Weaning requirements of larval mulloway, *Argyrosomus japonicus*

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**Abstract**

Mulloway (*Argyrosomus japonicus*) is an emerging aquaculture species in Australia, but there is a need to improve the production technology and lower costs, including those associated with larval rearing and live feeds. Three experiments were conducted to determine appropriate weaning strategies from live feeds, rotifers (*Brachionus plicatilis*) and *Artemia*, to cheaper formulated pellet diets. Experiment 1 examined the effects of feeding *Artemia* at different levels [0%, 50% or 100% ration of *Artemia* fed from 18 days after hatching (dah); based on current hatchery protocols] and a pellet diet from two larval ages (14 or 23 dah). In addition, rotifers were supplied to larvae in all treatments for the duration of the experiment (14–29 dah), at which time all larvae were successfully weaned onto the pellet diet. No significant (P > 0.05) differences existed between the growth of fish fed a 50% and 100% ration of *Artemia*; however, fish fed a 0% ration of *Artemia* had significantly (P < 0.05) reduced growth. The time of pellet introduction had no significant (P > 0.05) effects on the growth of larvae. Experiments 2 and 3 were designed to determine the size [total length (TL), mm] at which mulloway larvae selected *Artemia* equally or in preference to rotifers, and pellet (400 μm) equally or in preference to *Artemia* respectively. Each day, larvae were transferred from a holding tank to experimental vessels and provided with rotifers (2 mL⁻¹), *Artemia* (2 mL⁻¹) or a combination of rotifers (1 mL⁻¹) and *Artemia* (1 mL⁻¹) (Experiment 2), and *Artemia* (2 mL⁻¹), a pellet diet or a combination of *Artemia* (1 mL⁻¹) and a pellet diet that was broadcast every 15 min (Experiment 3). After 1 h, a sub-sample of larvae was randomly selected from each replicate vessel (n = 5) and the gut contents were examined under a light microscope. Mulloway larvae began selecting *Artemia* equally to rotifers at 5.2 ± 0.5 mmTL and selected pellets equally to *Artemia* at 10.6 ± 1.8 mmTL. Our results have led to the establishment of weaning protocols for larval mulloway, which optimize larval growth while reducing feed cost by minimizing the amount of *Artemia* used during production.

**Keywords:** *Artemia*, food selection, microdiet, mulloway, rotifers, weaning

**Introduction**

Mulloway (*Argyrosomus japonicus*) naturally occurs in near-shore coastal waters of the Indian and Pacific Oceans. They have been recognized with aquaculture potential in Australia and South Africa (Fielder, Bardsley & Allan 1999), and researchers are currently investigating the culture requirements of the species (see the review by Silberschneider & Gray 2008). Hatchery production, of mulloway and other finfish species in Australia, is limited in part by high production costs (Ballagh, Pankhurst & Fielder 2008; Fielder, Roberts-Thomson, Booth, Allan & Adlard 2008), with some of the most significant costs attributed to feeding, particularly those associated with live feeds, such as rotifers and *Artemia*, required to rear early larvae.

It is well documented that the cost and quality of *Artemia* can fluctuate over time as the supply is dependent on both the worldwide aquaculture demand and the weather patterns affecting the primary harvest areas (Sorgeloos, Dhert & Candreva 2001; Callan,
Jordaan & Kling 2003). In addition to the unstable nature of Artemia supply, the percentage of hatchery feed costs attributed to Artemia is substantial and it is therefore desirable to find alternative feeding strategies for rearing marine fish larvae.

While there is a strong argument to reduce reliance on Artemia, some risks are associated with early weaning and reduced Artemia use. Person Le Ruyet, Alexandre, Thébaud and Mugnier (1993) reported that up to 80% of Artemia costs could be saved by weaning sea bass (Dicentrarchus labrax) 15 days earlier than the usual protocol; however, the risks of doing so included a decrease in larval weight gain, increased larval size variation and skeletal abnormalities. Recent larval weaning studies have endeavoured to reduce feed costs and to overcome problems associated with decreasing the use of Artemia; however, many studies have suggested that some Artemia should be included in the weaning process to improve growth and survival (Callan et al. 2003; Curnow, King, Bosmans & Kolkovski 2006; Fletcher Jr, Roy, Davie, Taylor, Robertson & Migaud 2007).

The weaning success of any finfish species from live feeds onto a formulated microdiet is partly dependent on the composition of the diet and the ability of the larvae to select and digest a non-live food (Person Le Ruyet et al. 1993; Kolkovski 2001; Shaw, Pankhurst & Purser 2003). Stomach development and the production of digestive enzymes are regarded as indicators for the transition from live feeds to microdiets (Watanabe & Kiron 1994; Cahu & Infante 2001; Chen, Qin, Kumar, Hutchinson & Clarke 2006); however, the rate of ontogeny varies between species (Kolkovski 2001). It is considered that the composition of microdiets, including binders and proteins, makes digestion of the pellets difficult (Lindner, Eshel, Kolkovski, Tandler & Harpez 1995; Partridge & Southgate 1999). In addition, amino acids are freely available in live feeds and support larval digestion in marine fish larvae (Ronnestad, Thorsen & Finn 1999). The reduced ability of early larvae to digest protein in particular has been investigated in detail, with various studies reporting that microdiets containing pre-digested and soluble protein are more easily digested by marine larvae (Kvåle, Harboe, Espe, Naess & Hamre 2002; Tonheim, Nordgreen, Hogoy, Hamre & Ronnestad 2007).

Shifts in food type preference have been measured against larval age (Hung, Tuan, Cacot & Lazard 2002; Shaw et al. 2003), weight (Olsen, Attaramadal, Reitan & Olsen 2000) and length (Mayer & Wahl 1997), but the underlying factor that links these parameters is larval ontogeny. Sensory and visual development (Jones & Janssen 1992; Pankhurst 2008), locomotive ability (Blaxter 1986) and mouth gape (Fernández-Díaz, Pascual & Yúfera 1994) all play a role in prey detection, capture and consumption. Additionally, the prey taxa, size and abundance can influence larval preference (Pryor & Epifanio 1993; Mayer & Wahl 1997).

Weaning success and feeding efficiency can be improved by introducing new food sources at appropriate stages of larval development. At the Port Stephens Fisheries Institute (PSFI) mulloway hatchery, approximately 250 000 larvae are typically stocked into either a 2000 L intensive clear water tank or a 10 000 L green water tank (with resident algae). The density of live prey is increased as the larval density increases. Larval development is measured against length rather than age, and current weaning protocols use large strain rotifers (Brachionus plicatilis) from first feeding [2 days after hatching (dah), 2.5 mm total length (TL)] at a rate of 4 mL⁻¹ for a 10 000 L tank and up to 20 mL⁻¹ for a 2000 L tank. When larvae attain a mean length of 4–5 mm, rotifers are supplemented with enriched Artemia, which are initially introduced at a low rate (0.2–1 mL⁻¹). The Artemia density is then increased to accommodate the increased demand as larvae develop. A 400 μm (diameter) weaning diet is introduced when fish attain a length of approximately 8 mm, and the concentration of Artemia is gradually reduced until larvae are considered to be weaned (when all sampled fish are consuming mostly pellet).

Three experiments were conducted to examine whether the efficiency of mulloway weaning practices can be improved by decreasing the amount of Artemia required during the weaning process and determining the optimal time of Artemia introduction to reduce the costs of production. The first experiment aimed to investigate the effects of a range of live feed and pellet combinations on the growth and weaning success of larval mulloway. The other two experiments were short-duration feeding trials that aimed to investigate the transition by larval mulloway from rotifers to Artemia and then from Artemia to a pellet microdiet. The outcomes of the Artemia and pellet microdiet transition trial were then confirmed in a pilot commercial-scale production run.

**Methods**

**Fish and facilities**

Captive mulloway broodstock held at the PSFI, NSW, Australia, were induced to spawn using temperature
cues after exposure to a truncated photo-therm regime (described in Partridge, Jenkins & Frankish 2002). Fertilized eggs were collected on four different occasions, quantified and then treated with ozone [concentration (mg L$^{-1}$) × time (min) = 0.2] to disinfect eggs of pathogens, before being transferred to a 2000 L flow-through (1.5 L min$^{-1}$) clear water larval rearing tank for both Experiments 1 and 2, and an 8000 L flow through (5 L min$^{-1}$) clear water larval rearing tank for Experiment 3 and the confirmation trial. The rearing tanks were all maintained in clear water so that resident microalgae did not affect the feeding preferences.

**Water quality**

Water quality (mean ± SD; pH, dissolved oxygen [DO], temperature and salinity) parameters were measured daily in each experiment tank using a water quality meter (Horiba U-10, Kyoto, Japan). A rapid test kit (E. Merck, Model 1.08024, Darmstadt, Germany) was used to measure the total ammonium (NH$_4^+$ mg L$^{-1}$) (Table 1). Water quality variables were consistent across all treatment tanks for the duration of the experiments. The water quality in Experiments 2 and 3 was similar to that found in the holding tanks.

**Experiment 1**

Experiment 1 examined the effects of feeding six different combinations of live feeds and a 3/5 Proton pellet microdiet (INVE, Dendermonde, Belgium) on the weaning success and growth of larval mulloway. The 3/5 Proton diet was selected as it produced results comparable to two other commercially available diets for growth and survival in snapper (*Pagrus auratus*), another temperate marine fish (Fielder, Allan, Bardsey & Cheviot 2008). Mulloway larvae stocked at 125 fish L$^{-1}$ were fed enriched (Algamac 3050, Aquafauna Bio-Marine, Hawthorne, CA, USA) rotifers at 10 mL$^{-1}$ in a 2000 L holding tank until 13 dah (mean TL 4.9 ± 0.1 mm), when 800 larvae were stocked into each of 30, flow through (200 mL min$^{-1}$) experiment tanks (n = 5 replicate tanks/treatment; tank walls were black and conical floors were white) containing 100 L of 10 µm filtered estuarine water. The experiment treatments (Table 2) commenced on the following day (14 dah) and continued until the larvae in all the treatments were considered to be successfully weaned from live feeds to the pellet diet. Each experiment tank was siphoned daily to remove excess food and waste and the internal overflow screens were cleaned. Lights were set to turn on and off immediately without a dimming effect at 08:00 and 20:00 hours respectively (8 µmol s$^{-1}$ m$^{-2}$ in the light phase; LI-COR, model LI-1776, Lincoln, NE, USA). Enriched rotifers (maintained at 10 mL$^{-1}$) were fed to larvae in all treatments twice daily (09:00 and 15:00 hours) throughout the experiment (Table 1).

Nutritionally enriched Artemia were fed twice daily to four of the six treatments (Treatments 1, 2, 4, 5) from 18 to 27 dah and were maintained on either a standard PSFI ration (100%) or half the standard ration (50%). The standard ration began at 0.4 mL$^{-1}$ per feed at 18 dah (50 ± 0.13 mm, mean larvae length) and was doubled each day until 21 dah. The ration was then halved each day until 27 dah, which was the last time Artemia were fed to larvae. The residual concentration of live feeds was determined for each tank before the addition of each feed. The pellet microdiet was broadcast 4–6 times daily from either 14 dah (Treatments 4, 5, 6) or 23 dah (Treatments 1, 2, 3) until completion of the experiment (29 dah). The quantity of pellet broadcast at the start of the experiment was 0.2 g day$^{-1}$ and increased daily (approximately 17%) to ensure that the increase in feed demand was met.

Twenty larvae were sampled from each tank every 4 days and euthanized using a lethal dose of ethyl-p-aminobenzoate (100 mg L$^{-1}$; Sigma-Aldrich, Castle Hill, NSW, Australia). Larvae were examined immediately using a dissection microscope (M5 – 89734, Wild Heerbrugg, Switzerland) for TL (mm) from the top of

<table>
<thead>
<tr>
<th>Experiment</th>
<th>pH</th>
<th>DO mg L$^{-1}$</th>
<th>Temperature °C</th>
<th>Salinity (g L$^{-1}$)</th>
<th>Total ammonium (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>8.2 ± 0.1</td>
<td>6.7 ± 0.3</td>
<td>23.3 ± 0.8</td>
<td>35.3 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>8.1 ± 0.1</td>
<td>7.5 ± 1.6</td>
<td>20.1 ± 1.4</td>
<td>32.0 ± 0.4</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>8.1 ± 0.2</td>
<td>7.6 ± 0.2</td>
<td>22.5 ± 0.8</td>
<td>30.4 ± 0.8</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Confirmation Experiment</td>
<td>7.8 ± 0.1</td>
<td>10.1 ± 1.0</td>
<td>21.3 ± 0.3</td>
<td>35.7 ± 0.1</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>
Table 2 Feed treatments applied to fish throughout the feeding trial (n = 5 replicates) (Experiment 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rotifers (dah)</th>
<th>Artemia (dah)</th>
<th>Pellet diet (dah)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14–29</td>
<td>18–27a</td>
<td>23–29</td>
</tr>
<tr>
<td>2</td>
<td>14–29</td>
<td>18–27a</td>
<td>23–29</td>
</tr>
<tr>
<td>3</td>
<td>14–29</td>
<td>Nil</td>
<td>23–29</td>
</tr>
<tr>
<td>4</td>
<td>14–29</td>
<td>18–27a</td>
<td>14–29</td>
</tr>
<tr>
<td>5</td>
<td>14–29</td>
<td>18–27b</td>
<td>14–29</td>
</tr>
<tr>
<td>6</td>
<td>14–29</td>
<td>Nil</td>
<td>14–29</td>
</tr>
</tbody>
</table>

*a, 100% ration; b, 50% ration.
dah, days after hatching.

the snout to the end of the tail, and for food type consumed. The 20 fish from each tank were then pooled and dried (105 °C, 16 h) to obtain the mean dry weight (mg) of fish. Survival was calculated for each treatment replicate at the end of the experiment and excluded the fish removed for sampling [Per cent survival = (Final no. of fish/720) × 100].

Experiment 2

Experiment 2 was a short-duration feeding experiment that examined the transition of mulloway larvae from enriched rotifers to enriched Artemia as the larval length increased. The mean TL of larvae at the start of the experiment was 4.1 mm, which was smaller than the mean TL of fish at the start of Experiment 1 and allowed for a comparison of the results. Larvae for the experiment were sourced each day from a 2000 L tank (described for Experiment 1) and were maintained on rotifers at a density of 10 mL⁻¹. For three days leading up to and during the experiment, larvae in the holding tank were given one feed of Artemia each day at a density of 0.1 mL⁻¹, and so they were not naive to the new food source at the commencement of the experiment protocols. The tank water was exchanged at a rate of 1.5 L min⁻¹, which ensured the clearance of live feeds from the tank during the night and also ensured that the digestive tract of the larvae was cleared. Twenty fish from the holding tank were sampled daily until the mean TL reached 4.1 mm. On each morning thereafter, and before the addition of food in the holding tank, the sample of larvae (n = 20) was examined to confirm that no residual food remained in the digestive tract from the previous day. Then, 100 larvae were transferred every day to each of 15 randomly positioned blue experiment vessels containing 10 L of 10 μm filtered and lightly aerated static estuarine water (ambient light conditions, similar to the light conditions of the larviculture tank). Larvae were acclimated for 30 min before they were fed rotifers at 2 mL⁻¹ (Treatment 1), Artemia at 2 mL⁻¹ (Treatment 2) or both rotifers and Artemia at 1 mL⁻¹ each (Treatment 3). Larvae were given 1 h to feed (methods described by Shaw et al. 2003) and were then euthanized using a lethal dose of ethyl-p-aminobenzoate (100 mg L⁻¹) and immediately preserved in 10% buffered formalin for later dissection and stomach content assessment. A sub-sample of 20 fish from each replicate was examined using a dissection microscope and the stomach contents were teased apart to determine the food type and quantity consumed by individual larvae.

Experiment 3

Experiment 3 was a short-duration feeding experiment that examined the transition of mulloway larvae from enriched Artemia to a pellet microdiet (Proton 3/5, INVE) as the larval length increased. The mean TL of larvae at the start of the experiment was 8.3 ± 0.3 mm, which was smaller than the mean TL of fish observed to be feeding on pellets in Experiment 1 and allowed for a comparison of the results. Larvae for the experiment were sourced each day from an 8000 L flow-through holding tank, where they were maintained on Artemia at a density of 1 mL⁻¹. For three days leading up to and during the experiment, larvae in the holding tank were broadcast-fed approximately 8 g of a 300–500 μm pellet (3/5 Proton) once each day, and so they were not naive to the new food source. Once the mean TL of mulloway reached 8.3 ± 0.3 mm, 100 larvae were transferred each day to each of 15 randomly positioned blue experiment vessels containing 10 L of 10 μm filtered, lightly aerated static estuarine water, similar to those described in Experiment 2 (ambient light conditions, similar to that of the holding tank). Fish were transferred to the experiment vessels before feeding each day and the clearance of food from the guts of larvae was confirmed before transfer, as described in Experiment 2. Larvae were acclimated for 30 min before being offered either Artemia at 2 mL⁻¹ (Treatment 1), a 300–500 μm pellet broadcast at 15-min intervals (Treatment 2) or both Artemia at 1 mL⁻¹ and pellet broadcast at 15 min intervals (Treatment 3). The number of pellets provided was equivalent to the number of Artemia provided, which was determined by measuring the weight of a known number of pellets and then determining the required weight of pellets for the feeding trial. Larvae were allowed to feed for 1 h and were then euthanized using a lethal dose of
ethyl-p-aminobenzoate and immediately preserved in 10% formalin for later dissection and stomach content assessment. As in Experiment 2, a sub-sample of 20 fish from each replicate was examined using a dissection microscope, and the food type and quantity consumed by individual larvae was recorded.

The optimal feeding outcomes of Experiment 3 were then confirmed in a pilot commercial-scale production run at the PSFI. The mean TL of larvae selected for the start of this experiment was 9.8 ± 1.1 mm as this was smaller than the mean TL of fish that were feeding on pellets in Experiments 1 and 3 and enabled a comparison of the results. Mulloway larvae were housed in two larviculture tanks containing 8000 L of 10 μm filtered estuarine water and maintained on enriched Artemia at 1 mL\(^{-1}\). Once the average length of fish within the tanks was 9.8 ± 1.1 mm (mean ± SD), the larvae were offered a 300–500 μm pellet diet (3/5 Proton) in addition to the 1 mL\(^{-1}\) ration of Artemia. The ration of Artemia was reduced over the following 3 days to 0.8, 0.6 and 0.3 mL\(^{-1}\) respectively. The larvae were then maintained on Artemia at 0.3 mL\(^{-1}\) until all fish were observed to be feeding on pellets (7 days from the initial addition of pellet). Pellets were distributed to each tank using an automatic belt feeder and through regular manual broadcasting. Twenty larvae were sampled daily from each tank and the number of pellets and Artemia in the stomachs of individual larvae and TL were recorded. The TL was recorded for every fish, which differed from the initial feed selection experiments, where the mean TL of fish in the holding tank was recorded each day.

**Statistical analyses**

Statistical analyses were conducted using STATGRAPHICS Version 4.1 (STSC, USA). In Experiment 1, data were analysed for homogeneity of variance using Cochran's test. The experiment was designed for two-factor analysis of variance (ANOVA) to determine the effects of the Artemia ration size and the time of pellet introduction on weight, TL and survival. Where statistical differences were found, the means were separated using the Student–Newman–Keuls test (SNK). Where significant interactions were found, one-factor ANOVA and the SNK test were used to establish where the significant differences (P < 0.05) existed.

In Experiments 2 and 3, data in Treatment 3 were analysed for the mean number of food items consumed. Significant differences (P < 0.05) were determined using a two-sample paired t-test. Chesson's selectivity index (z) (Chesson 1978) was also used to compare the feeding preferences of mulloway larvae for each prey type.

\[
z_i = \frac{r_i/n_i}{\sum_{j=1}^{m} r_j/n_j}, \quad i = 1, \ldots, m
\]

where \(r_i\) is the number of items of prey type \(i\) in the larvae diet. \(n_i\) is the number of items of prey type \(i\) in the environment and \(m\) is the total number of prey types. Only larvae that had selected one or more prey items were used in the calculation of the selection index, to exclude non-feeding fish from the analysis.

Significant differences in selection (z) were determined using a t-test to compare z with neutral selection (z = 0.5) for each length using the equation (Chesson 1983)

\[
t = \frac{z_i - 0.5}{\sqrt{s^2/k}}
\]

where \(z_i\) is the sample mean and \(s^2\) is the sample variance of the \(k\) estimators of \(z_i\). Alpha levels of \(P < 0.01\) were considered to be significant for this analysis to reduce the risk of a type I error.

The percentage of fish feeding on each prey type was recorded for all treatments in Experiments 2 and 3 to determine the feeding ability of larval mulloway. The percentages of fish feeding in Treatments 1 and 2 were compared using a two-sample t-test, while in Treatment 3 (offered two food types), a two-sample paired t-test was used to compare the percentages of fish that were feeding. Those fish in Treatment 3 that were not feeding were excluded from the analysis.

**Results**

**Experiment 1**

No interactions existed (P > 0.05) between the two factors, time of pellet introduction (14 or 23 dah) and Artemia ration size (0%, 50% or 100% ration) for larval weight or TL at all sampling times. Also, no effects of the two levels of pellet introduction time were observed for larval weight or TL. Fish that were not fed Artemia performed poorly compared with fish in treatments that were fed Artemia. Significant differences (P < 0.05) existed between the mean weight or TL of fish in treatments that were not fed Artemia (i.e. Treatments 3 and 6) compared with those that were fed Artemia (Figs 1 and 2, Table 3) at completion of the experiment. However, no significant differences in the mean weight or TL were found between treatments fed a half ration (Treatments 2 and 5) and a
full ration (Treatments 1 and 4) of *Artemia* (Figs 1 and 2, Table 3). Fish in treatments that were not fed *Artemia* began consuming pellets earlier than fish that were fed *Artemia*. At 21 dah, two replicates from Treatment 6 (not fed *Artemia*) contained fish that were feeding on minimal amounts of pellet. The average TL (mean ± SEM) of fish in this treatment was 7.2 ± 0.1 mm (Fig. 2). At 25 dah, the mean TL of fish in Treatments 3 and 6 (not fed *Artemia*) was 8.8 ± 0.3 and 9.6 ± 0.3 mm, respectively, and all replicates contained fish that fed on pellets. At 29 dah, the mean TL of fish in all treatments fed on pellets.

A significant interaction (\(P < 0.05\)) occurred between the time of pellet introduction (14 or 23 dah) and *Artemia* ration size (0%, 50% or 100% ration) for survival (Table 3). This was caused by the significantly poorer survival in fish fed a 50% ration of *Artemia* and offered pellets from 23 dah than fish fed a 50% ration of *Artemia* and offered pellets from 14 dah. There were no significant differences in the survival of fish between the other feeding regimes (0% and 100% rations).

### Experiment 2

The percentage of feeding larvae in Treatment 1, offered only rotifers, increased over the duration of the trial from 21.0 ± 0.9% (mean TL 4.1 ± 0.3 mm) to 88.0 ± 1.0% (5.4 ± 0.5 mm) (Table 4). The percentage of larvae feeding in Treatment 2, offered only *Artemia*, also increased throughout the experiment from 11.0 ± 0.7% (mean TL 4.1 ± 0.3 mm) to 81.0 ± 0.9% (5.5 ± 0.4 mm) (Table 4). Larvae in Treatment 3 were offered both rotifers and *Artemia*, and as the mean TL of larvae increased, the acceptance of *Artemia* in preference to rotifers increased. A significantly greater

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**Table 3** Mean (± SEM) final dry weight, total length and survival of fish in each feeding treatment at the end of the experiment* † (Experiment 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Artemia (18 dah) (%)</th>
<th>Pellet diet (dah)</th>
<th>Weight (mg)</th>
<th>Length (mm)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>23</td>
<td>4.9 ± 0.5(^a)</td>
<td>14.4 ± 0.6(^b)</td>
<td>5.5 ± 3.0</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>23</td>
<td>4.5 ± 0.2(^b)</td>
<td>14.2 ± 0.2(^b)</td>
<td>1.0 ± 0.6(^a)</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>23</td>
<td>1.8 ± 0.1(^a)</td>
<td>10.0 ± 0.3(^a)</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td>4</td>
<td>100</td>
<td>14</td>
<td>4.5 ± 0.4(^b)</td>
<td>14.5 ± 0.5(^b)</td>
<td>3.7 ± 1.2</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>14</td>
<td>4.1 ± 0.3(^b)</td>
<td>13.7 ± 0.4(^b)</td>
<td>13.1 ± 4.1(^y)</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>14</td>
<td>2.6 ± 0.3(^a)</td>
<td>11.6 ± 0.4(^a)</td>
<td>7.4 ± 3.0</td>
</tr>
</tbody>
</table>

* A full ration (100%) of *Artemia* began at 0.4 mL\(^-1\) per feed at 18 dah and was doubled each day until 21 dah. The ration was then halved each day until 27 dah, which was the last feed of *Artemia*. The 50% ration was half of the full ration.
† Different letters in superscript within the same column indicate significant differences (\(P < 0.05\); two-factor analysis of variance. Student–Newman–Keuls test). \(^a\) and \(^b\) indicates the effects of *Artemia* ration; \(^x\) and \(^y\) indicates effects of pellet introduction, where significant interactions (\(P < 0.05\)) occurred.

dah, days after hatching.
significantly more rotifers than fish length (Fig. 3). Larvae in Treatment 3 consumed summed by mulloway larvae was also dependent on rotifers (Table 4).

The mean number of rotifers and Artemia consumed by mulloway larvae was also dependent on fish length (Fig. 3). Larvae in Treatment 3 consumed significantly more rotifers than Artemia until the mean larval TL reached 5.1 ± 0.1 mm, at which time no significant difference was observed between the mean number of rotifers and Artemia consumed. From 5.2 ± 0.1 mm, larvae began to consume significantly more Artemia than rotifers. The number (mean SEM) of Artemia consumed by larvae in Treatment 3 increased steadily from 0.1 ± 0.03 Artemia h⁻¹ for larvae of 4.1 ± 0.07 mm to 13.7 ± 0.31 Artemia h⁻¹ for larvae of 5.5 ± 0.1 mm.

Chesson’s selectivity index (α) showed a significant (P < 0.01) selection for Artemia for fish of 4.1 ± 0.3 and 4.9 ± 0.4 mm TL (Fig. 4). From 5.1 ± 0.5 to 5.4 ± 0.4 mm TL, there was no significant difference in selection between either rotifers or Artemia and neutral selection (α = 0.5). Larvae of 5.5 ± 0.4 mm TL showed significant (P < 0.01) selection for Artemia (Fig. 4).

### Table 4 The percentage (mean ± SEM; n = 5) of fish feeding on each food type, in each treatment, on experiment days a, b, c (Experiment 2)

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Treatment 1 – R</th>
<th>Treatment 2 – A</th>
<th>Treatment 3 – A</th>
<th>Treatment 3 – R</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 ± 0.3</td>
<td>21 ± 0.8</td>
<td>11 ± 0.7</td>
<td>91 ± 4.0</td>
<td>14 ± 5.9</td>
</tr>
<tr>
<td>4.4 ± 0.4</td>
<td>71 ± 1.7</td>
<td>67 ± 1.2</td>
<td>75 ± 4.7</td>
<td>40 ± 7.0</td>
</tr>
<tr>
<td>4.9 ± 0.4</td>
<td>76 ± 1.1</td>
<td>39 ± 1.1</td>
<td>91 ± 3.7</td>
<td>38 ± 6.7</td>
</tr>
<tr>
<td>5.1 ± 0.5</td>
<td>88 ± 0.9</td>
<td>57 ± 0.7</td>
<td>63 ± 7.8</td>
<td>54 ± 8.3</td>
</tr>
<tr>
<td>5.2 ± 0.5</td>
<td>81 ± 0.9</td>
<td>59 ± 1.2</td>
<td>55 ± 7.6</td>
<td>63 ± 7.4</td>
</tr>
<tr>
<td>5.4 ± 0.5</td>
<td>86 ± 1.0</td>
<td>56 ± 0.6</td>
<td>53 ± 3.4</td>
<td>64 ± 2.7</td>
</tr>
<tr>
<td>5.5 ± 0.4</td>
<td>86 ± 1.0</td>
<td>81 ± 0.9</td>
<td>24 ± 1.7</td>
<td>85 ± 1.5</td>
</tr>
</tbody>
</table>

*Lengths (mean ± SEM, n = 20 replicate fish) are those of fish in the holding tank on a given experiment day.†Fish in Treatment 1 were offered only rotifers (R), fish in Treatment 2 were offered only Artemia (A), and fish in Treatment 3 were offered both R and A. Treatment 3 is represented in the third and fourth rows as two food types were available.‡The mean percentages of fish feeding (excluding non-feeding fish) on each prey type in Treatment 3 were compared using a two-sample paired t-test. Different superscripts at the same length indicate significant differences (P < 0.05) between Treatments 1 and 2 (a and b), and between prey types within Treatment 3 (x and y).

### Figure 3 The number of food items (mean ± SEM; n = 5) consumed by fish in 1 h, for each treatment, on each sampling day of the experiment (Experiment 2). Lengths (means; n = 20) are those of fish in the holding tank on a given experiment day. Treatment 1 (only offered rotifers) and Treatment 2 (only offered Artemia) are represented by the bar graph. Treatment 3 (offered both rotifers and Artemia) is represented by the line graph, with each food type presented as a separate line. Diﬀerent letters between Treatment 3 values at the same length indicate signiﬁcant diﬀerences (P < 0.05; two sample paired t-test). A, Artemia and R, rotifers.

### Figure 4 Chesson’s selectivity index (α; mean ± SEM; n = 5) for fish fed a mixed diet containing 50% rotifers and 50% Artemia (Experiment 2). Fish that did not consume either prey were excluded from the analyses. Mean lengths displaying * indicate a signiﬁcant diﬀerence from neutral selection (P < 0.01; two-sample paired t-test).
**Table 5** The percentage (mean ± SEM; $n = 5$) of fish feeding on each food type, in each treatment, on consecutive experiment days*†,‡ (Experiment 3)

<table>
<thead>
<tr>
<th>Length (mm)</th>
<th>Treatment 1 – A</th>
<th>Treatment 2 – P</th>
<th>Treatment 3 – A</th>
<th>Treatment 3 – P</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.3 ± 0.3</td>
<td>95 ± 0.5‡</td>
<td>74 ± 0.5</td>
<td>97 ± 1.2‡</td>
<td>8 ± 2.0‡</td>
</tr>
<tr>
<td>8.8 ± 0.4</td>
<td>96 ± 0.4‡</td>
<td>55 ± 0.7†</td>
<td>98 ± 1.2‡</td>
<td>26 ± 3.3†</td>
</tr>
<tr>
<td>10.0 ± 0.3</td>
<td>96 ± 0.4‡</td>
<td>70 ± 0.7†</td>
<td>83 ± 2.7†</td>
<td>44 ± 8.4‡</td>
</tr>
<tr>
<td>10.6 ± 0.4</td>
<td>98 ± 0.2#</td>
<td>77 ± 1.7†</td>
<td>74 ± 4.6</td>
<td>79 ± 2.6</td>
</tr>
<tr>
<td>11.6 ± 0.2</td>
<td>100 ± 0.0#</td>
<td>86 ± 0.9§</td>
<td>53 ± 5.1†</td>
<td>81 ± 3.3‡</td>
</tr>
<tr>
<td>12.0 ± 0.4</td>
<td>98 ± 0.2#</td>
<td>81 ± 1.0§</td>
<td>78 ± 0.2</td>
<td>87 ± 0.2</td>
</tr>
<tr>
<td>12.5 ± 0.3</td>
<td>98 ± 0.2#</td>
<td>96 ± 0.5</td>
<td>78 ± 4.6</td>
<td>81 ± 0.4</td>
</tr>
</tbody>
</table>

*Lengths (mean ± SEM; $n = 20$ replicate fish) are those of fish in the holding tank on a given experiment day.
†Fish in Treatment 1 were offered only *Artemia* (A), fish in Treatment 2 were offered only pellets (P), and fish in Treatment 3 were offered both A and P. Treatment 3 is presented in the third and fourth rows as two food types were available.
‡The mean percentages of fish feeding in Treatments 1 and 2 were compared using a two-sample t-test. The mean percentages of fish that were feeding in Treatment 3 (excluding non-feeding fish) were compared using a two-sample paired t-test. Different superscripts at the same length indicate significant differences (P < 0.05) between Treatments 1 and 2 (a and b), and within Treatment 3 (x and y).

**Figure 5** The number of food items (mean ± SEM; $n = 5$) consumed by fish in 1 h for each treatment, on consecutive days of the experiment (Experiment 3). Lengths (means; $n = 20$) are those of fish in the holding tank on a given experiment day. Treatment 1 (only offered *Artemia*) and Treatment 2 (only offered pellets) are represented by the bar graph. Treatment 3 (offered both *Artemia* and pellets) is represented by the line graph, with each prey type presented as a separate line. Different letters between Treatment 3 values at the same length indicate significant differences (P < 0.05; two sample paired t-test).

**Experiment 3**

The percentage of feeding larvae in Treatment 1, offered only *Artemia*, ranged from 950 ± 0.5 (mean TL 8.3 ± 0.3) to 100.0 ± 0.0% (11.6 ± 0.2 mm) (Table 5). The percentage of larvae feeding in Treatment 2, offered only pellets, increased throughout the experiment from 250 ± 0.3 (mean TL of 8.3 ± 0.3) to 960 ± 0.5% (12.5 ± 0.3 mm) (Table 5). Larvae in Treatment 3 were offered both *Artemia* and pellets, and as the mean TL increased, the acceptance of the pellets in preference to *Artemia* increased. A significantly greater percentage of larvae consumed *Artemia* in preference to pellets until the mean TL reached 10.6 ± 0.4 mm, at which time no significant difference was observed between the percentages of fish consuming *Artemia* or pellets. Once the mean TL reached 11.6 ± 0.2 mm, a significantly greater percentage of fish consumed pellets in preference to *Artemia*. This significant difference was not, however, evident for fish with a mean TL of 12.5 ± 0.3 mm.

The mean number of *Artemia* and pellets consumed by mulloway larvae was also dependent on fish length (Fig. 5). Larvae in Treatment 3 consumed significantly more *Artemia* than pellets until the mean larval TL reached 10.6 ± 0.4 mm, at which time no significant difference was observed between the mean number of *Artemia* and pellets consumed. From a mean TL of 11.6 ± 0.2 mm, larvae began to consume significantly more pellets than *Artemia* (Fig. 5). The number (mean ± SEM) of pellets consumed by larvae in Treatment 3 increased steadily from 1.0 ± 0.2 pellets h$^{-1}$ for larvae of 8.3 ± 0.3 mm mean TL to 13.5 ± 0.6 pellets h$^{-1}$ for larvae of 11.6 ± 0.2 mm mean TL. Larvae with a mean TL of 12.0 ± 0.4 and 12.5 ± 0.3 mm did not continue to show an increase in the mean number of pellets consumed; however, they did continue to consume significantly more pellets than *Artemia* (Fig. 5).

Chesson’s selectivity index ($z$) showed significant (P < 0.01) selection of *Artemia* for fish of 8.3 ± 0.3 and 8.8 ± 0.4 mm mean TL (Fig. 6). Larvae of 10.0 ± 0.3 and 10.6 ± 0.4 mm mean TL were observed to have no significant difference in selection between *Artemia* or pellets and neutral selection ($z = 0.5$). Once the mean TL of larvae reached 11.6 ± 0.2 mm, significant selection for pellets was observed (Fig. 6). This significant difference was not, however, evident for fish with a mean TL of 12.5 ± 0.3 mm.
In the confirmation of these results (Experiment 3), larvae were observed to consume significantly more pellets than Artemia once the mean TL of fish was between 10.0 and 11.0 mm. No significant difference was observed between the mean numbers of food items consumed for fish of 8.0 ± 0.5 to 10.0 ± 0.5 mm TL. The percentage of fish that consumed pellets increased linearly (r = 0.936) from 47% in fish with a mean TL of 8.0 ± 0.5 mm to 100% in fish with a mean TL of 12.0 ± 0.5 mm. As expected, the percentage of fish consuming Artemia decreased consistently (r = 0.518) and ranged from 89.0% in fish with a mean TL of 10.0 ± 0.5 mm to 13.0% in fish with a mean TL of 15.0 ± 0.5 mm.

**Discussion**

This study has determined that mulloway larvae can be weaned directly from rotifers to the pellet microdiet tested without the use of Artemia (Experiment 1); however, this significantly reduces growth. Fish that were fed Artemia in combination with rotifers and pellets also weaned successfully but displayed better growth rates. These results suggest that the weaning process can be achieved more cost effectively by reducing the amount of Artemia currently used at the PSFI by half and is consistent with other studies that reported similar results when larvae were weaned onto pellet microdiets without the use of Artemia. Both Callan et al. (2003) and Fletcher et al. (2007) determined that it was possible to co-feed Atlantic cod (Gadus morhua) larvae a microdiet along with a reduced amount of Artemia without compromising growth and survival; however, if Artemia was excluded from the diet, growth and survival were reduced. Curnow et al. (2006) also found that the weaning process for barramundi (Lates calcarifer) should include some Artemia (5% of the previous industry ration) in conjunction with a pellet microdiet in order to stimulate feeding until the stomach is fully developed. The importance of including Artemia in the weaning process is apparent; however, the early introduction of pellets has also been reported to improve weaning success in marine larvae. Alves Jr., Cerqueira and Brown (2006) determined that fat snook (Centropomus parallelus) could be weaned onto a pellet microdiet earlier if a suitable co-feeding period was applied, while Brown, Wiseman and Kean (1997) discussed the importance of a co-feeding period to improve survival in Atlantic wolffish (Anarhichas lupus) larvae.

Some of the benefits of incorporating live feeds into the weaning process include improved larval digestion by stimulating enzyme secretion from the pancreas and activating zymogens in the gut to increase the overall enzymatic activity (Pedersen & Hjelme- land 1988; Person Le Ruyet et al. 1993). The reduced growth rates observed when mulloway larvae were not offered Artemia in conjunction with pellets may indicate that larval digestive enzymes were inadequate to digest and assimilate nutrients from this pellet diet. In addition, all fish (Experiment 1) not offered Artemia (Treatments 3 and 6) fed on pellets at mean lengths of 8.8 and 9.6 mm respectively. These lengths are larger than those determined to be appropriate to introduce Artemia, but smaller than was suggested to introduce pellets in feed selection Experiments 2 and 3 respectively. Again, this indicates that larvae in these treatments were ready to consume a larger food source, but digestive capacity at this time was inadequate to digest the pellet effectively. There is evidence to suggest that co-feeding early larvae both live feeds and inert diets can enhance the weaning success, growth and survival by pre-conditioning the larvae to accept the manufactured diets. Cañaveate and Fernández-Diaz (1999) determined that weaning success in sole (Solea senegalensis) was improved by co-feeding before metamorphosis rather than after, and discussed the difficulty in encouraging advanced larvae to accept an inert diet once they were accustomed to Artemia.
In Experiment 1, it is interesting that fish in all treatments fed on pellets once the minimum mean TL of 10.0 mm was attained. This is similar to the outcomes of feed selection Experiment 3, in which fish selected pellets equally to *Artemia* by 10.6 mm, and suggests that this length is suitable for the introduction of pellets to mulloway larvae. Furthermore, a similar study by Curnow et al. (2006) found that a reduction in live feed use in barramundi (*L. calcarifer*) could be facilitated by supplying a Gemma Micro diet (Skretting, Stavanger, Norway) but not with the Proton diet. It may be useful to investigate weaning success in mulloway using a range of pellet microdiets to determine whether further reductions in *Artemia* reliance are possible.

The reduced survival of fish fed pellets late (23 dah) rather than earlier (14 dah) when they were also offered a 50% ration of *Artemia* was likely to have been the result of the natural mortality that frequently occurs in larval experiments. This reduction in survival is not likely to be the result of treatment effects, but rather from handling stress.

In Experiment 2, the transition of food preference from rotifers to *Artemia* occurred once the mean size of mulloway larvae was 5.2 mm, at which time larvae selected *Artemia* equally to rotifers. Fish that were only fed *Artemia* consumed them at a greater rate than fish offered both rotifers and *Artemia*, which indicates that larvae were capable of feeding on *Artemia* but were preferentially selecting rotifers until they reached 5.2 mm in length. Additionally, the percentage of fish selecting *Artemia* increased steadily and was similar to the percentage of fish consuming rotifers once larvae reached 5.2 mm. The selection of smaller prey types up until larvae reached 5.2 mm can be explained on the basis of energy spent and profits gained. Mayer and Wahl (1997) discussed the prey preference of larval walleye (*Stizostedion vitreum*) in terms of profit as the fish selected strongly for prey types that improved growth and survival. Selective feeding has been described as an evolutionary tendency to maximize energy intake and must be an adaptive feature of larval fish to optimize energy consumption (Greene 1986; Schoener 1987). Mulloway larvae may have selected *Artemia* once swimming ability and sensory development had improved.

In Experiment 3, the transition of food preference from *Artemia* to the Proton pellet diet occurred once the size of mulloway was 10.6 mm. The fish that were only fed pellets consumed them at a much greater rate than fish offered a choice between *Artemia* and pellets, indicating that larvae were capable of consuming pellets but were preferentially selecting *Artemia* until reaching a mean length of 10.6 mm. Similarly, Chesson’s selectivity index indicated that the transition from *Artemia* to pellets occurred between 10.0 and 10.6 mm, after which selection of pellets was significantly greater than neutral selection. In addition, the percentage of fish selecting pellets increased with fish length and was similar to the percentage of fish consuming *Artemia* once larvae reached 10.6 mm. Olsen et al. (2000) found that less digestible prey types were not selected by Atlantic halibut (*Hippoglossus hippoglossus*), and it is possible that smaller mulloway larvae were not selecting pellets as they were more difficult to digest. Once mulloway larvae reached 10.6 mm, digestive capacity may have been sufficient to enable them to benefit from the more energy-rich pellets. This length is likely to be appropriate to reduce weaning difficulties that may arise from fish becoming accustomed to *Artemia*, as discussed by Cañavate and Fernández-Díaz (1999), but at the same time allow for larvae to benefit from the less digestible pellets.

The results of the confirmation pilot commercial-scale trial were similar to that of Experiment 3. It was demonstrated that mulloway larvae began to select pellets in preference to *Artemia* once the mean total larval length was between 10.0 and 11.0 mm. The initial experiment measured food preference against the average length of fish in the holding tank on a given day, while the confirmation trial measured food preference against individual larvae lengths. As the results of the two experiments were similar, it suggests that the methodology used in the initial experiment was sufficient to rely on the results obtained using average lengths each day and not individual lengths. The results of the three experiments examining the transition from *Artemia* to pellets were similar. In Experiments 1 and 3, and in the confirmation trial, larvae TLs at the time of pellet selection were 10.0, 10.6 and 10.0–11.0 mm respectively. The similarity in the results strongly supports the conclusion that 10.0–11.0 mm is an appropriate length to begin weaning larval mulloway from *Artemia* to the pellet microdiet.

These feeding studies have shown that the process for weaning larval mulloway onto a Proton microdiet needs to include some *Artemia* in order for the transition to be successful without compromising growth. The weaning process should begin with rotifers until the mean size of larvae reaches approximately 5.0 mm in length, after which, *Artemia* should be maintained until the mean fish TL is between 10.0
and 11.0 mm. The microdiet can then be introduced and the amount of *Artemia* can be gradually reduced over the following days, until all fish are considered to be successfully weaned. These protocols are expected to maintain growth rates while reducing costs by minimizing the amount of *Artemia* required for weaning and optimizing the time of introduction for each new food source.

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**References**


