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Effects of Temperature and Floating Materials on Breeding by the Paradise Fish (*Macropodus opercularis*) in the Non-reproductive Season

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Wen-Bin Haung and Fang-Lin Cheng (2006) Effects of temperature and floating materials on breeding by the paradise fish (*Macropodus opercularis*) in the non-reproductive season. *Zoological Studies* **45**(4): 475-482. The paradise fish, *Macropodus opercularis*, is native to Taiwan, and its reproductive season spans from Mar. to Oct. This experiment was conducted to examine paradise fish breeding in winter, a non-reproductive season, using different treatments of water temperatures (23, 27, and 31°C) and floating materials (floating ferns, green Styrofoam pieces, and no floating material). The fish built 1-3 bubble nests during the 20 d experimental period. A significant negative correlation was found between the temperature and the frequency of nest building, indicating that a high water temperature of 31°C was unfavorable for building nests. In the treatments with floating ferns and green Styrofoam pieces, the fish built more nests than in the treatment without floating materials. The sizes of the 1st bubble nests built were significantly larger at 27 and 31°C than at 23°C. Floating materials played an important role after the fish acclimated to the temperature. In the treatment with green Styrofoam pieces, the fish built smaller-sized 2nd nests than in the treatment without floating materials. One female in a tank treated at 27°C with green Styrofoam pieces laid 421 eggs during the 20 d experimental period. Two hundred and eighty larvae hatched the next day, for a hatching rate of 66.5%. In short, the paradise fish can breed at appropriate temperatures, such as 27°C, in winter, normally a non-reproductive season, and artificial floating materials are conducive to successful reproduction.
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Key words: Bubble nest, Floating fern, Formosan fighting fish, Anabantidae, Reproduction.

The paradise fish, *Macropodus opercularis* (Linnaeus), belongs to the family Anabantidae in which most of the members are "bubble-nest" builders (Forselius 1957). This species is characterized by the presence of a labyrinthiform organ, which is derived from the 1st gill arch and enables the fish to breathe in the air (Lagler et al. 1977). Floating plants are usually dominant in spawning areas of the fish (Jan 1994). Plants or other substrates are necessary to hold the nest in place (Degani 1989). The fish sexually matures at approximately 6 mo, breeds well in aquaria, and reaches a maximum size of approximately 80-100 mm in standard length (Forselius 1957, Hall 1968).

The paradise fish is native to China and nearby islands (Hall 1968). It is widely distributed in eastern Asia from the Yangtze River basin to Hainan I. in China, as well as Taiwan and North Vietnam (Freyhof and Herder 2002). The range of the fish's habitation is 20-30°N and 102-122°E, and its climatic temperature range of 16-26°C (Fishbase 2005) indicates that it is a subtropical and temperate fish. Indigenous to Taiwan, it is commonly known as the Formosan fighting fish, three-spot fish, and Chinese unicorn fish (Chen and Fang 1999, Shao and Chen 2003). Prior to the mid-1970s, the fish was widely distributed in lowland areas of Taiwan where it inhabited bodies

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of fresh water ranging in size from rice paddy fields to lakes (Tzeng 1990, Jan 1994, Young 1995, Shao and Chen 2003). *Salvinia natans*, *Lemna minor*, and *Pistia stratiotes* are floating plants to which the fish's bubble nests are commonly found to be attached in Taiwan (Jan 1994, Huang et al. 1998). The reproductive season of the fish spans from Mar. to Oct. in Taiwan (Jan 1994), and is particularly concentrated from May to July (Chen and Fung 1999, Shao and Chen 2003). In the last few decades, this fish has become rare in Taiwan (Shen 1993, Chen and Fang 1999). The construction of farmland irrigation canals and ditches and the heavy use of pesticides and insecticides in agriculture may be the main reasons why its abundance has fallen (Tzeng 1990, Young 1995). For the protection of the fish, it was listed as a rare and valuable species in the *Wildlife Conservation Law* on 31 Aug. 1989 by the Taiwanese government in order to ban the catching, killing, and selling of the species (ESRI 1996). However, to the present, studies of the conservation and enhancement of this fish species are still rare in Taiwan.

Temperature is one of the most potent environmental factors that influence the development and growth of fish (Herzig and Winkler 1986). Food intake, catabolism, and conversion rates of the food consumed by fish vary with body temperature (Lagler et al. 1977). The body temperatures of most fish are close to and do not exceed 1°C difference from the ambient water temperature, suggesting that water temperature plays an important role in the life of fish (Huang and Chiu 1993). In temperate regions, the timing of reproduction in annually spawning species is controlled by an endogenous cycle that in turn is entrained by environmental cues (Scott 1979, Bye 1990, Scott and Pankhurst 1992). Water temperature is one of the most important annual environmental cues (Bye 1990).

Numerous studies on the paradise fish have been conducted in the past. In the middle of the last century, most of the studies were concentrated at the family level of the fish. For example, the behavior, distribution, and endocrinology of several anabantoid species, *M. opercularis* included, were studied by Forselius (1957), the osteology and phylogeny were examined by Liem (1963), and the physical and physiological factors influencing spawning were evaluated by Hall (1966). At the end of the last century, studies of the fish mainly focused on behaviors associated with avoiding predation (Csanyi 1985 1986, Gerlai 1993, Miklosi et al. 1997, Miklosi and Csanyi 1999) and intraspecific aggression (Kassel and Devis 1975, Francis 1983, Miklosi et al. 1997), and the effects of environmental toxicants (Yin and Zhang 1986, Zhang and Yin 1986). To study the reproductive physiology, prostaglandin was injected into the paradise fish to study the effects of the hormone on its reproductive behavior by Villars et al. (1985) and Villars and Burdick (1986). As to the effect of temperature, Lindsey (1954) discussed the influence of temperature on the development of meristic characteristics in *M. opercularis*. Grodzinski (1971) indicated that the range of water temperature for the survival of paradise fish larvae was 8-41°C. However, our understanding of the physiological ecology of the fish, such as the effect of temperature on breeding, is limited. The effects of temperature and floating materials on the breeding of the fish in the non-reproductive season are still little understood.

In recent years, people in Taiwan have been begun to pay more attention to environmental education and teaching about native organisms. The paradise fish is one of the species native to the island. It likes to eat mosquito larvae and may be effective in helping prevent dengue fever (Shen et al. 1991); it is also easy to rear in captivity (Gerlai 1993, Young 1995). The reproductive behavior of the fish at 30°C was observed in Taiwan by Huang et al. (1998) with a video camera. The genetic diversity of the fish in Taiwan was examined by Wang et al. (1999) using mitochondrial DNA. Gaining a better understanding the effects of environmental factors on the reproduction and breeding of native fish is very important for their conservation.

The objective of this study was to examine the effects of temperatures and floating materials on the breeding of paradise fish during winter, normally a non-reproductive season. Two hypotheses were tested: (1) the paradise fish can breed by raising temperatures in winter, a non-reproductive season, and (2) floating materials are conducive to the reproductive success of the fish.

MATERIALS AND METHODS

The paradise fish used in this study were obtained from the Aquatic Breeding Institute of the Hualien County government in eastern Taiwan. The fish were bred and reared at the institute for about 1.5 yr. Males and females of approximately the same size were selected to reduce size effects in our study. After being brought to the laboratory on 13 Dec. 2004, male and female fish were maintained and acclimated in separate containers (60 x 30 x 35 cm) for 2 wk at 27 ± 1°C, with a 12: 12-h light: dark regime. They were fed frozen bloodworms twice a day (at 08:00 and 20:00).

After the acclimation period, 1 male and 1 female paradise fish were randomly selected from the separate containers and placed together in a round-shaped tank (with a radius of 9 cm and a height of 15 cm) on 26 Dec. 2004 (day 0). The water depth was kept at 10 cm, and the water volume was 2.5 L. The experimental period was in winter from 27 Dec. 2004 (day 1) to 15 Jan. 2005 (day 20), usually a non-reproductive period for the fish.

According to the current literature, the reproductive season of the paradise fish is particularly concentrated from May to July in Taiwan (Chen and Fang 1999, Shao and Chen 2003). The mean atmospheric temperature from May to July for 1971-2000 in lowland areas of Taiwan was 27.2 ± 1.5°C (mean ± SD), and ranged from 24.2 to 29.3°C (Central Weather Bureau 2005). This mean temperature (27°C) was used as the acclimation temperature in this study. Low-, middle-, and high-temperature treatments were then set at 23, 27 and 31 (± 1)°C using heaters in a cold room. For the purpose of imitating the natural habitat of the paradise fish, floating plants were placed in the tanks (Young 1995, Huang et al. 1998). The floating fern (*S. natans*), which is an endemic floating plant in Taiwan and is commonly used by paradise fish as material to which bubble nests are attached in their natural habitats, was used in this study, and the size of the area it occupied was controlled to about 3 x 6 cm in each tank. In addition, in order to examine whether floating materials are conducive to the reproductive behavior of the fish, a piece of green Styrofoam was used as a substitute for the floating fern of the same area (3 x 6 cm). There were 3 floating material treatments: floating ferns, green Styrofoam pieces, and no floating materials. Three replications were carried out for each of the temperature/floating-material treatments, resulting in 27 pairs of fish tested in this study. None of these fish were used more than once to avoid the order effect. The photoperiod (12: 12-h L: D) and feeding schedule (1.0 g frozen bloodworms per tank twice per day) were the same as those used during the acclimation period.

Because the fish alters its appearance after anesthetization (Kodric-Brown 1989), an additional 14 individuals of each sex were randomly selected and anesthetized for the purpose of body size measurement in this study. Standard lengths and body weights of the fish were measured to the nearest 0.1 mm and 0.1 g, respectively. The mean standard length (SL) and mean body weight (BW) of the male and female paradise fish used in this study were 58.4 ± 2.7 mm and 6.4 ± 1.0 g, and 55.5 ± 2.2 mm and 4.8 ± 0.5 g, respectively.

An inspection was made daily at 08:00 to observe and record the breeding of the paradise fish from days 1 to 20. Images of the bubble nests, if present, were taken with a digital camera using a standard scale. Sizes of the bubble nests were measured by calculating their area in the digital image using the software Motic Images 2000 vers.1.3. The number of eggs, which are light yellow and solid, spawned by the fish was counted from the image. The counts were repeated until the same count was obtained 3 times. Hatched larvae, if present, were counted and removed from the tanks by a dropper during the inspection.

The Pearson product-moment correlation coefficient was used to assess the relationship between the frequency of bubble nest-building and temperature treatments (Neter et al. 1989). In addition, analysis of covariance (ANCOVA) was used to analyze the effect of the floating-material treatments with temperature as the covariate. Two-way analysis of variance (two-way ANOVA) was used to determine the significance level of differences of the temperature and floating-material treatments (independent variables). The dependent variable was the size (area) of the bubble nest built, which was logarithmically transformed for normalization. The least significant difference test (LSD) was used as a post hoc procedure to test for differences among the treatment groups. The software used for the statistical calculations was SPSS (Chicago, IL, USA) for Windows vers. 10.0, and all statistical tests were considered significant at $p < 0.05$.

RESULTS

The paradise fish in this study built 1-3 bubble nests during the 20 d experimental period (Fig. 1). The frequency was negatively correlated to the temperature treatment ($r = -0.543$, $F_{1, 21} = 8.786$, $p = 0.007$). The frequency, which was adjusted with the temperature (ANCOVA: $F_{1, 19} = 14.500$, $p = 0.001$), significantly differed among the floating-material treatments ($F_{2, 19} = 8.257$, $p = 0.003$). The frequency was significantly higher in the floating-fern (3.00 ± 0.00) and Styrofoam (2.71 ± 0.49) treatments than that in the treatment without floating materials (2.13 ± 0.84) (LSD: $p < 0.05$).

In 20 of the 27 experimental tanks, bubble nests were found 1 d after the paradise fish were paired together. In 3 tanks, bubble nests were found 3 d after pairing. Aggression between fish was noted in the remaining 4 tanks, including 1 tank each for the treatment at 23°C without floating materials, at 23°C with Styrofoam pieces, at 23°C with floating ferns, and at 31°C with Styrofoam pieces. The sizes of the 1st bubble nests built significantly differed among the temperature treatments (two-way ANOVA: $F_{2, 14} = 10.466$, $p = 0.002$), but the effects of the floating-material treatments and their interactions were not significant ($p > 0.05$). The sizes (mean ± SD) of the nests built at 27 (38.2 ± 9.0 cm^2) and 31°C (31.5 ± 8.8 cm^2) were significantly larger than those at 23°C (20.2 ± 3.9 cm^2) according to the post hoc procedure (LSD, $p < 0.05$) (Fig. 2A).

There were 21 tanks in which a 2nd bubble nest was found 3-10 d after the 1st bubble nests were built (Fig. 2B). In the other 2 tanks, for which treatment consisted of 31°C without floating materials, no 2nd bubble nests were found during the 20 d experimental period. Since there was only 1 tank left treated at 31°C without floating materials, this treatment was excluded from the following

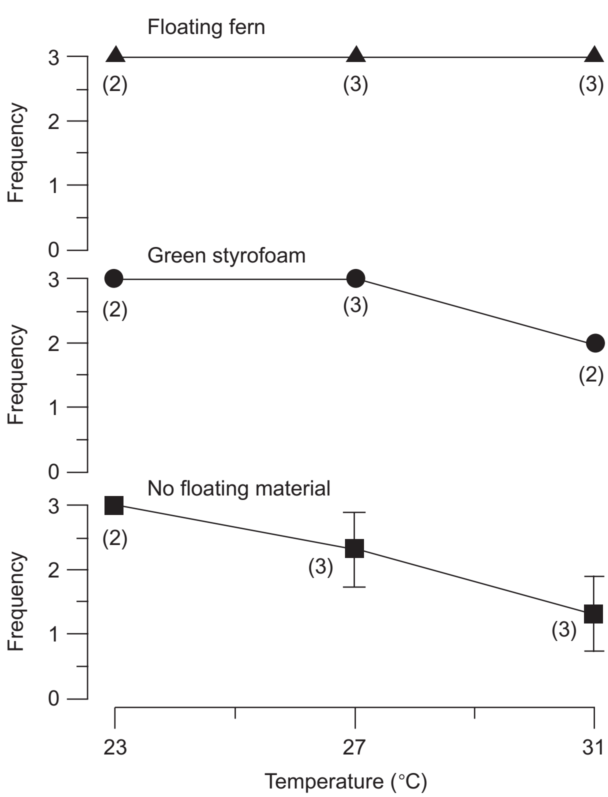

Fig. 1. Frequency of bubble-nest building in the different temperature and floating-material treatments by the paradise fish. Numbers in parentheses are the numbers of fish pairs. The length of the perpendicular line indicates the standard deviation.

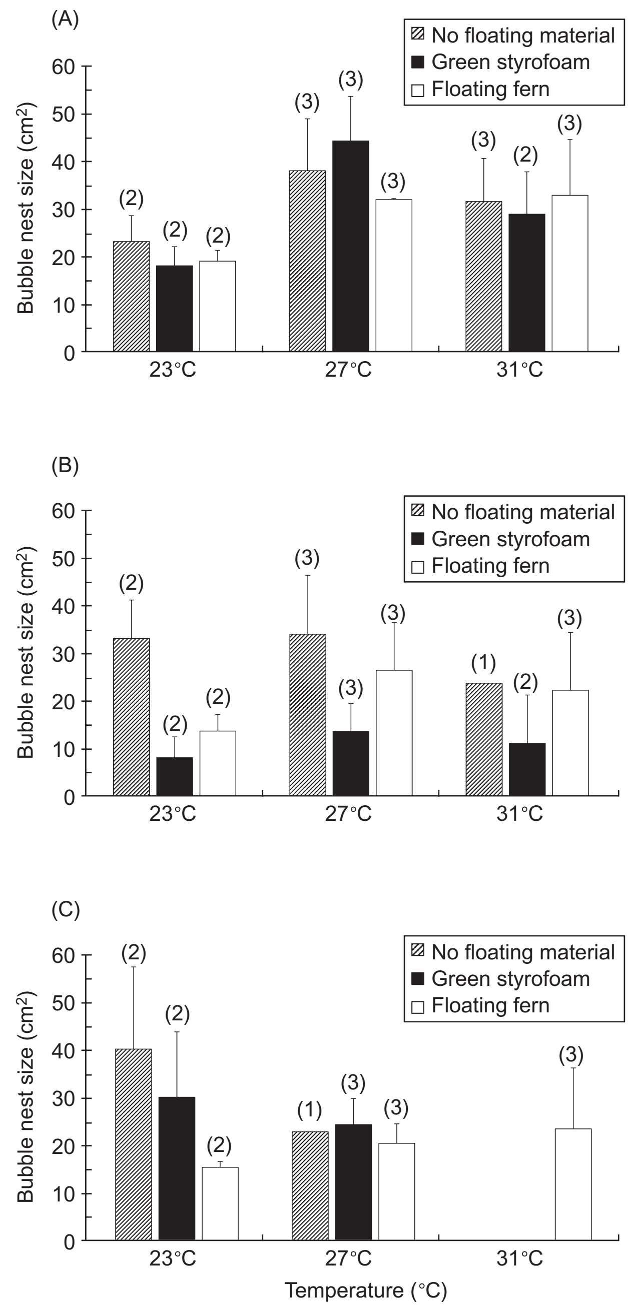

Fig. 2. Sizes of the 1st (A), 2nd (B), and 3rd (C) bubble nests built in the different temperature and floating-material treatments by the paradise fish. Numbers in parentheses are the numbers of fish pairs. The length of the perpendicular line indicates the standard deviation.

analysis. The areas of the 2nd bubble nests significantly differed among the floating-material treatments (two-way ANOVA: $F_{2, 12} = 5.402$, $p = 0.021$), but the effects of the temperature treatments and their interactions were not significant ($p > 0.05$). The areas (mean ± SD) of the bubble nests built in the tanks with the green Styrofoam pieces (12.0 ± 6.6 cm^2) were significantly smaller than those in the tanks without floating materials (31.6 ± 9.4 cm^2) according to the post hoc procedure (LSD, $p < 0.05$) (Fig. 2B). The area (20.0 ± 11.4 cm^2) of the bubble nests built in the tanks with the floating ferns did not differ from those in treatments without floating materials or with the green Styrofoam pieces (LSD, $p > 0.05$).

There were 16 tanks in which 3rd bubble nests were found 3-9 d after the 2nd bubble nests were built (Fig. 2C). In the remaining 5 tanks, treated at 31°C without floating materials (1 tank), at 31°C with Styrofoam pieces (2 tanks), and at 27°C without floating materials (2 tanks), no 3rd bubble nests were found during the 20 d experimental period. Because there were 3 floating-material/temperature treatments in which fewer than 2 tanks had 3rd bubble nests, one-way ANOVA was employed in place of two-way ANOVA to determine the significance level of the differences of the temperature treatments in the groups with floating ferns and floating materials at 23°C. The areas of the 3rd bubble nests did not significantly differ among the temperature treatments in the group with floating ferns (one-way ANOVA: $F_{2, 5} = 0.349$, $p = 0.721$) or among the floating-material treatments at 23°C (one-way ANOVA: $F_{2, 3} = 3.078$, $p = 0.188$) (Fig. 2C).

One female in a tank treated at 27°C with Styrofoam pieces laid 421 eggs on the 9th day. Two hundred and eighty larvae hatched the next day, for a hatching rate of 66.5%.

DISCUSSION

Influence of temperature on reproductive behaviors

Initiation of bubble-nest building by the paradise fish indicates that the reproduction of the fish is commencing (Tsai 1992, Huang et al. 1998). Bubble-nest building is the 1st step of the fish ethogram defined by Hall (1968). In our study, 81.5% (20 of 24) pairs of fish built their 1st bubble nest 1 d after a male and female were placed together during the winter, which suggests that reproduction can proceed in the non-reproductive season under the conditions used in this experiment. The acclimation temperature of 27°C, which is the mean atmospheric temperature of the main reproductive season for the fish in lowland areas of Taiwan, may be favorable for reproduction of the fish and for maintaining its testes in a reproductively active state. Bubble-nest building was triggered when a male and female were put together at this temperature.

A bubble nest will daily decrease in size if there is no supplementation from subsequent bubble-nest building. A larger bubble nest means more-efficient oxygenation, and a larger bubble nest is able to support more eggs and larval fish (Jaroensutasinee and Jaroensutasinee 2001). If the male fish wants to achieve higher reproductive success, it will maintain or enhance the size of the nest as best as it can. The male fish were in good condition in our study; therefore, they built 2nd and sometimes 3rd bubble nests.

A significant negative correlation ($r = -0.543$) was found between the frequencies of bubble-nest building and temperature within the range of 23 to 31°C (Fig. 1). Habitat temperatures of the paradise fish, a subtropical and temperate fish, range 16-26°C (Fishbase 2005). In Taiwan, the monthly mean atmospheric temperature during the main reproductive season, from May to July, of the fish ranged from 24.2 to 29.3°C for 1971-2000 (Central Weather Bureau 2005). These figures indicate that temperatures higher than 30°C are unfavorable for paradise fish bubble-nest building. However, the sizes of the nests built at 27 and 31°C were significantly larger than those at 23 °C (Fig. 2A). Significantly, a temperature of 27°C was the mean atmospheric temperature during the main reproductive season of the fish in lowland areas of Taiwan from 1971 to 2000. These results suggest that temperatures close to 27°C may be optimal for paradise fish bubble-nest building. Similarly, a mean water temperature of 29.9°C was found in rice paddy fields of the wild Siamese fighting fish *Betta splendens* in the reproductive season (Jaroensutasinee and Jaroensutasinee 2001), and a water temperature of 30.0°C was the optimal temperature for breeding and egg development (Innes 1950, Gordon and Axelrod 1968, Hoedeman 1974).

In tropical regions, temperature and day length are the two main reproductive cues for fish (Hails and Abdullad 1982). Jaroensutasinee and Jaroensutasinee (2001) suggested that warm water may decrease egg-hatching times and consequently reduce egg predation risks and the costs of male parental care. For the Siamese fighting fish, the frequency of bubble-nest building and female spawning increased in warm water of 30°C in the field (Gordon and Axelrod 1968). However, Degani (1989) indicated that temperature is not the reproduction trigger for the blue gourami, *Trichogaster trichopterus*, since no relationship was found between temperature and nest size or the number of larvae within the range of 23 to 29°C. No plants were used in Degani's study. In our study, the higher temperature was found to be unfavorable for the building of additional bubble nests by the paradise fish, significantly so in the treatment without floating materials (Fig. 1). Floating ferns were advantageous to the building of the 3rd bubble nests at the high temperature (Fig. 2C). Floating plants, as well as species-specific differences, may play important roles in the temperature effect.

Influence of floating materials on reproductive behaviors

In natural habitats of the blue gourami, there are many plants and the water is opaque (Forselius 1957, Degani 1989). In the laboratory, reduction of light by dense plant growth had a positive effect on nest building of the blue gourami (Degani 1989). The habitat being sheltered by hydrophytes may help reduce predation mortality of post-flexion larvae of the paradise fish in the wild (Young 1995). In our study, the frequency of bubble-nest building in the sheltered treatments, using floating ferns and green Styrofoam, was significantly higher than that in non-sheltered treatments, i.e., treatment without floating materials (Fig. 1). These results suggest that shelter materials, such as hydrophytes, in the reproductive area may play an important role in the reproduction of the fish.

The sizes of the 2nd bubble nests built significantly differed among the floating-material treatments (Fig. 2B). The fish had been acclimated to the temperature for several days when they built the 2nd nests, and then the temperature effect may have been reduced and the floating-material effect on the nest size more significant. Water plants or other substrates are necessary so the fish can attach the bubble nests to them (Degani 1989). If there were no place to attach the mucous bubble nests, the nest would spread out on the water. The sizes of the bubble nests built in the treatments without floating materials were significantly larger than those in tanks with the green Styrofoam pieces (Fig. 2B).

The sizes of the 3rd bubble nests did not significantly differ among the temperature treatments in the group with floating ferns or among the floating-material treatments at 23°C (Fig. 2C). This may have been due to the small sample size and a high variance among the individual fish in this study.

Eggs and hatching larvae

The number of eggs spawned per spawning sequence by the paradise fish has been documented to be as low as 100-200 (Tsai 1992) to as high as 900 (Hall 1966). In general, the number of eggs ranges from 300 to 500 (NMMBA 2005). One female in a tank treated at 27°C with Styrofoam pieces laid 421 eggs on the 9th day of the 20 d experimental period. Two hundred and eighty larvae hatched the next day, for a hatching rate of 66.5%. We also noted 1 female which laid 483 eggs, of which 344 larvae hatched (for a 71.2% hatching rate) in another experiment of 23-27°C fluctuation with Styrofoam pieces in the non-reproductive season (unpubl. data). These results show that the paradise fish can successfully reproduce in the non-reproductive season at an appropriate temperature. A similar result was found in Degani's study (1989) that the blue gourami can reproduce year round in the laboratory at an appropriate temperature and with live food available.

In general, the female paradise fish (Huang et al. 1998), as does the Siamese fighting fish female (Bronstein 1982), lays its eggs within 24 h after mature males and females are placed together. Females of the blue gourami mostly lay their eggs by the 2nd day after being placed together for breeding in light conditions, but began more slowly in dark conditions (Degani 1989). However, males of the blue gourami, which is territorial, built nests even in the smallest containers with 0.05 m^2 of water, but only 1 nest in that size container led to successful reproduction. Also, there was only 1 tank in which the paradise fish female laid eggs and larvae hatched in our study. The water surface area of the tank was 0.025 m^2. Consistent with their territorial characteristic, it is likely that the fish need a larger area (Degani 1989). Every fish pair of the blue gourami requires approximately 0.5 m^2 of water for breeding (Forselius 1957).

In conclusion, the results of this study show that if water temperature is appropriate, near

27°C, the paradise fish can proceed with normal reproductive behaviors of mating, laying eggs, and larval hatching in winter, normally a non-reproductive season. In addition, artificial floating materials in the spawning areas are conducive to successful reproduction. Green Styrofoam, a substitute for floating plants, worked to boost the reproduction of the fish. If the results of the treatments with green Styrofoam at 27°C in our study are applied to breeding the paradise fish for biological education in schools, the reproduction experiment of the fish can be carried out year round with simple equipment.

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REFERENCES

Bronstein PM. 1982. Breeding, paternal behavior, and their interruption in *Betta spledens*. Anim. Learn. Behav. **10:** 141-151.

Bye VJ. 1990. Temperate marine teleosts. *In* AD Munro, AP Scott, TJ Lam, eds. Reproductive seasonality in teleosts: environmental influences. Boca Raton, FL: CRC Press, pp. 125-144.

Central Weather Bureau. 2005. The climate and weather in Taiwan area. Available at http://www.cwb.gov.tw/V4/index.htm.

Chen IS, LS Fang. 1999. The freshwater and estuarine fishes of Taiwan. Pingtung, Taiwan: National Museum of Marine Biology and Aquarium. (in Chinese)

Csanyi V. 1985. Ethological analysis of predator avoidance by the paradise fish (*Macropodus opercularis* L.) I. Recognition and learning of predators. Behaviour **92:** 227-240.

Csanyi V. 1986. Ethological analysis of predator avoidance by the paradise fish (*Macropodus opercularis* L.) II. Key stimuli in avoidance learning. Anim. Learn. Behav. **14:** 101-109.

Degani G. 1989. The effect of temperature, light, fish size and container size on breeding of *Trichogaster trichopterus*. Isr. J. Aquacult-Bamid. **42:** 67-73.

ESRI. 1996. A field guide to the conservation wildlife. Nantou, Taiwan: Endemic Species Research Institute, Council of Agriculture, Executive Yuan.

Fishbase. 2005. A global information system on fishes. Available at http://fishbase.sinica.edu.tw/home.htm.

Forselius S. 1957. Studies of anabantid fishes. Zool. Bidrag Fran Uppsala. **32:** 93-597.

Francis RC. 1983. Experiential effects on agonistic behavior in the paradise fish, *Macropodus opercularis*. Behaviour **85:** 292-313.

Freyhof J, F Herder. 2002. Review of the paradise fishes of the genus *Macropodus* in Vietnam, with description of two species from Vietnam and southern China (Perciformes: Osphronemidae). Ichthyol. Explor. Freshwat. **13:** 147-167.

Gerlai R. 1993. Can paradise fish (*Macropodus opercularis*, Anabantidae) recognize a natural predator ? An ethological analysis. Ethology **94:** 127-136.

Gordon M, HR Axelrod. 1968. Siamese fighting fish. Neptune, NJ: TFH Publications.

Grodzinski Z. 1971. Thermal tolerance of the larvae of three selected teleost fishes. Acta Biol. Croacov. (Zool.) **14:** 289-298.

Hails AJ, Z Abdullah. 1982. Reproductive biology of the tropical fish *Trichogaster trichopterus* (Regan). J. Fish Biol. **21:** 157-170.

Hall DD. 1966. Physical and physiological factors influencing spawning in two species of anabantoid fishes (Pisces, Belontiidae). Tex. J. Sci. **18:** 104-107.

Hall DD. 1968. A qualitative analysis of courtship and reproductive behavior in the paradise fish, *Macropodus opercularis* (Linnaeus). Z. Tierpsychol. **25:** 834-842.

Herzig A, H Winkler. 1986. The influence of temperature on embryonic development of three cyprinid fishes, *Abramis brama*, *Chalcalburnus chmento* and *Vimba vimba*. J. Fish Biol. **28:** 171-181.

Hoedeman JJ. 1974. Naturalist's guide to fresh-water fish aquaria fish. New York: Sterling Publ. Co. Inc.

Huang WB, TS Chiu. 1993. The relationship between rearing fish and its environment. China Fish. Month. **492:** 7-15. (in Chinese)

Huang YH, TH Lee, YC Liao, SP Yo. 1998. Observation on the reproductive behavior of the paradise fish, *Macropdus opercularis*. Chinese Biosci. **41:** 21-28. (in Chinese with English abstract)

Innes WMT. 1950. Cause of failure in breeding Bettas. Aquaria **19:** 404-408.

Jan JP. 1994. Fishes in the Tachia River, Taichung. Taichung, Taiwan: Taichung County Culture Center. (in Chinese)

Jaroensutasinee M, K Jaroensutasinee. 2001. Bubble nest habitant characteristics of wild Siamese fighting fish. J. Fish Biol. **58:** 1311-1319.

Kassel J, RE Davis. 1975. Early behavioral experience and adult social behavior in the paradisefish, *Macropodus opercularis* L. Behav. Biol. **15:** 343-351.

Kodric-Brown A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol. **25:** 393-401.

Lagler KF, JE Bardach, RR Miller, DRM Passino. 1977. Ichthyology. New York: J Wiley.

Liem KF. 1963. The comparative osteology and phylogeny of the Anabantoidei (Teleosti, Pisces). Urbana, IL: Univ. of Illinois Press.

Lindsey CC. 1954. Temperature-controlled meristic variation in the paradise fish, *Macropodus opercularis* (L.). Can. J. Zool. **32:** 87-98.

Miklosi A, V Csanyi. 1999. The ontogeny of antipredator behaviour in paradise fish larvae III. Size-related avoidance of predator models. J. Fish Biol. **54:** 328-337.

Miklosi A, J Haller, V Csanyi. 1997. Learning about the opponent during aggressive encounters in paradise fish (*Macropodus opercularis* L.): when it takes place? Behav. Process. **40:** 97-105.

Neter J, W Wasserman, MH Kutner. 1989. Applied linear regression models, 2nd ed. Homewood, IL: Richard D. Irwin, Inc.

NMMBA. 2005. The paradise fish/natural conservation. Pingtung County, Taiwan: National Museum of Marine Biology and Aquarium. Available at http://study.nmmba.gov.tw/upload/Resource/conserv0058.htm.

Scott DBC. 1979. Environmental timing and the control of reproduction in the teleost fish. Symp. Zool. Soc. Lond. **44:** 105-128.

Scott SG, NW Pankhurst. 1992. Interannual variation in the reproductive cycle of the New Zealand snapper *Pagrus aurate* (Bloch and Schneider) (Sparidae). J. Fish Biol. **41:** 685-696.

Shao GZ, JI Chen. 2003. A field guide to fishes. Taipei, Taiwan: Yuan-Liou Publishing. (in Chinese)

Shen SC, CS Tzeng, CY Shoang. 1991. Studies on the mosquito control by using endogenous fishes. Taipei, Taiwan: Institute of Zoology, National Taiwan Univ. (in Chinese)

Shen SJ. 1993. Fishes of Taiwan. Taipei, Taiwan: Department of Zoology, National Taiwan Univ. (in Chinese)

Tsai YN. 1992. The Formosan fighting fish and fighting fish family. Taipei Zoo Quart. **47:** 4-7. (in Chinese with English abstract)

Tzeng CS. 1990. The manual of Taiwan wildlife resource investigation (3) The freshwater fishes in Taiwan (I). Taipei, Taiwan: Council of Agriculture, Executive Yuan. (in Chinese)

Villars TA, M Burdick. 1986. Rapid decline in the behavioral response of paradise fish (*Macropodus opercularis*) to prostaglandin F sub (2 alpha) treatment. Gen. Comp. Endocr. **63:** 157-161.

Villars TA, N Hale, D Chapnick. 1985. Prostaglandin-F sub (2 alpha) stimulates reproductive behavior of female paradise fish (*Macropodus opercularis*). Horm. Behav. **19:** 21-35.

Wang TZ, CS Tzeng, SC Shen. 1999. Conservation and phylogeography of Taiwan Paradise Fish, *Macropodus opercularis* Linnaeus. Acta Zool. Taiwanica **10:** 121-134.

Yin Y, F Zhang. 1986. Toxic effect of butyl sodium xanthate on fishes. II. Toxic effect of butyl sodium xanthate on *Macropodus opercularis*. Trans. Chin. Ichthyol. Soc. **5:** 159-163.

Young SS. 1995. Notes on the early developmental stages of paradise fish (*Macropodus opercularis* (L.)) in captivity. Acta Zool. Taiwanica **6:** 83-89.

Zhang F, Y Yin. 1986. Toxic effect of butyl sodium xanthate on fishes. I. Toxic effect of butyl sodium xanthate on fish embryos. Trans. Chin. Ichthyol. Soc. **5:** 153-158.