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INFLUENCE OF TEMPERATURE ON SURVIVAL, GROWTH AND PLASMA LEVELS OF FALSE PERCULA CLOWN FISH, *AMPHIPRION OCELLARIS*

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ABSTRACT

The changes in water temperature in clown fish Amphiprionocellaris were reared in captive conditions at different water temperatures. Assisting the physiological and ecological responses of marine fishes to the change in climate on water gives a dramatic response. The results illustrated that water temperature has drastic change on the survival, growth and plasma levels of *A. ocellaris*. Specific growth rate (SGR), FCR (feed conversion ratio) increased with the increase of water temperature from 23 to 33 ^{o}C and sudden decrease was observed at extreme high temperature treatment (37 ^{o}C) which was noted as thermal stress in clown fish *A. ocellaris*. The plasma level concentration at different water temperature also deliberately increased with the increase of temperature. This study put forward that the reduce growth, survival and feeding of juveniles at lower temperature which have ecological impacts on clown fish juveniles in settlement and population replacement in the wild. This study also reveals that the physiological response of juveniles of clown fish to the change of water temperature and substantiated that water temperature influenced juvenile growth, survival feeding and plasma levels significantly.

Keywords: false percula clown fish, FCR, SGR, plasma levels, climate change, water temperature.

INTRODUCTION

Water temperature undoubtedly plays an important role in the vertical distribution of marine ornamental fish within coral reef habitats (Wexler et al., 2007). The survival of marine fish larvae during the first week after hatching is dependent on predator or prey interactions, but may also be dependent on the temperatures, salinity and oxygen levels encountered within these habitats where spawning occurs (Fiedler and Talley 2006; Glynn, et al., 2001). The marine fish larvae during the yolk sac stage are dependent on endogenous reserves for survival until they attain pigmentation and completely developed for feeding (Margulies et al., 2001). And the duration that take place from the yolk sac stage until the first feeding is completely temperature dependent (Margulies et al., 2007). Development of various internal organs (Takashi et al., 2006), metabolism in fish (Sclafani et al., 1997) and the ontogenetic changes in larval density relative to the ambient sea water temperature are probably components controlling their ability to migrate within the water column (Schaefer, 2001).

The early life stages of fish have been identified as the most liable to environmental stress both natural and anthropogenic (Rosenthal and Alderdice, 1976). Mortality is greatest and most variable from year to year during the embryonic and larval phases (Jeanne et al., 2011). Many of the ocean habitats in which marine ornamental fish are found to be characterized by decreases or increases in water temperature and salinity which are allied with seasonal changes in thermocline depth and upwelling (Lauth and Olson, 1996; Ekau et al., 2010).

Temperature, salinity, oxygen concentration, photoperiod and pH are environmental factors which are well known to have significant impacts on the performance of fish larvae and juveniles, such as survival, growth, feeding and metamorphosis, for instance temperature can influence larval and juvenile metabolism rate of marine fishes from temperate and subtropical waters (Peck et al., 2008;Tombaugh, 1997) and consequently influence their physiological performance such as growth, development and behavior (Houde, 1989; Martinez-Palacios et al., 1879; Koumoundouros et al., 2001;Koumoundouros et al., 2002).

A number of reef species utilize near shore and estuarine areas for settling in sea grass and mangrove habitats before migrating to reefs as they mature (Chester and Thayer, 1990; Allman and Grimes, 2002). Different stages of many reef fishes undergo a defined ontogenetic sequence of environmental requirements, often associated with distinct habitat features (Nagelkerken et al., 2000). In addition, abiotic environmental factors are also important for modifying the energetic (i.e. feeding, metabolism, growth rate) and ultimate production of reef fish (Fry, 1971).

Marine ornamental fish displays a wide range of color and very important in the aquarium and due its different color it attracts the aquarists and trading this fishes is more elite and moreover adapted in captive conditions (Ajith Kumar et al., 2012a, 2009). Due to demand in ornamental fish, artificial breed was popularized and admired focus to rear these tiny species in controlled conditions (Ajith Kumar et al., 2012b;

Dhaneesh et al., 2011 and Arvedlund, 2000). Several studies on the breeding of these have done in the premises of India that given a boost up in trade of ornamental fish especially the clowns (Ajith Kumar et al., 2011; Ajith Kumar et al., 2009).

Due to the climate change, water temperature is steadily increasing, which in turns gives direct and indirect effects on the population of coral reef fishes. To enhance these effects, to find out at what level this water temperature may exceeds and effects the biological life of the coral reef fish. So, the present study is framed in a concept of climate change with special reference to the temperature an effect to the clown fish. The main focus of this study were to investigate the effects of temperature on survival, growth, feeding and plasma levels of the of clown fish *Amphiprionocellaris*in captivity and to understand the physiological and ecological responses of clown fish to the change of temperature in confinement.

MATERIALS AND METHODS

Study species:

*A. ocellaris*is collected from the broodstock bank for marine ornamental fish, Annmalai University and the present study was also done. Average total length (TL) of all the anemone fish were measured about 8.5 ± 0.1 cm and they were placed in 500L glass tanks. Estuarine water was drawn from the Vellar estuary (Lat.11°29′N; Long. 79°45′ E) with the help of 5 Hp pump during high tide and allowed to reconcile in a sump for two days and then water was passed through sand and UV filters and finally stocked in a storage tank installed with a canister filter from their water was taken for hatchery operations. The water quality parameter such as salinity is around 24-26 ppt, pH is around 7.8-8.0 and temperature is around 26 °C– 28 °C was maintained.

Experimental design:

By adopting the method Akatsu et al (1983), five water temperature groups (23, 26, 29, 33 and 37) were established with the help of thermostat and each had triplicates. The optimum temperature 29°C is taken as the control tank during the experimental period. The experiment was conducted in January – April, 2014and terminated when more than two-thirds of clown fish juveniles in a group attained their marketable size.

In this study, 3000 numbers of juveniles (J) of *A. ocellaris*were randomly assigned into 15 FRB tanks measuring 150L. Water temperature of three buckets was subsequently reduced to 26°C and 23°C ± 0.5°C and another six tanks were increased to a temperature of 33°C ± 0.5°C, and 37°C ± 0.5°C within ten days of period and checked for further acclimation of clown fish in different tanks and controlled water temperature is maintained at 29°C ± 0.5°C.

Gentle aeration was supplied to experimental tanks and fed with *Artemia*sp. And they were maintained at 15 – 20 individuals/ml and 12hr light and 12 darkness of controlled photoperiod is maintained.

Measurement of A. ocellaris (length and weight):

Metamorphic juveniles of *A. ocellaris*were sampled randomly and measured for total length (mm) and body weight (mg) at beginning of the experiment. Thrice in a week, 3 juveniles from each tank were sampled randomly and measured for TL and BW upto end of the experiment.

Experimental feeding:

*Artemia*nauplii were given initially for a period of days that is followed with a different time intervals such as 9:00 am, 11:00 pm, 16:00 pm and 18:00 pm. Each feeding lasted for 15 minutes. The numbers of juveniles fed *Artemia*nauplii were calculated after each feeding, finally the total number of *Artemia*naupli fed and number of naupli remained after feeding were calculated in each of the experimental tank.

Statistical analysis:

Weight gain = Final weight (g) - Initial weight (g) Specific Growth Rate (SGR) = 100 (ln W_2 - ln W_1)/T where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period. Feed Conversion Ratio (FCR) = feed intake (g) /weight gain (g). Survival rate (SR) was calculated from SR (%) = N_T/ (N_0 - N_S) x 100, where N_T is the number of marketable juveniles at the end of the experiment, N_0 is the number of metamorphic juveniles at the beginning of the experiment and N_S is the number of juveniles sampled for measurement during the experiment. Growth performance of the fish was determined by following the standard formula (Mohsen *et al.*, 2008). Daily growth rate was calculated from GR (mm/day) = TL_t– TL_o/t, where TL_t and $TL_o$are the total length at the end of the experiment and beginning of the experiment, and t is the days for the experiment.

Plasma levels determination:

Plasma levels were measured by the immunoassay following extraction with ethyl acetate using the reagents and protocols described by Pankhurst and Conroy (1987) and Hofmann et al (2000). A 300µl aliquot of plasma was extracted with 1.5 ml ethyl acetate and 200 µl of added to each assay tube for evaporation. Remains in the tube were resuspended in assay buffer (450 µl) with the reagent and mixed with ready gel (Ecolite, Global Export Services). Then blood samples were extracted from the fish through 23 – gauge hypodermic needle by anaesthetized fish. Blood samples were immediately transferred for 2.5 ml fluoride oxalate tubes, mixed gently and stored on ice until transported at 14,000 rpm for 10 min at 4 ^{o}C. Upon collection of the blood samples, the fish were sacrificed and weight and other measurements were taken for further analysis.

Data were subjected to one-way ANOVA to test the effect of temperature on growth performance, survival rate of different clown fish. Data have been expressed as mean values ±S.D. All statistical analyses were made using the statistic software SPSS version 16.0 as described by Sokal and Rohlf (1981).

RESULTS

Amphiprion ocellaris:

Effect of water temperature on clown fish, A. ocellaris on its survival rate (%):

With the increase of water temperature from 23°C to 37°C, the water temperature influences the survival of *A. ocellaris*significantly and the juveniles that were reared at high water temperature of 33°C had highest survival rate (75 %) at end of the experiment (P < 0.05) illustrated in Fig. 1. Survival rates in all the temperature groups had significantly different and were between 51 % at 23°C, 60 % at 26°C, 63 % at 29°C and 65 % at 37°C (P < 0.05). Survivals were temperature dependent during all the experimental period. The juveniles of *A. ocellaris*raised at 23 °C and 26 °C showed a progressive mortality from day 3 onwards. On contrary, mortalities at 29, 30, 33 and 37 °C have less mortality and were considerably reduced. As shown in Fig. 1, differences in survival patterns were even more evident when effective, when the water temperature increased from 23°C to 33 °C and a sudden decrease in the survival rate at 37 °C that influenced the juvenile survival which are significantly different. At low temperature treatment tank (23 and 26 °C) and extreme high temperature (37 °C) the survival rates decreased significantly (P < 0.05).

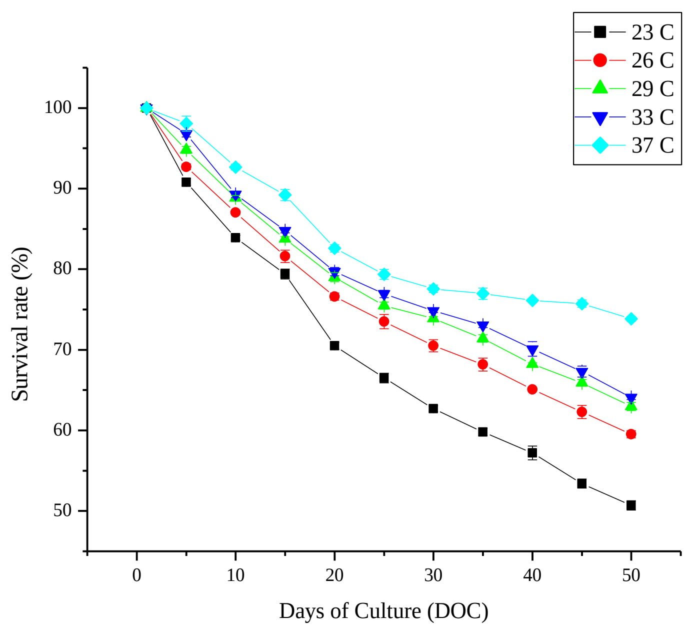

Figure 1: Effect of temperature on survival rates of clown fish *A. ocellaris* that were reared at different water temperature. The water temperature is significantly different among each treatment groups (P < 0.05).

Effect of water temperature on growth rate of A. ocellaris:

The growth rate of *A. ocellaris*juveniles during the experimental trial is shown in Fig.1. The maximum growth response in terms of specific growth rate (SGR) and final body weight was observed at 33°C (26 mm ± 0.361 in TL) (Table. 1). The final weight of the fish, weight gain and SGR increased significantly (P <0.05) with the increase in water temperature. Growth rate at 33°C temperature was significantly different from that of all other temperature groups (P <0.05). With the increase of water temperature from 23°C to 37°C, there is a significant differences in the growth rate was observed which clearly states that the water temperature influences the growth rate of *A. ocellaris*. Growth rates of all the temperature groups had significantly different and were between 15.43 mm ± 0.262 at 23°C, 17.73 mm ± 0.273 at 26°C, 20.09 mm ± 0.122 at 29°C and 21.21 mm ± 0.423 at 37°C. As shown in Fig. 2, growth rates are significantly different and more effective, when the water temperature increased from 23°C to 37 °C and also influences the growth rates of *A. ocellaris*.

Particulars	23 °C	26°C	29°C	33°C	37°C
Initial Weight (g)	0.041 ± 0.009	0.041 ± 0.003	0.042 ± 0.002	0.041 ± 0.001	0.042 ± 0.012
Final Weight (g)	0.105 ± 0.018	0.129 ± 0.016	0.255 ± 0.011	0.425 ± 0.032	0.324 ± 0.034
Weight Gain (g)	0.063 ± 0.014	0.096 ± 0.014	0.212 ± 0.024	0.382 ± 0.016	0.280 ± 0.024
Survival Rate (%)	51 ± 0.136	60 ± 0.244	63 ± 0.546	75 ± 0.125	65 ± 0.543
FCR	0.071 ± 0.01	0.058 ± 0.04	0.056 ± 0.002	0.057 ± 0.034	0.048 ± 0.026
SGR (%/day)	1.03 ± 0.006	1.24 ± 0.006	0.324 ± 0.008	2.45 ± 0.034	1.89 ± 0.014

Table 1: Specific growth rate (SGR) and feed conversion efficiency (FCR) of *A. ocellaris*(mean ± SE, *n* = 3) reared at different water temperatures of 26, 28, 30, 32 and 34 °C.

Effects temperature on plasma levels of *A. ocellaris:*

Few modules in the plasma concentrations were been observed in the low temperature groups, and as the temperature in the water increases the plasma concentrations levels are also increased with a limit to the thermal stress (Fig. 2)

Plasma levels of the fish were steadily increased with the water temperature up to an extent of increasing water temperature. Plasma levels of were illustrated in Fig.2. The plasma levels were high in the increasing temperature in the experimental temperature treatment at 33 °C. And low levels of plasma concentrations were observed in low temperature treatments (23 and 26 °C) and sudden decrease in the concentrations of plasma were seen after 33 °C, and it was observed low levels were notified in the water temperature treatment 37 °C. Moderate levels of plasma concentrations were appeared in the 29 °C water temperature treatment group.

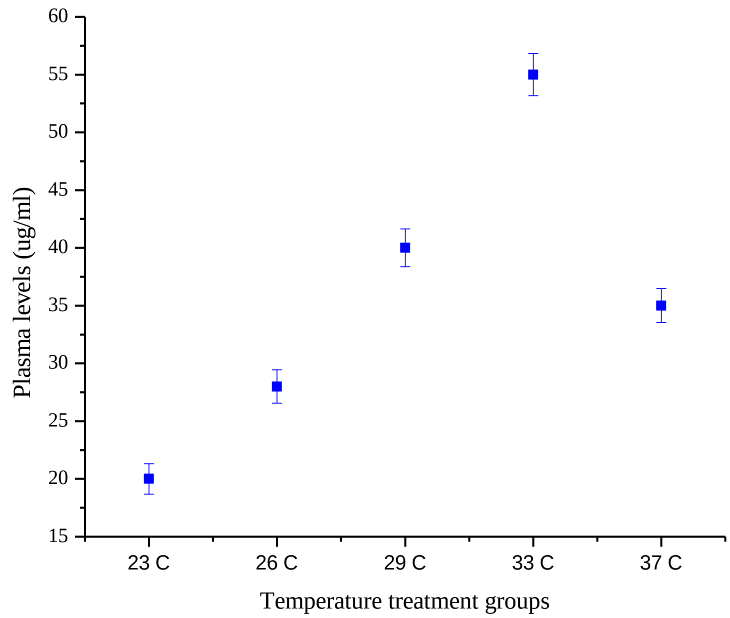

Figure 2: Plasma levels of clown fish *A. ocellaris* maintained at 23, 26, 29, 33 and 37 °C water temperature treatments for a minimum of 60 days. Values are mean + SE (*n*) that were significantly different among each treatments ($P < 0.05$).

DISCUSSION

This study demonstrated that the change of water temperature had significantly influences on physiological responses of the metamorphic juveniles of *A. ocellaris,* which are widely distributed marine fish species crop up from the tropical to temperate waters. Various developmental phases of the fish in their life cycle were prejudiced by many environmental factors in wild condition (Koumoundouros et al., 2001) with metamorphic stage is one of the most sensitive phases to change of environmental conditions (Ferron and Leggett, 1993). Developmental rates of fish embryos, larvae and young juveniles were significantly affected by the water temperature (Benoit and Pepin, 1999; Bjornsson et al., 2001; Von et al., 1996).

In the present study, all the experimental fish exposed to low water temperature (23 °C and 26 °C) had significantly higher mortality, low growth rates and low survival rates. Moderate growth rates and survival rates have been observed when the fishes are exposed to the 29 °C. Growth rates and survival rates have been noticed higher when the clown fish were exposed to 33°C. And sudden decrease in the growth rates and survival rates were observed after 33 °C. It may due to the ability to loss effect from temperature; the juveniles of *A. ocellaris*are not able to adapt with that extreme temperature, moreover that result in the high mortality and less growth rates. It is also known that fish juveniles live in low water temperature reduce swimming ability, eventually led to slow capability of fish juveniles to avoid predators, low ability to catch prey and low survival rate (Miller et al., 1988; Von et al., 2001).

Results from the present study states that, Temperature had strong effects on the feeding and growth rates of clown fishes under laboratory conditions. The response to temperature was not constant through all the treatment levels, increasing more rapidly through the lower portion of the range, and leveling off at higher temperatures. Fish showed an increase in growth, feeding, metabolism rate, food digestion and absorptionunder optimal water temperature and craving with the increase of water temperature (Zhang et al., 2001). In the present study, *A. ocellaris*grew faster when reared at higher water temperature. Rao et al (2013), clearly says that increasing temperature boost up the energy ability of the fish. And the present study also explained that, the temperature influences the growth and survival rates of clown fish *A. ocellaris*.

While comparing with the results with Rao et al 2013, it is showed that different clown fish (*P. biaculeatus* and *A. clarkii*) in their experiment, lower growth and survival rates when they were reared at 34 ^{o}C and gradually reduced when they were reared at 26 ^{o}C than the other clown fish. And the present results also showed that at 33 ^{o}C water temperature clown fish SGR, feeding capacity was increased and growth rates deliberately increased. Ye Le et al. (2011) evidently showed that, change of water temperature influenced the larval survival and growth rates, the results from the present study also clearly states that, the water temperature influenced the juveniles growth and survival rates. Thus, the growth potential is greater at highest water temperature, providing that Maximum ingestion requirements are met to promote survival (Houde, 1989). To conclude, the present study clearly shows that increasing water temperature influences the growth and survival rates of the clown fish *A. ocellaris*. In the different experimental groups from the present study, *A. ocellaris*showed significantly higher growth rates and survival rates when they were reared at 33^{o}C temperature and low growth rates and low survival rates are predominantly appeared when they were reared under 23 and 26 ^{o}C temperature.

The present results also signify that FCR has significantly influence on growth of the juveniles of different species of clown fish. The adaptive methods subsist which can regulates the fish capacity for utilization of feed for growth above a range of temperature which was universally accepted (Cui and Wootton, 1988).

Archosargusrhomboidalis and *Chiruslineatus*(Houde, 1974) are some of the sub tropical marine fish species which are having less growth rates when they were reared under different temperatures, the present study revealed that temperature has influenced the growth rates. Temperature effects of different clown fish showed maximum growth rates than that of *A. melanopus* (Green and Fisher, 2004), *Brevoortiatyrannus*and*Gadusmorhua* (Fitzhugh and Nixon, 1997).

Green and Fisher, 2004 showed that A. melanopus reared at low temperature did not show a better swimming ability, and in the present study water temperatures (23 and 26 ^{o}C) did not showed the swimming performance than the other water temperature treatments. And this is also in agreement with the studies of Fujiman 1991; Ojanguren and Brana, 2000).

Plasma levels in the fish increased with the increasing temperature, the clown fish *A. ocellaris*reared at high temperature 33 °C water temperature increased and decreased rather after this water temperature this may be due to the internal ability of the fish that was metabolized the Na^+ K^+ ATPase activity in the cells (Fig. 2). Hence from the present it can be concluded that, temperature had significant effect on the plasma levels of the fish. And the present study also agrees with the studies of Hofmann et al (2000). Low plasma levels were obtained in the low water temperature treatments (23 and 26 °C). Hofmann et al (2000) clearly states that the Na^+ K^+ ATPase activity and plasma levels in the juveniles of salmons decreased when the water temperature are extremely high and extremely low. And by observation from the present study of ours, the *A. ocellaris*can adapt to external stress even at high and low temperature with slight changes.

Findings of the present study also supports the studies done by RonzaniCerqueira, (1991); Fielder et al., (2002) who investigated that the temperature can also affect feeding efficiency of the juveniles by influencing processes such as metabolism, oxygen consumption, behavior, swimming speed and gut evacuation time.

This study suggested that the effect of temperature on growth and survival rates of A. Ocellaris, false percula clown fish. which occurs in both tropical and temperate waters. It is important to note that this study has traditionalized the fact that high water temperature influenced the growth and survival rates of the clown fish juveniles. In spite of the limitations, temperature tolerance described herein should provide a base for future studies on clownfish and help in achieving conservation. It will be of great interest to compare the effect of temperature with more different species of clownfish from different latitude in future studies.

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