Southern Bluefin Tuna, *Thunnus maccoyii* 12

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Introduction

The southern bluefin tuna (SBT) (*Thunnus maccoyii*, Castelnau) is one of two species of 'bluefin' tunas and the key tuna species currently being farmed in Australia. Reported at sizes of up to 220 cm in length and 200 kg in weight, the SBT is one of the largest members of the Scombridae (mackerels and tunas) (Fig. 12.1). Its close relative, the northern bluefin tuna (NBT) (*Thunnus thynnus*) is the only species in the family that grows to a larger size (Kailola *et al.*, 1993). The NBT is the species that predominates in the developing tuna aquaculture industries in the Mediterranean, North America and Japan.

Tunas are unusual among teleosts because they are warm-bodied (Carey and Teal, 1969). They are able to maintain body temperatures above ambient water temperature through the use of counter-current heat exchangers. Bluefin tunas have the most advanced heat-exchange systems of all the tunas, and are able to thermally insulate a number of organs and tissues (e.g. brain, viscera and muscles) from ambient water and from other tissues (Stevens and McLeese, 1984). SBT are high-level to apex predators even as juveniles, and have very broad dietary ranges (Young *et al.*, 1997).

The SBT aquaculture industry runs seasonally. Each year, beginning in the Australian summer (December–February), juvenile fish of approximately 20 kg (2–4 years of age) are caught by purse-seine nets in the Great Australian Bight, off the southern Australian coastline. The fish are then transferred to specially designed towing pontoons and slowly towed back to Boston Bay near Port Lincoln in South Australia. During the next 4–8 months, through autumn, winter and into spring, the tuna are primarily fed baitfish to improve both their condition and their biomass. By October, most of the fish have been harvested and the industry begins preparations for the return of the fish to the Great Australian Bight during the following summer.

Fig. 12.1. Southern bluefin tuna on the market floor at Tsukiji market in Japan.

The product is sold almost exclusively to the Japanese sashimi markets, the largest and most influential of which is Tsukiji market in Tokyo (Fig. 12.2). Other key markets for farmed tuna include Osaka, Nagoya and Yokohama, though most regional markets will service some quantities of farmed tuna (Glencross, 1999).

Diet development for SBT has been particularly challenging for many reasons. The costs of research with this species are extremely high, having to maintain operations of numerous sea-cages, boats, crews and associated infrastructure. Each of the 1000 SBT used annually for research have been worth close to US$600. The annual cost of diet for research is approximately US$60,000–100,000. Working with SBT presents many problems other than the financial costs. The sheer size of the fish involved (usually around 1 m in length and 20 kg in weight) and the nature of its physical requirements demand that experiments be conducted in sea cages and that handling be kept to an absolute minimum. For similar reasons, a large number of replicates to minimize experimental variance and assist the design of statistically sound experiments has been limited. Because of these difficulties several alternative strategies have been developed to examine some growth parameters, such as levels of tissue and blood nucleic acids and protein, and *in vitro* digestibility studies (Carter *et al.*, 1998, 1999). Although these methods hold much promise, to date they have not been able to replace growth trials as the key form of performance assessment.

Fig. 12.2. Tsukiji wholesale market floor – fresh chilled product, October 1998.

Nutrient Requirements

The difficulties associated with conducting structured and highly replicated trials with SBT have prevented the determination of nutritional requirements through standard dose–response techniques (Glencross *et al.*, 1999a). As a consequence relative requirements have been determined based on the modelling of the performance of SBT fed various practical diet formulations. This approach can provide useful insights that can be applied in the early development of artificial feeds within the constraints of a restricted research environment. To date, it has contributed to the rapid development of a moist feed that supports performance equivalent to that obtained with SBT fed baitfish under research conditions (i.e. small pontoons, low stock density and controlled feeding).

Protein and amino acids

No specific studies examining protein utilization by SBT have been attempted to date. Despite this, several alternative and/or indirect methods of measuring protein utilization by and amino acid requirements (Table 12.1) of SBT have been examined (van Barneveld *et al.*, 1997; Carter *et al.*, 1998, 1999; Glencross *et al.*, 1999a).

Considerable effort has been made to develop both *in vitro* assessment techniques and surrogate assessment methods (Carter *et al.*, 1998, 1999; Bransden *et al.*, 1999; Table 12.2). Results from these studies have proved useful in the refinement of moist-diet development for SBT (Table 12.3). More recent studies have examined growth rate as a function of protein intake, taking into consideration the effect of water temperature and dietary energy (Glencross *et al.*, 1999b). These interpretations suggest that optimal daily protein intake for growth is no greater than 10 g day^{-1} kg^{-1} of biomass.

The capacity for protein synthesis (mg ribonucleic acid (mg (RNA) g^{-1} protein) provides a key indicator of protein metabolism and a value of 8 has been measured in SBT for approximate maintenance (Carter *et al.*, 1998). This value is at least four times higher than that predicted for a fish based on the weight, temperature and feed intake of the SBT (Houlihan *et al.*, 1995) but very similar to that of an unfed mammal (Milward *et al.*, 1973). A consequence would be significantly higher energy demands associated with protein turnover in SBT than in other fishes, even at zero growth. This suggests a close relationship between protein metabolism and energy requirements in SBT.

Table 12.1. Essential amino acid composition of key muscle types (g kg^{-1} on a dry-matter basis) of SBT and a proposed ideal amino acid balance, relative to lysine (data from van Barneveld *et al.*, 1997).

Amino acid	White muscle (g kg^{-1})	Red muscle (g kg^{-1})	Ideal balance (%)
Methionine	10.9	11.0	35
Threonine	17.2	18.2	56
Valine	19.2	20.4	63
Isoleucine	17.1	18.7	57
Leucine	27.6	29.3	90
Phenylalanine	14.9	15.1	47
Lysine	30.7	32.5	100
Histidine	30.3	30.5	96
Arginine	22.3	23.9	73

Table 12.2. *In vitro* digestibility of key feed ingredients by SBT digesta enzymes (data derived from Carter *et al.*, 1999).

Ingredient (nitrogen %)	Digestible protein	Digestible organic matter
Fish-meal – Tasmanian (11.1%)	81.7 ± 2.01%	74.7 ± 0.45%
Fish-meal – Chilean (9.8%)	65.8 ± 0.65%	54.7 ± 1.66%
Pilchards (*Sardinops sagax*) (12.2%)	82.3 ± 1.53%	78.3 ± 2.73%
Squid meal (10.6%)	36.7 ± 1.08%	27.2 ± 1.74%
Casein (13.3%)	98.3 ± 0.19%	97.8 ± 2.03%
Wheat gluten (11.5%)	94.7 ± 2.39%	71.4 ± 1.90%
Soybean meal (6.9%)	45.1 ± 3.48%	49.2 ± 3.19%
Wheat flour (1.8%)	88.3 ± 1.02%	58.31 ± 0.98%

The indispensable amino acid requirements of SBT have also been estimated based on the composition of both red and white muscle (Table 12.1). From this an ideal amino acid balance has been estimated (van Barneveld *et al.*, 1997). Similar to other fish species lysine has been estimated as the key limiting amino acid. As a consequence, diets have generally been formulated to consider this limitation within the scope of key ingredients used.

Energy

Based on the non-parametric analysis of data from several moist diets and baitfish diets fed under various feeding regimes during the 1998 season trials, a response surface model was developed. This model suggested that both protein and energy were key response factors in the diet of SBT, but that dietary energy had a greater influence than dietary protein (Glencross *et al.*, 1999b).

Modelling of the nutritional value of feed with respect to the effects of dietary energy on feed conversion suggested that dietary fat levels were a key factor in influencing the energetic value of the diets to SBT. This aspect will be discussed further in the following section on lipids.

The apparent enormous dietary energy requirement of SBT is consistent with what is known of their general physiological requirement to thermoregulate. The energetic processes required to generate metabolic heat in an environment that is constantly against a thermal gradient is likely to place an enormous energetic demand on these fish (Carey *et al.*, 1984).

Table 12.3. Fatty acid composition of SBT muscle lipid (data derived from Nichols *et al.*, 1998).

Fatty acid	% of total fatty acids
16:1n-7	0.5
18:0	11.1
18:1n-9	7.8
18:2n-6	0.9
18:3n-3	0.1
20:1n-9	0.2
20:4n-6	3.0
20:5n-3	4.8
22:1n-11	0.0
22:6n-3	44.1
SFA	34.2
MUFA	11.5
PUFA	54.3
n-6	4.4
n-3	50.0

PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; MUFA, mono-unsaturated fatty acids.

The decline in diet intake throughout a production season and the slowing of growth has provided a key indicator to levels of dietary intake required for maintenance. At the maintenance level, where growth has ceased, an average of about 150 kJ of gross energy (GE) was consumed per kilogram of fish per day. In most cases, this was under conditions where ambient water temperatures were less than 15°C. Based on the data from Glencross *et al.* (2002), the energetic requirement for 1 kg of gain, at a temperature of about 16°C was 56 MJ of GE. This was about half of that reported from earlier studies (Glencross *et al.*, 1999b), though still close to twice that estimated for Atlantic salmon, *Salmo salar* (NRC, 1993). This variability highlights the dramatic improvements made in diet performance and nutritional assessment over a few years.

Similar to the recent studies examining growth rates as a function of average protein intake has been the evaluation of GE intakes as a function of growth. As would be anticipated, GE intakes were consistent with protein intake assessments (Fig. 12.3). The plateauing of growth in this case was observed with a daily energy intake of about 300 kJ kg^{-1} day^{-1}. As with the previous interpretations of protein intake it should be noted that there was no compensation for variation in ambient water temperatures.

Lipids and fatty acids

Lipids

Dietary lipids play an important part in the nutrition of SBT, primarily because of their apparent enormous need for energy. To date this has been demonstrated by the relationship between the fat content of a range of moist pellets and baitfish, and the food conversion ratio (FCR) of these feeds on a dry-matter basis (Fig. 12.4). This interpretation suggests that the nutritional performance of the feed can be improved by increasing the dietary fat content (Glencross *et al.*, 1999b).

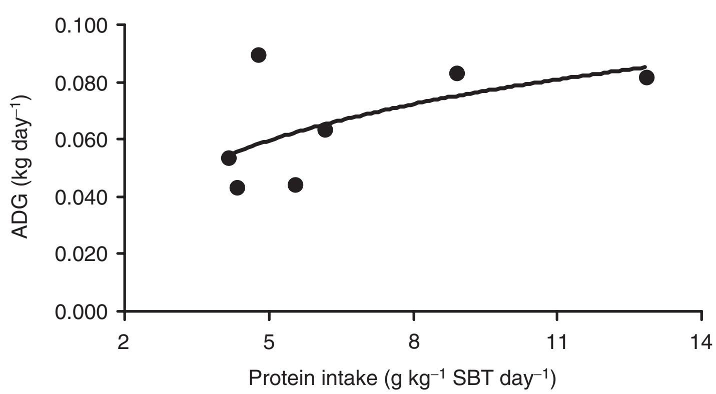

Fig. 12.3. Average daily gain (ADG) as a function of daily dietary protein intake (g kg^{-1} SBT day^{-1}). (Data derived from Glencross *et al.*, 2002.)

Fatty acids

Examination of the fatty acid profile of lipid from flesh of wild-caught SBT (Table 12.3) reveals very high levels of the long-chained, n-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) (22:6n-3) (Nichols *et al.*, 1998). Levels of eicosapentaenoic acid (EPA) (20:5n-3) are notably low, as are other long-chained PUFAs. The high level of DHA and a high n-3 : n-6 ratio suggest that SBT may have high requirements for the n-3 series of fatty acids, notably DHA.

Carbohydrates

Characterization of the digestive enzyme capabilities of SBT has identified some α-amylase activity, though not enough to be considered as viable for large-scale carbohydrate digestion (van Barneveld *et al.*, 1997). As a consequence of this finding, starch has been used as a filler/binder in some experimental diets.

Vitamins and minerals

No studies have yet examined the vitamin or mineral requirements for SBT. Supplementation of vitamins to baitfish used as feed has been undertaken by some sectors of the industry, despite no conclusive proof as to its value. Vitamin fortification has been attempted by either coating the baitfish in a vitamin-containing gel or by direct injection of the baitfish with a vitamin premix. Anecdotal evidence suggests that vitamin fortification is only of value when poorer-quality feeds (e.g. unfresh and rancid fish) were used and/or the SBT

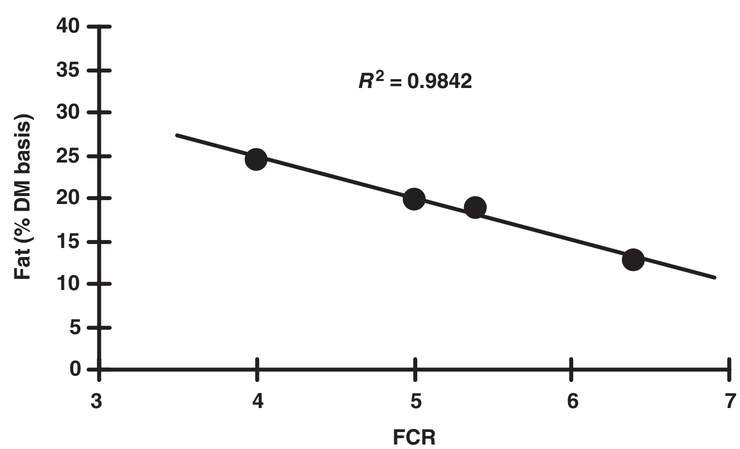

Fig. 12.4. Food conversion ratio (FCR) response to varying levels of fat (dry-matter (DM) fat) in various diets, modelled on a dry-matter consumption basis (data derived from Glencross *et al.*, 1999a).

were in poor condition. Vitamin premixes have been based primarily on those formulated for salmonids (van Barneveld *et al.*, 1997).

Supplementation of baitfish feeding with a vitamin-enriched moist pellet has been attempted by both the R&D and industry sectors as a pilot initiative (Glencross *et al.*, 1998). This practice showed considerable improvements in survival of SBT, though more studies are needed. The use of vitamin-fortified diets has also been shown in pilot studies to be beneficial in fortifying the α-tocopherol content of the flesh.

Practical Diets

The key practicalities of diets for the SBT aquaculture industry have been dictated by the need for large quantities of readily consumed, nutritious diet. Baitfish of various species have proved to be an ideal food, though the risks associated with using baitfish diets are many (Clarke *et al.*, 1997). To date, satisfactory manufactured diets for commercial use are available, but need further improvement. Though considerable progress has been made in recent years with the development of specialized moist pellets, the practicalities of using these feeds have not seen them gain widespread acceptance. Development of better bound and more attractive moist pellets and/or development of semi-dry or soft-dry pellets, primarily through extrusion, appears to hold the key to future progress in diet development for this species.

Currently the SBT aquaculture industry exclusively uses baitfish as the prime diet source. The actual species fed to the juvenile SBT varies throughout the year and from year to year, depending on availability of local and international baitfish stocks. Primarily, the pilchard (*Sardinops sagax*) has been the feed of choice. Origins, quality and composition of this feed, however, have varied considerably; they range from local Australian stocks to those from California and/or Japan. On some farms, Atlantic herring (*Clupea harengus*) and small blue mackerel (*Scomber scombrus*) have also been fed. Some farmers have used whole squid as a supplement. Some vitamin supplements have also been used.

To facilitate the weaning process of the juvenile SBT, a moist (400 g kg^{-1} water content) pellet has been fed to the fish during the initial phases of the production cycle in midsummer, when the fish feed extremely vigorously. The moist pellets typically have a water content in excess of 30% (Table 12.4). These have been primarily made through the addition of a formulated meal to ground baitfish to form a moist, doughlike mash, which is screw-pressed to make pellets. Under research conditions, growth performance of SBT fed moist diets is similar to that of fed baitfish (Fig. 12.5).

Most dry diets trialled to date have been hard-extruded products. While the proximate (relative protein and fat contents) composition of these feeds has not differed dramatically from that of the other feeds (Table 12.4), progress with them has been slow. The development of dry manufactured diets has been limited, primarily due to the problems encountered in weaning the juvenile SBT on to a dry artificial diet. Though weaning on to moist diets has been

Table 12.4. Proximate composition (protein, fat and moisture) of key experimental diets. Values are means ± SEM (n = 3).

	Pilchards	Dry pellets	Moist pellets
Protein (%)	17.7 ± 1.8	46.4 ± 1.1	35.9 ± 1.1
Fat (%)	7.4 ± 2.6	13.1 ± 0.6	12.2 ± 1.0
Water (%)	68.9 ± 2.1	6.0 ± 0.3	38.4 ± 0.8

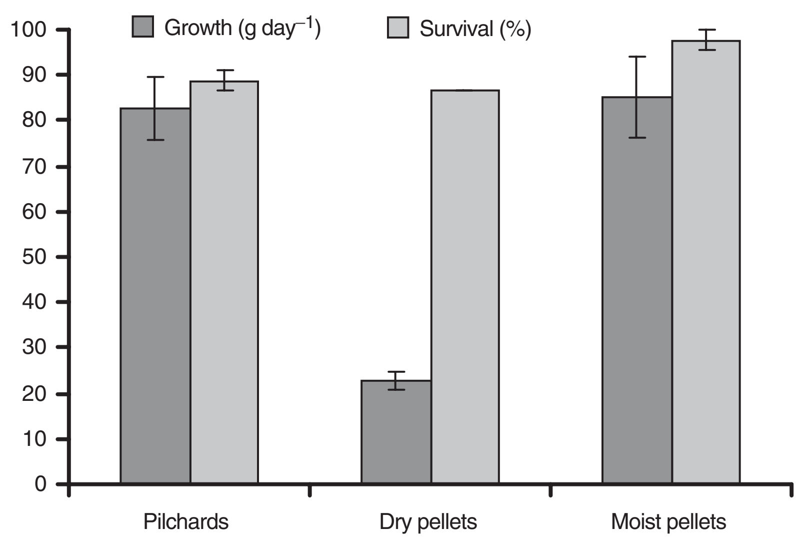

Fig. 12.5. Performance of juvenile SBT fed one of three generic feed types (Glencross *et al*., 1999a).

particularly successful, similar levels of success have not yet been achieved with dry pellets.

Feeding Practices

The feeding practices associated with SBT aquaculture have developed primarily to accommodate the tuna's huge appetite and minimize the labour intensity required. The key feeding practices vary depending on the type of food being fed.

Feeding with baitfish

Despite their generalist diet in the wild, SBT exhibit a high degree of selectivity when feeding in captivity. Preferred food appears to be clupeoids, the pilchard (*Sardinops sagax*) and other related species. Squid and other species of fish have been used with varying success. If SBT have been fed pilchards when first acclimatized into the farms, it has been very difficult to wean them on to other food types (including moist pellets).

Baitfish are fed to SBT either after thawing or in frozen blocks. Feeding of thawed baitfish is carried out by hand, requiring the shoveling of thawed baitfish into the sea cages. Some farmers have used pumps to move baitfish from boat holds to the cages via pipes and hoses. In contrast, frozen-block feeding involves placing 30 kg frozen blocks of baitfish into floating feed cages within each sea cage. As the blocks of baitfish thaw, the thawed baitfish fall through the mesh of the feed cages, where they are then consumed by the SBT. Frozen-block feeding gained considerable popularity in the late 1990s as a means of feeding almost continuously through the day, while minimizing labour inputs. Many operators currently use a combination of frozen-block and thawed feeding to ensure optimal FCR.

Feed intakes of SBT fed baitfish vary seasonally. During periods of high water temperatures (> 20°C), daily feed intake has been observed to be as high as 15% of their body weight, though 10–12% is more common. At lower water temperatures, feed intake is reduced to a level as low as 2% of their body weight (Glencross *et al.*, 2001).

Feeding with pellet diets

To date, all pellet feeding has been carried out by hand, although recent developments have examined the use of mechanized feeders. Typically this has been carried out twice daily, reducing to a single feeding during the later stages of the growing season, in response to slower feeding activity (Glencross *et al.*, 2002). Hand-feeding of moist pellets has been carried out in a similar fashion to that of baitfish, usually by shovelling chilled (not frozen) pellets to the feeding fish. Feeding has usually been regulated by visual assessment of feeding activity. Since 1998, underwater surveillance cameras have also been used to assist in determining the feeding activity and estimating feed losses on the floor of the sea cages (Glencross *et al.*, 2002). Total diet intake of pellets has always been less than that of baitfish, though it is greater on a dry-matter basis (Glencross *et al.*, 1999b).

Feeding frequency

Research over the last 3 years has indicated that feeding SBT 7 days a week does not result in significantly higher growth rates or better condition factors than a feeding strategy in which the fish are fed on 5 or 6 days a week. The industry now uses a variety of feeding strategies, with most farmers feeding 5–6 days a week during the summer growing season and less during winter.

By tracking the variations in visceral temperature using archival tags (Gunn *et al.*, 1994), it has been possible to record over periods of weeks to months when SBT have eaten in the farms (Fig. 12.6). Archival tag data have been used to compare the feeding patterns of fish fed under different feeding strategies to answer the key question of whether fish eat at every opportunity and, if not, is this related to how often they are fed? In 1999, the feeding frequency of fish fed to satiation, twice daily, 4, 5 and 6 days per week was compared, once in summer and again in winter. Table 12.5 shows the proportion of days on which food was offered and feeding took place. These show that, during summer, there was very little difference between treatments in the frequency of feeding. However, in winter, there is clear evidence that the fish that were fed only 4 days per week were feeding more frequently than those fed 5 and 6 days. During the summer, fish fed 5 and 6 days a week ate more and as a result they gained more weight than those fed 4 days per week. During the winter, fish fed 4 days per week fed more often and ate more at each feeding than those fed 5 and 6 days per week. As

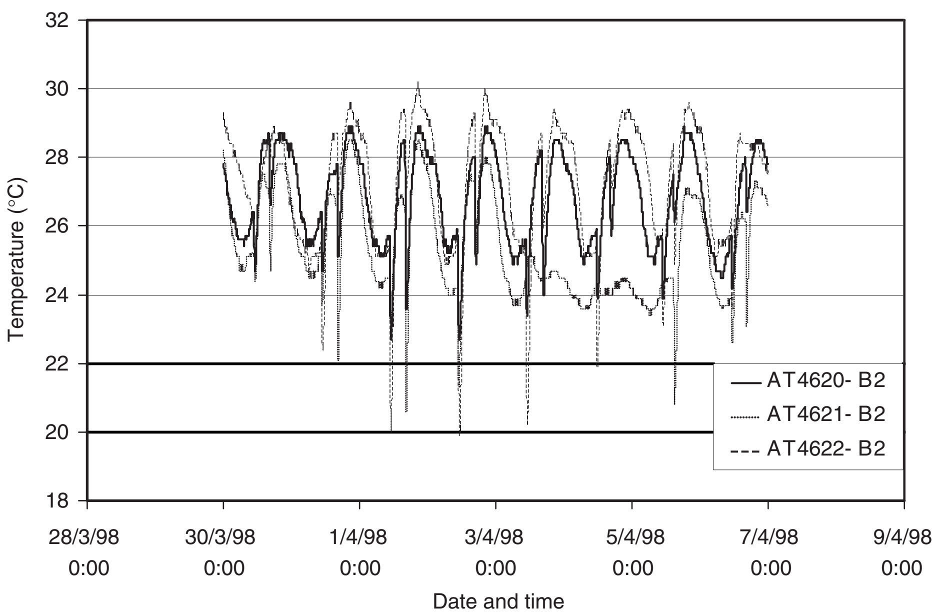

Fig. 12.6. Variation in visceral temperature in three SBT fed twice each day, 7 days per week. Note the differences in the visceral temperature and the similarity in the timing of feeding in each fish as indicated by the sharp rise in visceral temperature.

Table 12.5. Proportion of days on which a fish was offered food and a feeding event was recorded in the archival tag data.

	4 days week^{-1}	5 days week^{-1}	6 days week^{-1}
Summer	95.7%	95.3%	95.6%
Winter	78.3%	55.6%	50.60
Number of fish	13	11	9
(number of days fed summer/winter)	(46/49)	58/59)	(70/69)

a result, they either grew faster or held their weight, whereas the fish fed 5 and 6 days per week lost weight. By the end of the two experiments, there were no significant differences in the overall growth rates of fish in the three treatments.

Acknowledgements

We acknowledge the support of the Australian Fisheries Research and Development Corporation (FRDC), the Cooperative Research Centre for Aquaculture (CRC), the Tuna Boat Owners Association of South Australia, PIVOT Ltd, Ridley AgriProducts, the South Australian Research and Development Institute and the Commonwealth Scientific and Industrial Research Organization Division of Marine Research.

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