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The effect of stocking density and repeated handling on the growth of juvenile mulloway, *Argyrosomus japonicus* (Temminck & Schlegel 1843)

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Abstract The effect of stocking density on the growth of mulloway, *Argyrosomus japonicus*, was tested with 17 g fish stocked at 4.08, 8.16, or 16.32 kg m^{-3} in 50 l aquaria. Weight checks were carried out every 2 weeks to track performance. Each density treatment was also compared to a nonhandled control group to establish if handling during weight checks influenced the growth of mulloway. Mulloway performed poorly at the lowest density and, under the current experiment conditions, growth did not appear to be negatively affected by regular handling.

Keywords Mulloway · *Argyrosomus japonicus* · Stocking density · Growth · Handling

Introduction

Mulloway, *Argyrosomus japonicus*, are a commercially and recreationally important sciaenid species in Australia and efforts in recent years have focused on improving production techniques for wild-stock enhancement and aquaculture of the species (Fielder and Bardsley 1999; Fielder et al. 1999). As a new aquaculture species relatively little is known of the effects that various environmental factors have on the growth of mulloway. Stocking density is one of the most important biotic factors influencing growth and feed intake of fish in culture (Kestemont and Baras 2001), directly modifying feeding behavior (Boujard et al. 2002), social interactions (Barcellos et al. 1999), and water quality (Ellis et al. 2002), and has also been shown to influence sexual dimorphism (Davis et al. 2002). Stocking densities of 15 kg m^{-3} at harvest have been achieved for mulloway (Quartararo 1996)

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however the relationship between stocking density and growth of mulloway is currently unknown.

The primary objective of this study was to identify the effects of stocking density on the growth of juvenile mulloway as evidenced by survival, body weight and length, condition factor, size heterogeneity, and feeding efficiency. This information will be of use in determining appropriate stocking densities of mulloway for both future growth studies and aquaculture.

During growth studies on fish it is common practice to track performance (growth) over time by sampling periodically and measuring some physical parameter, e.g., weight, length, etc. Anaesthetics are commonly used to minimize the stress response when handling fish; however, anesthesia can itself produce a stress response (Ortuno et al. 2002a, b) and can also have a negative effect on growth (Hoskonen and Pirhonen 2006). Each stocking density treatment in this study was therefore also compared to a nonhandled control group to identify if the growth of mulloway was compromised from routine handling during regular weight checks.

Materials and methods

The effect of density on the growth of mulloway was tested over 37 days using 17 ± 3.5 g, 4-month-old F2 juvenile fish from broodstock held at the New South Wales Department of Primary Industries, Port Stephens Fisheries Centre (PSFC). Fish were sedated by using 20 mg l^{-1} benzocaine (ethyl *p*-aminobenzoate) and stocked into 50 l aquaria at one of three stocking densities: 4.08, 8.16, or 16.32 kg m^{-3} (12, 24, or 48 fish $aquaria^{-1}$), nominally low (LD), medium (MD), and high (HD) densities, respectively. There were four replicate aquaria for each density treatment. The control (nonhandled) group consisted of an additional four replicate aquaria for each of the three stocking densities. Once stocked, the control fish were not handled until the completion of the experiment. In this experiment the combined effects of anaesthesia and handling cannot be separated and therefore the terms 'handling' or 'handled' are used to denote both.

The experiment system consisted of 24 × 50 l replicate acrylic aquaria integrated via a semi-recirculating biofiltration unit. A moderate flow-through rate allowed twice daily renewal of water to the system. Flow to each aquarium was approximately 2 l min^{-1}, ensuring similar water quality between all treatment aquaria. Ranges and means (± standard deviation [SD]) for water quality parameters were: temperature (°C) 19.6–22.5, 20.8 (0.9); NH_4^+ (mg/l) 0.1–0.8, 0.4 (0.1); DO (mg/l) 5.3–7.2, 6.1 (0.3); pH 7.8–8.3, 8.0 (0.1); salinity (ppt) 26.0–32.3, 29.4 (1.4). Black plastic sheets were placed between each aquarium and across the front to minimize disturbance. All aquaria were exposed to a 12L:12D photoperiod using fluorescent lighting (<1 μE m^{-2} s^{-1} at aquaria surface).

Analysis of variance (ANOVA) of initial weights ($F_{2,21} = 3.35$; $P > 0.05$) and initial CV ($F_{2,21} = 0.76$; $P > 0.1$) demonstrated no significant difference between treatments. An additional 100 individuals were also measured for weight and total length (L_T) for initial condition factor (K) comparison. Table 1 summarizes the initial data.

Weight checks were carried out every 2 weeks on the handled treatment group. To ensure that handling protocols during weight checks remained consistent between all density treatments, fish in the highest density were sampled first and the exposure time to handling and benzocaine per aquarium noted. This time (approximately 15 min) was then applied to the remaining densities and also to subsequent weight checks.

Table 1 Summary of initial and final data

Treatment	Survival (%)	Weight (g)	Length (mm)	Condition (K)	CV (%)	FE
Initial	–	16.5 (1.9)	117 (2.9)	1.04 (0.06)	11.2 (1.4)	–
Final						
LD	92.8 (3.3)	23.8 (0.7)^{a}	131 (0.7)^{a}	1.03 (0.02)	24.5 (2.3)	0.45 (0.04)^{a}
MD	88.0 (2.5)	28.7 (0.8)^{b}	139 (0.9)^{b}	1.05 (0.01)	28.5 (1.9)	0.84 (0.03)^{b}
HD	85.7 (1.9)	27.5 (0.7)^{b}	138 (0.9)^{b}	1.03 (0.01)	26.9 (1.2)	0.90 (0.04)^{b}

Initial data are means ± SD. Final data are pooled mean values (± SE; $n = 8$) for each density tested. Means sharing lower-case letter superscripts are not significantly different ($P > 0.05$) according to Tukey–Kramer test between densities

Fish were fed by hand twice daily (08:30 and 15:00) to apparent satiation with a commercial barramundi (*Lates calcarifer*) diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; reported nutrient composition: 50% crude protein, 12% crude fat, 2.5% fibre, 18 MJ kg^{-1} gross energy), which was reground and repelleted (3 mm) to sink.

Aquaria were inspected daily and any mortalities were replaced with similar size fish in order to maintain treatment densities. Replacement fish were fin clipped (left pectoral) for ease of identification and were not used in the final analyses; all data were derived from the tank means of the remaining original fish. Faeces and feed debris were siphoned from tanks daily. Shoaling and feeding behavior and responses to routine aquaria maintenance were observed daily; however, these were not quantified.

Coefficient of variation (CV) of weight (%), condition factor (K), and feeding efficiency (FE) were calculated as:

$$\mathrm{CV} = s \cdot \bar{x}^{-1} \times 100$$

$$K = [W/L_{\mathrm{T}}{}^{3}] \times 100$$

where W = wet weight (g) and L_{T} = total length (cm).

$$\mathrm{FE} = \text{wet weight gain}(g)/\text{total feed intake}(g).$$

A two-way ANOVA was used to determine density and handling effects on the dependent variables survival (%), final weight (W_{f}), final length (L_{f}), FE, CV, and K. Cochran's C test was used to test homogeneity of variances. Tukey–Kramer test was used for a posteriori multiple comparison of means on significant terms. Probability of type I error was set at $\alpha = 0.05$ for all analyses.

Results

There was no significant interaction or handling main effect between densities for all variables (survival (%), W_{f}, L_{f}, K, CV, and FE) (Table 2). The handling term was therefore removed and all subsequent analyses performed as a single-factor ANOVA on pooled data.

Mean individual weights were significantly different between density treatments from the first weight check 2 weeks after initial stocking ($F_{2,9} = 6.35$; $P < 0.02$) (Fig. 1). At week two MD fish were larger than LD fish but not significantly different from the HD fish. By week four both the MD and HD fish were larger than the LD fish ($F_{2,9} = 8.05$; $P < 0.01$) (Fig. 1). The effect of stocking density was also significant on final weight

Table 2 Two-factor analysis of variance for survival, final weight, final length, condition (K), CV, and FE

Term	DF	Survival (%)			Weight_f			Length_f			Condition (K)			CV			FE		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
A. Handled vs. control	1	3.99	0.07	NS	1.39	0.31	NS	12.14	2.07	NS	0.00	0.05	NS	1.43	0.05	NS	0.01	0.60	NS
B. Density	2	105.06	1.79	NS	51.99	11.55	**	118.39	20.17	**	0.001	0.67	NS	30.13	1.08	NS	0.47	40.42	**
AB	2	61.65	1.05	NS	2.99	0.66	NS	1.79	0.30	NS	0.001	0.71	NS	43.39	1.55	NS	0.01	0.85	NS
Residual	18	58.56			4.50			5.87			0.001			27.99					

NS indicates not significant at $P < 0.05$, * significant at $P < 0.05$, ** significant at $P < 0.01$

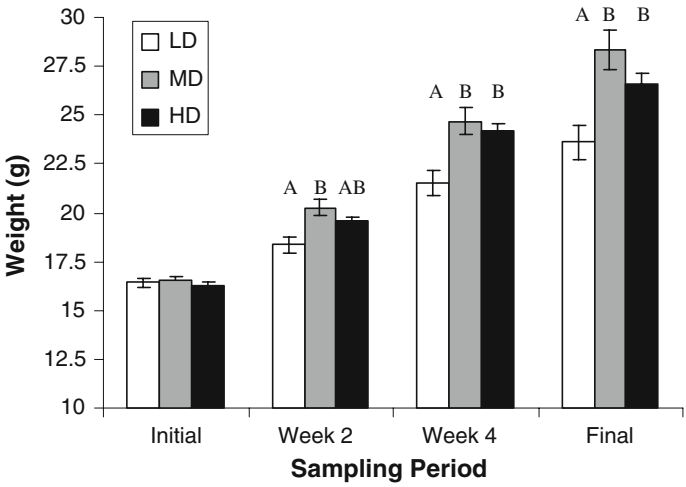

Fig. 1 Initial mean stocking weight (g) and mean weight of the handled group over time (±SE; $n = 4$): LD = 12, MD = 24, and HD = 48 fish aquaria^{-1}. Means sharing letters are not significantly different ($P > 0.05$) according to Tukey–Kramer test between densities

($F_{2,21} = 12.35$; $P < 0.001$) and final length ($F_{2,21} = 20.48$; $P < 0.001$) with MD and HD fish being larger than LD fish (Table 1). Stocking densities (±SD; $n = 8$) at the conclusion of the experiment were 5.7 (0.5), 13.8 (1.1), and 26.4 (1.9) kg m^{-3} for LD, MD, and HD, respectively.

Total overall survival was 88%. There was a trend for greater survival with decreasing density; however, this was not statistically significant ($F_{2,21} = 1.87$; $P > 0.1$) (Table 1).

Final FE was significantly poorer for the LD treatment than for the MD and HD treatments (Table 1). CV increased from initial stocking (Table 1); however there was no significant difference between final density treatments ($F_{2,21} = 1.07$; $P > 0.2$; Table 1).

Initial and final condition coefficients were similar (Table 1). Stocking density did not have a significant effect on final K ($F_{2,21} = 0.72$; $P > 0.5$). Heterogeneity of variances could not be removed from final K data; however, ANOVA was still performed. The result is valid as heterogeneous data increases the chance of type I error (Underwood 1997) and, in this case, there were no significant differences.

No agonistic behaviour was observed during feedings or at other times in any of the aquaria. LD fish appeared to be quite timid for the first 2 weeks; often staying in the back corner of the aquaria, huddled together, and taking longer to approach food. In contrast, MD and HD fish were evenly dispersed throughout the aquaria. Fish did not appear to be disturbed by daily siphoning of the aquaria. Lights switching on and off startled the fish, causing them to swim rapidly for several seconds and collide with the aquaria surfaces; however, normal behavior appeared to resume quite quickly after each event.

Discussion

The results indicate an appropriate initial (~17 g fish) lower stocking threshold for mulloway of above 4.08 kg m^{-3}, while growth at the MD and HD stocking densities was similar, suggesting that an initial stocking density in excess of 16.32 kg m^{-3} may be

achievable. While direct extrapolation of the MD or HD stocking densities used in this experiment to commercial-scale culture or different size classes of mulloway may not be appropriate, it is important to note that this study demonstrated the significant negative effect of low stocking density on the growth of mulloway after only 2 weeks.

Under the current experimental conditions mulloway were not negatively affected by regular handling. Negative growth responses to anesthesia may be anesthetic specific (e.g., Hoskonen and Pirhonen 2006) and in this case mulloway appear to be able to tolerate regular weight checks using benzocaine. It should be noted however that exposure to a repeated stressor can potentially reduce the ability of fish to respond to an additional acute stressor (Barton 2002). It is unclear to what extent, if any, the daily switching on and off of lights (repeated stressor) masked the additional effect of handling (acute stressor) on the growth of mulloway in this experiment. Growth of MD mulloway in this experiment were however comparable to those of juvenile mulloway in intensive culture using 10,000 l tanks ($\sim$0.35 g day^{-1}) (Booth, Allan & Losordo, unpublished data, 2002).

LD fish fed erratically; they were reluctant to feed when food was introduced into the aquaria, then darted over to pellets, often stirring them up. MD and HD fish in contrast fed well from the experiment outset. The FE value of the LD treatment should be regarded with some caution as the erratic feeding behavior of the LD fish made accurate quantification of feed intake difficult. However; the low FE value for this group does provide an indication of the overall inefficient feeding behaviour of mulloway at low densities.

Qualitative observations during the present study did not identify any obvious agonistic behaviour among any of the density treatments while the similarity of growth heterogeneity between the density treatments reinforced this observation. This implies a moderate social hierarchy independent of the stocking densities used in this experiment (Brett 1979). This also occurred despite the introduction of replacement fish to maintain density values.

One of the primary functions of shoaling behavior in fish is predator avoidance (Pitcher 1986), and the size of the shoal has been shown to directly influence the behavior of individuals (Magurran and Pitcher 1983). Magurran (1986) proposed that, as a fish shoal increases in size, "corporate vigilance" for predators decreases. This relationship is not unique to fish and has been documented extensively in many animal behavioral studies (e.g., birds, Pulliam 1973; wild boar, Quenette and Gerard 1992; rabbits, Roberts 1988; also see the reviews by Lima and Dill 1990; Roberts 1996). The results and observations from this study indicate that a lower threshold of stocking density may also apply to mulloway; we hypothesize that, at a certain density, a social cohesiveness forms which encourages a reduction in corporate vigilance and a change to normal feeding and behavior. Below this threshold mulloway may become increasingly skittish and vigilant for (perceived) predators, increasing general activity and inefficient feeding behaviour. Growth and feeding studies combined with quantifiable behavioral data would test this hypothesis.

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