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SHORT COMMUNICATION

In vitro hybridization of coral trouts, *Plectropomus leopardus* (Lacepède, 1802) and *Plectropomus maculatus* (Bloch, 1790): a preliminary investigation

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Coral trouts of the genus *Plectropomus* are members of the serranid subfamily Epinephelinae, which are commonly known as groupers. Two of these species, *Plectropomus leopardus* (Lacepède, 1802) and *Plectropomus maculatus* (Bloch, 1790), are among the most popular and valuable of the groupers, and both species are major targets of reef fisheries which operate throughout the Indo-West Pacific region (Williams 2002; Sadovy, Donaldson, Graham, McGilvray, Muldoon, Phillips, Rimmer, Smith & Yeeting 2003). Much of the catch is exported to Hong Kong, where the retail price of live specimens ranges from US$ 50 to 75 per kg (Sadovy *et al.* 2003). This high value has resulted in a strong demand for coral trouts and an upward trajectory in their total annual harvest (Williams 2002; Sadovy *et al.* 2003).

Fishing pressure has significantly decreased the abundance of coral trouts in parts of Australia and Southeast Asia (Sadovy *et al.* 2003; Evans & Russ 2004); thus, there is a need to develop more sustainable methods of supplying coral trouts to the market. Although there have been many previous attempts to culture these fish in Australia and Southeast Asia, most have been unsuccessful, largely due to problems associated with the acceptance of live feeds by early larval stages (Rimmer, Garrett & Samoilys 1994; Sadovy *et al.* 2003).

One mechanism by which the survival and performance of cultured fish can be enhanced is to 'cross' or hybridize two closely related species. For example, the hybrid offspring of white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*) outperform (in terms of growth and survival) the purebred offspring of both parental species when cultured in captivity (Smith 1988). This phenomenon, known as heterosis, results from the combination of desirable dominant genes accumulated by each parental species, as well as an increase in the total level of heterozygosity (Kirpichnikov 1981).

In some hybrid fish, heterosis manifests as accelerated growth, improved environmental tolerance, increased disease resistance and enhanced overall survival (reviewed by Bartley, Rana & Immink 2001). Not surprisingly, hybrid fish can confer substantial benefits to aquaculture enterprises. In many other fish, however, hybridization results in impaired development, such that they are of no use to aquaculture (Hulata 1995). It is impossible to predict whether hybridization will benefit or detract from a fish's performance in captivity, and hence hybrids need to be evaluated on a case-by-case basis.

In this study, we investigated the utility of hybridization between coral trouts, and compared the performance of purebred and hybrid larvae in captivity. We measured growth, heart rate and survival as indicators of larval performance (Glamuzina, Glavic, Skaramuca, Kozul & Tutman 2001; Paschos, Nathanailides, Perdikaris & Tsoumani 2004; Schwerte, Voigt & Pelster 2005).

Mature *P. leopardus* and *P. maculatus* were collected from Orpheus Island (Great Barrier Reef, Australia) during the new moon periods of late spring when

coral trouts aggregate to spawn (Frisch, McCormick & Pankhurst 2007). Owing to the biased sex ratio and parapatric distribution of coral trouts, it was extremely difficult to obtain ripe individuals of both sexes and both species simultaneously. Consequently, experimental trials were performed on two occasions, with one hybrid and one purebred cross initiated during each trial. As gametes from only a single pair of parental fish were used to initiate each cross, our results are indicative of trends rather than conclusive.

Mature fish were collected by spearfishing just before dusk (17:00–18:40 hours). Eggs were immediately stripped and divided equally into two separate 100 mL vials. Sperm was obtained via the same method and added directly to the eggs, such that each vial contained one of the following crosses: *P. leopardus* ♀ × *P. maculatus* ♂; *P. leopardus* ♀ × *P. leopardus* ♂; *P. maculatus* ♀ × *P. maculatus* ♂; and *P. maculatus* ♀ × *P. leopardus* ♂. A small volume (ca. 50 mL) of seawater was added to each vial, followed by gentle mixing. After 10 min, eggs were rinsed on a 210 μm screen and placed in a plastic bucket containing 3 L of aerated seawater for transport (ca. 4 h) to a larval rearing facility at James Cook University, Townsville.

After 6 h, a random sample of approximately 200 eggs was collected from each bucket for microscopic examination (× 5–50 magnification). Proliferating blastodermal cells were observed among all of the gamete combinations, thus demonstrating successful fertilization. However, fertilization rates were highly variable, ranging from 44% to 90% (Table 1). This result is not uncommon for artificial fertilizations involving wild-caught fish (Rimmer *et al.* 1994) because the gametes taken from different individuals may be at slightly different stages of maturation.

Floating, fertilized eggs were separated (as per Glamuzina *et al.* 2001) and transferred into 200 L larviculture tanks supplied with recirculated seawater (temperature 27–28 °C; salinity 0.35 g L^{-1}; dissolved oxygen 8–9 mg L^{-1}; exchange rate 50% day^{-1}) and a 12:12 artificial photoperiod (light intensity 900 lx). Only four tanks were utilized (one per cross). Live unicellular algae (*Nannochloropsis occulata*) and rotifers (*Brachionus rotundiformis*, SS strain) were added daily to achieve densities of 5×10^5 cells and 10 individuals mL^{-1} respectively (Tucker 2003). Three larvae were collected from each tank and subsequently placed in a petri dish for microscopic examination (× 10 magnification). Total length was determined using an eyepiece micrometer, and heart rate (beats min^{-1}) was estimated by counting the number of cardiac contractions over a period of 30 s. Larvae were sampled every 24 h until all fish were dead.

After 24 h, newly hatched larvae (hybrid and purebred) were observed swimming near the water surface. Hybrid larvae appeared to be morphologically similar to purebred larvae, with no apparent differences in size at hatching or subsequent growth (Fig. 1). Similarly, there were no apparent chronological differences in the acquisition of developmental features such as fins, mouth and teeth. Furthermore, the heart rates of hybrid and purebred larvae were not distinctly different (Fig. 2). Together, these results suggest that hybrid and purebred larvae have broadly similar developmental rhythms when reared under captive conditions.

One group of hybrid larvae (*P. leopardus* ♀ × *P. maculatus* ♂) lived longer than both groups of purebred larvae (Table 1). This result is promising, and may reflect the enhanced fitness of hybrid larvae reared under culture conditions. However, it may also reflect differences between parent fish, as larval quality is influenced by parental condition (Kerrigan 1997) and only one parental fish of each species was used in each cross.

Table 1 Comparison of egg fertilization rates and larval survival times for purebred and hybrid crosses of *Plectropomus leopardus* and *Plectropomus maculatus*

Cross (♀ × ♂)	Date of trial	Approximate number of eggs collected*	Fertilization rate (%)	Maximum survival time (DAH†)
L × M	3/10/05	33 100	90	8
L × L	3/10/05	33 100	78	7
M × M	2/11/05	27 600	44	5
M × L	2/11/05	27 600	54	5

*Estimated volumetrically from a sample of 200 eggs.
†L, *P. leopardus*; M, *P. maculatus*; DAH, Days after hatching.

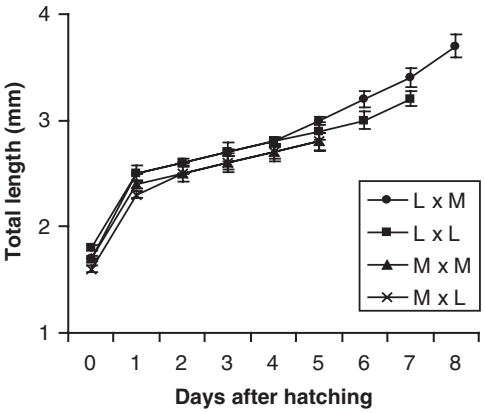

Figure 1 Growth of purebred and hybrid *Plectropomus* larvae maintained in captivity. Data are presented as mean total length ± standard error of three larvae per sample. Samples were taken every 24 h until all larvae were dead. Each cross is listed as ♀ × ♂. L, *Plectropomus leopardus*; M, *Plectropomus maculatus*.

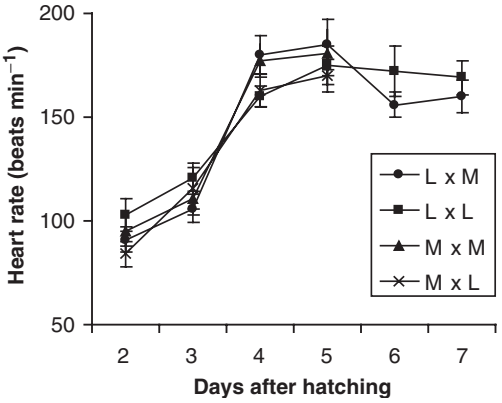

Figure 2 Heart rate (beats min^{-1}) of purebred and hybrid *Plectropomus* larvae maintained in captivity. Data are presented as mean heart rate ± standard error of three larvae per sample. Samples were taken every 24 h until all larvae were dead. Each cross is listed as ♀ × ♂. L, *Plectropomus leopardus*; M, *Plectropomus maculatus*.

The short (≤ 8 days) lifespan of larvae from all crosses made it difficult to assess the performance of hybrid versus purebred forms. Although rotifers and microalgae were observed in the gut of larvae from all four crosses after 5 days, we suspect that the high mortality of larvae was largely the result of inadequate nutrition (i.e. inappropriate size, type and availability of food items). This is supported by the fact that larval growth rates declined noticeably (see Fig. 1) when the yolk and oil droplet were absorbed (2 days after hatching) and larvae became dependent on exogenous food sources. The transition from endogenous to exogenous nutrition is a critical phase in the culture of small fish larvae with limited yolk reserves (Kuo 1995; Tucker 2003). Accordingly, the problems associated with first feeding of coral trout larvae must be resolved before the overall performance of hybrid larvae can be accurately assessed. Nonetheless, it is apparent from our results that *in vitro* hybridization of coral trouts is possible and that hybrid larvae develop in a manner similar to purebred larvae (at least during early stages) when reared in captivity.

The literature presents only four previous attempts to hybridize groupers, none of which involved coral trouts. Like the present study, however, most of these investigations ended prematurely (Tseng & Poon 1983; Glamuzina, Kozul, Tutman & Skaramuca 1999; Glamuzina *et al.* 2001). In contrast, James, Al-Thobaiti, Rasem and Carlos (1999) successfully reared hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus polyphekadion*) to harvest size (700 g). In doing so, they found that the hybrid fish outgrew the purebred fish of both parental species, thus suggesting that if other groupers (e.g. coral trout) could be successfully reared in captivity, then hybridization may enhance the culture of these species.

In summary, this study demonstrated that *in vitro* hybridization of gametes from heterospecific coral trouts yielded free-swimming larvae, and that the performance of hybrid and purebred larvae was similar during early ontogenetic stages. Future attempts to hybridize coral trouts should commence once suitable feeding protocols have been developed for these species.

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