The gradual loss of lipid components was also discovered by Roy to possibly be related to toxicity and death when the fish were exposed to anionic detergent (Roy 1989b). Sublethal concentrations of LAS were discovered by Zaccone et al. (1985b) to lead to the rupture of some club cells (which appeared in the gill epithelium and were released into intercellular space). LAS did damage lamella during this present histological examination on gill epithelium. Other effects of toxicity require further study in the future.

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清潔劑LAS對花身雞魚呼吸功能之影響

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爲評估合成清潔劑LAS (Linear Alkylbenzene Sulfonate)對花身雞魚(*Terapon jarbua*)呼吸功能之毒性作用，本實驗針對三方面主題(半致死劑量，呼吸曲線及鰓之病理變化）進行探討。

依死亡或然率(Probablity mortality)模式化求得LAS 24小時之半致死劑量濃度爲3.28 ppm；由其呼吸曲線檢定其呼吸速率，致死溶氧值及存活時間之顯著變化均出現在LAS濃度3.5 ppm和5.0 ppm之間；病理組織切片顯示鰓部之上皮組織(epithelium)出現腫脹，剝落及壞死，利用無母數的統計檢定法(Non-parametric statistic method)比較鰓部之病理變化，其顯著變化在2.5 ppm和5.0 ppm之間。

因此，清潔劑LAS對河口性花身雞魚造成鰓部組織之病變，呼吸受阻，甚或致死。

關鍵字：半致死劑量，耗氧曲線，組織病變，河口魚種。

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Zoological Studies

A New Aulopid Species, *Aulopus formosanus* (Aulopiformes: Aulopodidae) from Taiwan*

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Sin-Che Lee and Wei-Chen Chao (1994) A new aulopid species, *Aulopus formosanus* (Aulopiformes: Aulopodidae) from Taiwan. *Zoological Studies* **33**(3): 211-216. The unidentifiable species of *Aulopus* sp.1 of Masuda et al. (1984) is described and named based on observations and electrophoretic comparisons of specimens collected from Kaohsiung, Taiwan. The new species differs from all other members of the genus by lower gill-raker counts; and, among males the second dorsal ray is an extended filament. The two morphological forms are confirmed as members of the same species.

Key words: Sexual dimorphism, Electrophoresis, Congeneric species.

Aulopodidae is one of twelve families within the Order Aulopiformes, it is distinguished by having two supramaxillae, an orbitosphenoid bone, and fulcral scales on the caudal region. Only one genus *Aulopus*, with 7 species, is found throughout the tropical and subtropical seas excluding the eastern Pacific (Nelson 1984).

In Taiwan, *Aulopus japonicus* has long been regarded as the only indigenous species within the genus and was first recorded, without description, by Liang in 1951. The subsequent records of this species by Chen (1954 1956 1969 1986) and Shen (1984a,b) are made accordingly. A second species of *A. damasi* was reported by Kao and Lin in 1986. During our recent examination of the specimens stored in National Taiwan University, we found that some specimens had been misidentified as *A. japonicus*. In addition, the species identified as *A. japonicus* by Shen (1984b) is identical to the *Aulopus* sp.1 described by Masuda and his colleagues in 1984. This raises an interest in pursuing a further clarification of the systematic status of the entire *Aulopus* species, since other species may have been included in the previous records by mistake.

After a close examination of 30 specimens (16 males and 14 females) collected from Kaohsiung, we found that none of them fit the typical *A. japonicus* description; both sexes have fewer gill-rakers and only the 16 males had a second dorsal ray extending into a filament. The above characteristics quite agree with those shown in pl.61-c of Masuda et al. (1984) as *Aulopus* sp.1.

Since the two morphological types of *Aulopus* are found together in Taiwan, to avoid possible confusion of the 30 specimens with respect to the presence or absence of a dorsal filament, we employed electrophoresis for enzymes and none enzymatic muscle proteins. Thus, the purpose of this paper is to describe a new species and biochemically confirm whether or not the two morphological types belong to one species.

MATERIALS AND METHODS

A typical *Aulopus japonicus* specimen used here for comparison is preserved and was collected from Nanfanao, Taiwan in November, 1970 (ASIZP 054460; SL. 155.3 mm; male). The 30 specimens used in this study were collected from the coastal waters off Kaohsiung, Taiwan from a depth of 30 m. They were frozen immediately after capture and brought to the laboratory deep freezer (– 75°C)

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as soon as possible. The skeletal muscle, eyes, and liver of each specimen were removed and were homogenized for electrophoresis. The following enzyme system (and loci scored) were analyzed by starch gel electrophoresis: asparte aminotransferase (*AAT-A, B*), creatine kinase (*CK-A*), glucosephosphate isomerase (*GPI-A, B*), isocitrate dehydrogenase (*IDH-A*), lactate dehydrogenase (*LDH-A, B, C*), malate dehydrogenase (*MDH-A, B*), malic enzyme (*ME-1*), phosphoglucomutase (*PGM-A, B*), and general muscle proteins (*Gp-1, Gp-2, Gp-3*). The enzyme and buffer system employed are listed in Table 1. The electrophoresis procedure follows that of Tsoi et al. (1987). The TC buffer running time is 4-5 hours while that for the EBT buffer, about 12 hours.

The remaining body was fixed and preserved in 10% neutral formalin for further inspection of external characteristics including morphometrics. The body proportion measurements and meristic counts follow Lee (1980). Locus nomenclatures follow Shaklee et al. (1990).

RESULTS

Aulopus formosanus sp. n.

(Figs. 1-2; Tables 2-3).

Aulopus sp.1.: Masuda et al., 1984: 60.

Aulopus japonicus (non Gunther): Shen. 1984b: 15.

Holotype: ASIZP 056190, male, 169.4 mm SL, August 13, 1987, Kaohsiung.

Paratypes: ASIZP 056153, male, 138 mm SL, March 13, 1987, Kaohsiung; ASIZP 056142, female, 190.9 mm SL, April 1987, Kaohsiung. Other 30 unregistered paratypes (16 males and 14 females), 148.9-248.6 mm SL, April to August 1987, Kaohsiung.

Diagnosis: Meristic data and morphometric data based on holotype and 30 paratypes. D.14-16 (modally 16), A.8-10 (modally 9), P.11, branched caudal rays 17, L1.39-44 (modally 42), Ltra.5-6 (modally 5), vertebrae 40-43 (modally 42), GR (developed) 4-5 + 10-12 = 14-17. Body moderately elongated, depth 4.51-5.89 in standard length (or 22.2-17% of SL). Orbit larger than snout measured 31.0-37.5% of HL, maxilla with two well developed supramaxillae. Second dorsal ray longer, extending into a rather long filament in male only, measuring 32.4-51.9% of SL in contrast to 14.4-18.0% in female. Caudal region with fulcral scales. Pyloric caeca absent. Color when fresh, pinkish red with irregularly scattered black spots on back and two rows of violet red spots on lateral side. Dorsal fin with yellowish spots in male and reddish spots in female. Anal fin with a median yellowish stripe in male only.

Description of holotype: Counts and measure-

Table 1. Enzyme system tested and electrophoretic buffers employed, with a list of loci scored and allelic frequencies of two morphological *Aulopus* sp. types

Enzyme (abbr.)	E.C.No.	Buffer	Tissue exam.	Locus	Allele	Morph A male ($n = 14$)	Morph B female ($n = 16$)
Aspartate aminotransferase (AAT)	2.6.1.1	TC	liver	*AAT-1**	*100*	1.00	1.00
				*AAT-2**	*100*	1.00	1.00
Creatine kinase (CK)	2.7.3.2	EBT	muscle	*CK-A**	*100*	1.00	1.00
Glucosephosphate isomerase (GPI)	5.3.1.9	EBT	muscle	*GPI-A_2**	*100*	1.00	1.00
				*GPI-B_2**	*100*	1.00	1.00
Isocitrate dehydrogenase (IDH)	1.1.1.42	TC	heart	*IDH-A**	*100*	1.00	1.00
Lactate dehydrogenase (LDH)	1.1.1.27	TC	muscle	*LDH-A_4**	*100*	1.00	1.00
			heart	*LDH-B_4**	*100*	1.00	1.00
			eye	*LDH-C_4**	*100*	1.00	1.00
Malate dehydrogenase (MDH) (NADsuperanatant)	1.1.1.37	TC	muscle	*S-MDH-A**	*100*	1.00	1.00
			eye	*S-MDH-B**	*100*	1.00	1.00
Malic enzyme (ME)	1.1.1.40	TC	heart	*ME-1**	*100*	1.00	1.00
Phosphoglucomutase (PGM)	2.7.5.1	EBT	muscle	*PGM-A_2**	*100*	1.00	1.00
				*PGM-B_2**	*100*	1.00	1.00
General proteins		EBT	muscle	*Gp-1**	*100*	1.00	1.00
				*Gp-2**	*100*	1.00	1.00
				*Gp-3**	*100*	1.00	1.00

Fig. 1. *Aulopus formosanus* sp. n., holotype, ASIZP 056190, male, 169.4 mm SL, August 13, 1987, Kaohsiung.

Fig. 2. *Aulopus formosanus* sp. n., paratype, ASIZP 056142, female, 190.9 mm SL, April 1987, Kaohsiung.

Table 2. Counts and measurements of *Aulopus formosanus* sp. n.

	Holotype	Paratypes	
		Morph A Range (mean)	Morph B Range (mean)
Standard length (mm)	169.4	147.5-229.5	152.8-205.6
Sex	male	male	female
No. fish examined	1	16	14
In % standard length			
Head length	32.5%	30.7-33.2% (31.9%)	31.1-33.4% (32.4%)
Body depth	20.4%	17.0-22.2% (19.5%)	17.6-21.5% (19.5%)
Longest dorsal ray	36.3%	32.4-51.9% (39.6%)	14.4-18.0% (16.8%)
Pectoral fin	19.5%	19.2-22.5% (21.0%)	18.6-20.5% (20.0%)
In % head length			
Eye diameter	37.5%	31.0-36.6% (33.8%)	32.7-35.3% (33.9%)
Snout length	26.7%	24.2-27.3% (25.6%)	23.8-27.7% (25.2%)
Dorsal rays	16	15-16(mode 16)	14-16(mode 16)
Anal rays	10	9-10(mode 9)	8-9(mode 9)
Ventral rays	9	9	9
Pectoral rays	11	11	11
Branched caudal rays	17	17	17
Scales on lateral line	41	39-41	40-44
Scales from origin of dorsal fin to lateral line	5	5-6	5-6
Total gill rakers (lower limb)	15 (12)	14-17 (10-12)	14-16 (10-12)
Second dorsal rays filamentous	yes	yes	no
Coloration of spots on dorsal fin	yellow	yellow	red
A median yellowish stripe on anal fin	present	present	absent

Table 3. Comparisons of some critical characteristics among *Aulopus formosanus*, *A. japonicus*, *A. microps*, and *A. damasi*

	A. formosanus $n=30$	*A. japonicus*[1] $n=11$	*A. microps*[1] $n=33$	*A. damasi*[2] $n=1$
Dorsal rays	16	16-17	16-17	14
Anal rays	8-10(9)	9-10	10-11	8-9
Pectoral rays	11	11	11	13
Second dorsal ray prolonged in male	yes	no	no	no
Second dorsal ray prolonged in female	no	no	no	no
Pyloric caeca	0	9-11	0	not examined
Total gill rakers	14-17	17-25	21-23	20
Scales on lateral line	39-44	41-44	43-45	36
Vertebrae	40-43	42-43	41-43	not examined
Size of orbit larger or smaller than snout	larger	larger	smaller	smaller

[1]Based on Parin and Kotlyar 1989.
[2]Based on Kao and Lin 1986.

ments of the holotype and paratypes are shown in Table 2.

Body moderately elongated, slightly compressed laterally, greatest depth 20.4% SL. Orbit slightly larger than snout, 37.5% of HL, interorbital space concave, about half the width of orbit. Mouth terminal, very wide, maxilla reaches slightly beyond the posterior margin of pupil, with two well developed supramaxillary bones. Small conical tooth bands on jaws and palatines. The palatine tooth band is fused with those on the vomer and pterygoids. Body covered with ctenoid scales, except those on the caudal region which appear fulcral; forty one scales on lateral line and 5 between the origin of the dorsal and lateral line. Adipose fin present, base of dorsal fin long, 1.65 times as long as the distance between rear end of dorsal fin and origin of adipose fin, 16 rays, the anterior two rays simple, the second extending into a filament, measuring 36.3% of SL. The predorsal length is much greater than the distance between the posterior end of the dorsal fin and the anterior margin of the adipose fin. Anal base short, 10 rays. Pectoral fin in lower position, originating slightly before the dorsal fin, 11 rays. Ventral fin thoracic, with 9 rays, the anterior four slightly thickened. Caudal fin deeply forked with 17 branching rays. Number of gill-rakers on lower limb of first left arch 12 (total 15), longer. Gill-rakers on the second arch shorter while those on the third and fourth arches are bulb-like, consisting of spinule patches. Pyloric caeca absent.

Color when fresh pink with several irregularly scattered black spots on the back and two rows of purplish red spots on the lateral side. Dorsal fin with yellowish spots in male and reddish spots in female. Anal fin transparent, with a yellowish median stripe in male, absent in female. Caudal fin yellowish with pink spots in both sexes.

Etymology: The name for this species is given because of its predominancy in Taiwan (Formosa).

Electrophoretic comparisons between two types of *Aulopus formosanus*:

In order to confirm the hypothesis that among our *Aulopus formosanus* specimens the 14 females have a shortened second dorsal ray and the 16 males a filamentous second dorsal ray, genetic implications were taken into account. As indicated in Table 1, the 17 loci with 17 alleles scored in both sexes are shown to be identical, giving evidence to support that they belong to same species. All the loci investigated are monomorphic among all individuals.

DISCUSSION

The status of genera *Aulopus* and *Hime* were discussed previously by several authors: *Hime* was separated as a valid genus from *Aulopus* in 1924 by Starks based on its weaker or absence of ossification in the anterior interorbital region (orbitosphenoid), and a longer dorsal base. The most recent authors, Parin and Kotlyan (1989) agree with this classification. However, Mead (1966), Nelson (1984), and the present authors recognized *Hime* as a generic synonym for *Aulopus*. Regarding the *Aulopus* fauna in Taiwan, the two other types

frequently encountered in Taiwan along with the existing *Aulopus japonicus* and *A. damasi* is the subject specimen. We conclude from electrophoretic comparison that the subject specimens are *Aulopus formosanus*, and that the presence of a filamentous ray and a yellowish median stripe on the anal fin, are simply a sexual dimorphism. Still, in the case of two morphologically similar Hawaiian bone-fish *Albula* (Shaklee and Tamura 1981) and three Hawaiian lizard fish, *Saurida* (Waples 1982), revealed a high level of fixed allelic differences. This is applicable to the two *Aulopus* sp. morphs; no differences were found. Consequently, both forms belong to the same species.

Though *Aulopus japonicus* is the earliest recorded Taiwan species, it is much less common than the present new species, *A. formosanus*. Although not available as fresh material for electrophoretic comparison with *A. formosanus*, an old preserved specimen of *A. japonicus* proved readible for morphological inspection. The following characteristics seem to illustrate some differences between the two species: male *A. formosanus* has a filamentous second dorsal ray; wheareas, male *A. japonicus* has no filaments. *A. formosanus* has fewer gill-rakers, 10-12 on the lower limb as opposed to that of 15 in *A. japonicus*. Pyloric caeca is absent in *A. formosanus* but present in *A. japonicus*. Moreover the anterior portion of the dorsal fin has a large reddish blotch in male *A. japonicus*. The present new species can also be separated from *A. filamentosus* of the Mediterranean Sea and the Atlantic Ocean, and *A. purpurissatus* of Australia. *A. filamentosus* classified by Gunther (1864:402) has both second and third dorsal ray filaments, and 11-12 anal rays. *A. purpurissatus* calssified by Gunther (1864:403) has the second and third dorsal ray filaments too, and has more dorsal rays (19-22) and anal rays (12-14). This new species is more closely related to *A. japonicus* than *A. filamentosus* and *A. purpurissatus* based on some morphological characteristic. *A. formosanus* differs from *A. damasi* in that the latter species has fewer dorsal rays (14) and a smaller orbit (shorter than the snout). When compared with the recently published *Hime microps* Parin and Kotlyan (1989) (= *Aulops microps*), *A. formasanus* is the most closely-related. It shares the following characteristics: absence of pyloric caeca, similar fin rays counts, lateral line scales, and vertebrae. However, they are readily recognizable by lower gill-raker counts (14-17 vs. 21-23), the prolonged second dorsal ray in male, and larger oribit (31.9-37.5% vs. 22.5-24.3% of head length).

In the case of female *A. formosanus, A. japonicus, A. microps*, and *A. damasi*, though resembling one another in external appearance, they differ in gill-raker counts, orbit size, and the presence or absence of pyloric caeca (Table 3).

It is concluded that to date there are three aulopid species in Taiwan, namely *A. formosanus, A. japonicus*, and *A. damasi*. Their distinctive characteristics are summarized in the following key:

1. Dorsal rays 14; orbit shorter than snout ..... *A. damasi*
   Dorsal rays 16-17 (mostly 16); orbit equal or larger than snout 2
2. Second dorsal ray of male prolonged into a filament; gill-rakers on lower limb of first arch 10-12; pyloric caeca absent ..... *A. formosanus*
   Second dorsal ray of male not prolonged; gill-rakers on lower limb of first arch 14 or more; pyloric caeca 9-13 ... . *A. japonicus*

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台灣產新種仙女魚(*Aulopus formosanus*)之記述

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益田氏等1984年書中所記之未定名仙女魚*Aulopus* sp.1，經本作者確定爲一有效新種，並經命名爲台灣仙女魚(*Aulopus formosanus*)。該種魚類之有別於同屬之其他仙女魚在於雄性第二背鰭軟條呈顯著之絲狀延長，且鰓耙數亦較少。與具絲狀延長鰭條之雄魚同時採獲之不呈絲狀延長之雌魚，經同功異構酶及肌蛋白之電泳分析比較結果，證實二者同屬一種。

關鍵詞：性徵，電泳，屬內種間比較。

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